Effects of pre-ensiling treatments on quality of lucerne silages, *in vitro* rumen fermentation and microbiota composition

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Thomas Dietmar Said Hartinger, MSc

aus

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First supervisor:	Prof. Dr. Karl-Heinz Südekum
Second supervisor:	Prof. Dr. Jürgen Hummel
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SUMMARY

Effects of pre-ensiling treatments on quality of lucerne silages, *in vitro* rumen fermentation and microbiota composition

Lucerne (*Medicago sativa* L.) silages (LS) are commonly fed to ruminants. Due to substantial proteolysis during ensiling, the majority of crude protein (CP) in LS is non-protein nitrogen (NPN), which is rapidly degraded to ammonia in the rumen and to a great extent ultimately lost in urine, meaning increased environmental pollution. Post-harvest conditions strongly affect LS properties, notably the CP fraction by decelerating proteolysis, hence resulting in silages with less NPN and finally a potentially increased ruminal N utilization from LS. Therefore, the present thesis investigated the effects of the pre-ensiling treatments dry matter (DM) concentration, wilting intensity and sucrose addition on LS quality, *in vitro* rumen fermentation and microbiota composition. The first article of this thesis provides a detailed literature review on major nitrogenous compound-degrading rumen microbes and options to manipulate their activity and abundance in the rumen. The next study measured the effects of the aforementioned pre-ensiling treatments on LS quality with the novel aspect of wilting intensity, which has not been considered so far for lucerne ensiling. All three pre-ensiling treatments reduced the NPN concentration in the LS and combining high-intensity wilting to 35% DM concentration with sucrose addition resulted in highest true protein preservation, in which sucrose addition further enhanced lactic acid fermentation. The subsequent article presents the rumen fermentation characteristics during LS incubation in a long-term *in vitro* rumen simulation system. A higher DM concentration and the addition of sucrose increased the concentration of total volatile fatty acids and gas production, but reduced fibre degradability. High-intensity wilting increased hemicellulose degradability but decreased the degradability of organic matter, whereas most variables of ruminal N utilization were less affected. The last study focuses on how the pre-ensiling treatments affected the microbiota composition in the rumen simulation system. Sucrose addition was the main driver for alterations in the microbial community, but also adaption time of the microbes to the LS clearly shaped the composition. Overall, this study demonstrates that pre-ensiling treatments can substantially support a sufficient fermentation and true protein preservation of LS during ensiling. Although these beneficial effects were not completely rediscovered during *in vitro* rumen fermentation, promising results on fibre degradability encourage further investigations. The microbiological analysis revealed first insights on how the pre-ensiling treatments affect the ruminal microbiota composition, which might help to understand alterations in the rumen fermentation. In conclusion, the applied pre-ensiling treatments improve the quality of LS and have potential to increase the ruminal N utilization from LS. Efforts in this direction should be pursued and refined in the future.

ZUSAMMENFASSUNG

Einflüsse verschiedener Vorbehandlungen auf die Qualität von Luzernesilagen, *in vitro* Pansenfermentation und die mikrobielle Gemeinschaft

Luzernesilagen (*Medicago sativa* L.; LS) werden häufig in der Wiederkäuerfütterung eingesetzt. Aufgrund umfangreicher Proteolyse während der Silierung besteht der größte Teil des Rohproteins (RP) in LS aus Nicht-Protein-Stickstoff-Verbindungen (NPN), die im Pansen schnell zu Ammoniak abgebaut und schließlich ausgeschieden werden, was zu erhöhten Emissionen führt. Die Nachernte-Bedingungen haben großen Einfluss auf die Eigenschaften von LS, insbesondere auf das RP, da verlangsamte proteolytische Prozesse in LS mit geringerem NPN-Anteil und möglicherweise einer verbesserten ruminalen N-Nutzung resultieren. In der vorliegenden Arbeit wurden daher die Effekte der Erntegut-Behandlungen Trockenmasse-(TM)-Konzentration, Anwelkintensität und Saccharosezugabe auf die LS-Qualität sowie die Pansenfermentation und Mikrobiotazusammensetzung *in vitro* untersucht. Der erste Teil der Arbeit ist eine Literaturübersicht über die wichtigsten am ruminalen RP-Abbau beteiligten Mikroben und zeigt Möglichkeiten, ihre Aktivität und Präsenz im Pansen zu beeinflussen. Anschließend werden die Auswirkungen der oben genannten Erntegut-Behandlungen auf die LS-Qualität unter besonderer Beachtung der Anwelkintensität erörtert. Alle drei Behandlungen reduzierten den NPN-Anteil in den LS und die Kombination von intensivem Anwelken auf 35 % TM mit Zugabe von Saccharose führte zur höchsten Reinproteinkonservierung, wobei die Saccharosezugabe zudem die Milchsäuregärung verstärkte. Die anschließende Studie untersucht die Pansenfermentationsprofile während der LS-Inkubationen in einem Langzeit-*in vitro*-Pansensimulationssystem. Eine höhere TM-Konzentration und die Zugabe von Saccharose erhöhten die Gesamtkonzentration der flüchtigen Fettsäuren und die Gasbildung, verringerten jedoch den Faserabbau. Intensives Anwelken erhöhte den Abbau von Hemicellulosen, verringerte jedoch den Abbau der organischen Masse, während die meisten Kenngrößen der ruminalen N-Nutzung nur geringfügig beeinflusst wurden. Die letzte Studie befasst sich mit den Auswirkungen der Erntegut-Behandlungen auf die Zusammensetzung der Mikrobiota im Pansen-simulationssystem. Dabei konnte Saccharose als Hauptursache für Änderungen in der mikrobiellen Gemeinschaft ausgemacht werden, wobei auch die Anpassungszeit der Mikroben an die LS einen klaren Effekt auf die Zusammensetzung hatte. Insgesamt zeigt die vorliegende Arbeit, dass Behandlungen des Ernteguts vor der Silierung eine ausreichende Kohlenhydratfermentation und Reinproteinkonservierung in LS maßgeblich unterstützen können. Obwohl die *in vitro* Pansenfermentation dies nicht vollständig widerspiegelt, fordern die Ergebnisse zum Faserabbau zu weiterer Forschung auf. Die mikrobiologische Analyse gab einen ersten Einblick, wie die Behandlungen auf die Mikrobiota wirken, was zum Verständnis der Änderungen in der Pansenfermentation beitragen kann. Die hier angewandten Behandlungen

verbessern die Qualität von LS und haben ein beträchtliches Potenzial, die ruminale N-Nutzung bei LS zu verbessern. Bestrebungen in diese Richtung sollten verfolgt und weiterentwickelt werden.

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ABBREVIATIONS

*	$P < 0.05$
**	$P < 0.01$
#	$0.05 < p < 0.1$
25HI, 250HI	250 g kg^{-1}, high-intensity wilting and no sucrose addition
25HISA, 250HISU	250 g kg^{-1}, high-intensity wilting and sucrose addition
25LI, 250LI	250 g kg^{-1}, low-intensity wilting and no sucrose addition
25LISA, 250LISU	250 g kg^{-1}, low-intensity wilting and sucrose addition
35HI, 350HI	350 g kg^{-1}, high-intensity wilting and no sucrose addition
35HISA, 350HISU	350 g kg^{-1}, high-intensity wilting and sucrose addition
35LI, 350LI	350 g kg^{-1}, low-intensity wilting and no sucrose addition
35LISA, 350LISU	350 g kg^{-1}, low-intensity wilting and sucrose addition
AA	Amino acid
ADFom	Acid detergent fibre after incineration
ADL	Acid detergent lignin
Ammonia-N_{blank}	Ammonia-nitrogen in the ruminal fluid-buffer solution without sample substrate
Ammonia-N_{rumen}	Ammonia-N concentration in the rumen
Ammonia-N_{sample}	Ammonia-nitrogen in the ruminal fluid-buffer solution with sample substrate
An. fungi	Anaerobic fungi
aNDFom	Neutral detergent fibre after incineration and amylase treatment
AS	Alfalfa silage
B.	*Butyrivibrio*
BCVFA	Branched-chain volatile fatty acid
Cl.	*Clostridium*
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
CT	Condensed tannin
d	day
DM	Dry matter
DML	Dry matter level

EC	European Commission
e.g.	Exempli gratia
En.	*Entodinium*
ET	Early time point
Eu.	*Eubacterium*
FIR	First time point
GABA	Gamma-aminobutyric acid
h	hour
HAB	Hyper-ammonia producing bacteria
HI	High-intensity wilting
i.e.	Id est
Ig	Immunoglobulin
L.	*Lactobacillus*
LI	Low-intensity wilting
LS	Lucerne silage, Luzernesilage
LT	Late time point
M.	*Megasphaera*
MCPF	Microbial crude protein attached to the feed residue
N	Nitrogen
NC	Nitrogenous compound
NH_3	Ammonia
NSC	Non-structural carbohydrate
Ncm.	*Neocallimastix*
NGS	Next-generation sequencing
NPN	Non-protein nitrogen, Nicht-Protein-Stickstoff
NS	Not significant
N_{sample}	Nitrogen added to the syringe through the sample substrate
OM	Organic matter
OTU	Operational taxonomic unit
P.	*Prevotella*
PBLC	Plant bioactive lipid compound
PCoA	Principal co-ordinate analysis

PD	Phylogenetic diversity
pH_{rumen}	pH value in the rumen
PPO	Polyphenol oxidase
Psc.	*Peptostreptococcus*
qPCR	Quantitative real-time polymerase chain reaction
R^2	Coefficient of determination, R-square
Rb.	*Ruminobacter*
RDA	Redundancy analysis
RP	Rohprotein
RUP	Rumen-undegraded dietary crude protein
Rusitec	Rumen-simulation technique
S.	*Saccharomyces*
SA	Sucrose addition
SCFA	Short-chain fatty acid
SEC	Second time point
Sel.	*Selenomonas*
SEM	Standard error of the mean
Staph.	*Staphylococcus*
Strep.	*Streptococcus*
SU	Sucrose addition
TP	True protein
uCP	Utilizable crude protein at the duodenum
uCP2	Effective utilizable crude protein at the duodenum to passage rate of 0.02 hr^{-1}
uCP5	Effective utilizable crude protein at the duodenum to passage rate of 0.05 hr^{-1}
uCP8	Effective utilizable crude protein at the duodenum to passage rate of 0.08 hr^{-1}
VDLUFA	Association of German Agricultural Analytic and Research Institutes
VFA	Volatile fatty acid
WI	Wilting intensity
WSC	Water-soluble carbohydrate

CHAPTER 1

General introduction

The agricultural sector significantly contributes to environmental pollution, the anthropogenic climate change and an intense usage of scarce resources (Tamminga, 1996; Bouwman et al., 1997; Powell et al., 2010). In response to public and political pressure, the reduction of environmental pollution from livestock farming is one of the main challenges for modern agriculture and apart from methane and phosphorus-containing substances, nitrogenous compounds emitted by ruminants (Tamminga, 1996) are in the focus of attention (Powell et al., 2010). Nitrogenous compounds like ammonia and nitrate contaminate soil and water (Tamminga, 1996) or in case of nitric oxide contribute to the anthropogenic greenhouse effect (Flachowsky et al., 2017). Over the last two decades, the global beef and milk consumption has increased by 21% and 39% (Food and Agriculture Organization of the United Nations, 2019), respectively, and is still expected to maintain rising (Thornton, 2010). This will aggravate the problems of livestock-related emissions on the environment (Flachowsky et al., 2017, 2018) and sustainable solutions, which allow the efficient input of resources, are mandatory to decrease nitrogen (N) emissions from beef and milk production.

Concurrently, the world population will constantly increase (Thornton, 2010; Meale et al., 2014) causing an ever-growing competition between the use of crops directly for human nutrition or as feedstuffs for animals (Thornton, 2010; Flachowsky et al., 2017). In this regard, ruminants possess the advantage of being able to utilize plant biomass indigestible for humans (Meale et al., 2014) and thus are not in direct food competition with humans, but provide valuable foodstuffs. The rumen is the predominant place, where feedstuffs are degraded and fermented by a complex microbial community of bacteria, archaea, protozoa and anaerobic fungi (Puniya et al., 2015), which altogether enable the host the energetic and nutritious utilization of protein-N, non-protein-N (NPN) as well as carbohydrates with α- and ß-glycosidic linkages (Van Soest, 1994). To maintain rumen health and avoid digestive dysfunctions, a sufficient provision with physically effective fibre is required, which implies roughages to be a main constituent of ruminant rations. Besides grasses, also forage legumes rank among roughages and they are characterized by high crude protein (CP), but low water-soluble carbohydrate (WSC) concentrations (McDonald et al., 1991; Lüscher et al., 2014). Depending on vegetative stage, CP comprises about 20% of dry matter (DM) in legumes, which is facilitated by their unique ability to symbiotically fix atmospheric N – up to 500 kg N per ha and year – which further on improves soil fertility and can partly or fully replace the application of mineral N fertilizers (Lüscher et al., 2014). Consequently, legumes

can be important components of ruminant rations as they constitute an inexpensive on-farm produced protein source that provides sufficient amounts of fibre and simultaneously can supply a considerable part of the animals' demand for amino acids (AA). Additionally, societal concerns regarding deforestation, cultivation and import of genetically modified plants (Alvensleben, 2001; Flachowsky and Aulrich, 2001) favour the usage of home-grown forage legumes as protein rich feedstuffs for dairy and beef cattle in future.

Lucerne (*Medicago sativa* L.) is the most commonly used forage legume in cattle and particularly dairy rations (Barros et al., 2017; Broderick, 1995b; Kornfelt, 2012), but as the annual vegetative growth periods are limited in most geographical regions, lucerne must be preserved to allow a continuous feeding throughout the year (Huhtanen et al., 2010). Ensiling is a widely applied conservation method for roughages and less weather dependent compared to field drying that bears a high risk for feed losses due to inappropriate atmospheric conditions (Broderick, 2018; Rotz, 1995). Although artificial drying would offset this risk, it also means substantial costs for energy and machinery equipment (Mandell et al., 1989; Rotz, 1995), therefore reducing the competiveness and sustainability of this conservation method. The principle of ensiling is the spontaneous fermentation of sugars to lactic acid under anaerobic conditions by hetero- and homofermentative lactic acid bacteria (McDonald et al., 1991). The formed lactic acid reduces the pH and inhibits the activity of spoilage microorganisms, e.g. clostridia and enterobacteria (Hoedtke et al., 2010), and therefore preserves the feed value of the roughage. Concerning forage legumes, however, high buffering capacities and low WSC concentrations (Lüscher et al., 2014) make them difficult to ensile and a considerable part of the CP undergoes massive degradation resulting in large amounts of low-molecular-weight CP. These CP degradation processes can be ascribed to the synergetic effect of plant-derived and microbial enzymes before and during ensiling, respectively (Hoedtke et al., 2010). As a result, between 50 and 87% of total CP in lucerne silages is NPN (Broderick, 1995a; Guo et al., 2008; Coblentz and Grabber, 2013), i.e. "oligopeptides, free amino acids, ammonium compounds, and other small molecules" (Broderick, 1995a).

The CP fraction of lucerne silages is highly rumen-degradable (Coblentz and Grabber, 2013) and its large proportion of low-molecular-weight CP is rapidly broken down to ammonia by rumen microorganisms (Tamminga, 1979). Although ammonia constitutes the main and partly even sole N source for rumen microbes (Russell et al., 1992), excessive ruminal proteolysis and deamination result in inordinate amounts of ammonia that exceed the microbial fixation capacity (Annison, 1956). The majority of the oversupplied ammonia will then be absorbed from the rumen, converted to urea in the liver and predominantly excreted via the urine (Coblentz and Grabber, 2013), leading to increased environmental pollution,

poor AA provision to the host and a higher metabolic burden that may reduce animal performance (Coblentz and Grabber, 2013). This scenario will be particularly true for feeding rations with moderate or high CP concentrations as the ammonia originating from ureolysis primarily returns to the blood rather than mixing with the ruminal fluid (Lu et al., 2014). Consequently, the efficiency of CP utilization from lucerne silage-based rations is low (Broderick, 2018) and enhanced nitrogenous emissions are the outcome. When feeding such rations, however, ruminants particularly rely on an efficient microbial N utilization in the rumen and efforts must be made to maximise the N utilization from lucerne silages. Thereby, two main strategies can be pursued.

First, the synchronized supplementation of concentrate, meaning rapidly available dietary energy (Owens et al., 1998), to enhance the energy-dependent ruminal N fixation may constitute an option. However, this also means higher feeding costs and workload, an increased nutrient input into the farming cycle as well as a greater risk for rumen acidosis (Owens et al., 1998), which in total limits the provision of concentrate to ruminants. Thus, adequately meeting the microbial energy demand for fixing the available N emerging from rapid NPN degradation seems hardly feasible – unless depressed ruminal fibre degradation, potential harmful alterations of the microbiome as well as impaired rumen health and animal welfare (Zebeli and Metzler-Zebeli, 2012) are recklessly accepted.

The second option is the direct manipulation of the CP fraction in lucerne silages targeting a decelerated proteolysis in the silo, therefore preserving higher true protein (TP) proportions in the silages and accordingly less rapidly rumen-degradable NPN. Various approaches have been investigated so far, including the application of organic acids (Muck, 1988), bacterial inoculants (Seale et al., 1986), protein-binding compounds like tannins (Tabacco et al., 2006) or different pre-ensiling treatments (Seale et al., 1986; Owens et al., 1999), i.e. diverse DM concentrations, wilting or the addition of a WSC source. Studies evaluating the effect of tannins on silage CP composition have shown decreased concentrations of NPN and less ruminal CP disappearance compared to untreated silages (Tabacco et al., 2006). Likewise, by altering the solubility and protein structure (Waltz and Stern, 1989) along with rapidly decreasing the pH (Muck, 1988), organic acids preserve TP in silages (Muck, 1988). Albeit, the application of organic acids cause costs for acquisition and maintenance of corroded materials (Lorenzo and O'Kiely, 2008), while tannins often reduce feed intake (Vasta et al., 2010) due to reduced palatability and lower ruminal degradation rates (Makkar et al., 1995; Tabacco et al., 2006), which may outweigh the advantages of protein preservation. Additionally, although the protein-tannin-complexes are cleaved in the abomasum, the risk of re-complexing or tannin-binding to host enzymes in lower gut sections remains present (Jones and Mangan, 1977).

Ensiling conditions strongly affect the CP composition and general quality of the emerging silages (Muck, 1988). Therefore, by shaping these conditions via different pre-ensiling treatments, lucerne silage properties may be manipulated in a beneficial way. First, higher DM concentrations, i.e. above 30%, should be targeted as below this threshold, plants are extremely susceptible to clostridial fermentation (Kung and Shaver, 2001), which results in elevated pH as well as high butyric acid loads from deamination processes (Hoedtke et al., 2010). Regarding legumes, however, it is particularly important to not exceed certain DM levels as mechanical losses during harvest (Rotz, 1995) and therewith associated nutrient losses may otherwise nullify the potential advantages on silage quality.

It has been shown that wilting per se affects the CP composition in lucerne silages, for instance by reducing ammonia-N concentrations (Zheng et al., 2017). Thus, applying high-intensity wilting treatments may even be more effective for stabilizing TP concentrations as it rapidly reduces the activity of water-dependent plant-derived proteases, which otherwise degrade proteins already before ensiling (Hoedtke et al., 2010). Moreover, high-intensity wilting may preserve WSC from degradation by plant enzymes or respiration losses, which is both decreased by a rapid moisture loss of the plant (Wylam, 1953). Hitherto, wilting intensity has not received much attention during silage preparation, but should be taken into account as prior grass silage-based research indicated that not solely the final DM concentration affects the silage composition and subsequent intra-ruminal N recycling, but also under which conditions this DM is achieved (Edmunds et al., 2014). Edmunds et al. (2014) observed increased proportions of moderately and slowly ruminally degradable TP and less NPN with both higher DM concentrations and higher wilting intensity. Likewise, NPN concentrations in lucerne increased with higher levels of shade during wilting (Owens et al., 1999).

The low WSC concentration and simultaneously high buffering capacity of lucerne (McDonald et al., 1991) often hampers a successful ensiling of this forage species. Thus, even with the addition of lactic acid producing bacteria, the resulting lucerne silage quality may be deficient as there is a lack of fermentable substrate for these microorganisms (Seale et al., 1986). Thus, a further provision of WSC is highly recommended to ensure a fast and sufficient acidification in the silo, which decreases ammonia-N formation and establishes a stable silage fermentation (Muck, 1988; Seale et al., 1986).

Finally, the quality of the lucerne silage CP entering the rumen is altered and also the dietary energy provision may be different, which both should affect the intra-ruminal N recycling and therefore the efficiency of N utilization in the rumen. So far, studies investigating the effect of lucerne silages prepared with different pre-ensiling treatments on rumen fermentation are not available, but grass silages with high concentrations of free AA (> 300 mmol/kg DM) and less

TP resulted in higher concentrations of ammonia along with a lower efficiency of N assimilation into microbial protein by liquid-associated bacteria *in vitro* (Gresner et al., 2015). Accordingly, high-intensity wilted grass silages had higher ruminally undegraded CP concentrations during *in situ* and lower ruminal ammonia concentrations during *in vitro* incubations (Edmunds et al., 2014). Both studies provide indications for a favourable manipulation of the intra-ruminal N recycling by pre-ensiling treatments and further point out to alterations in the rumen microbiome, namely its composition and metabolic activity. With an altered quality of lucerne silage CP, adaptions may occur within the CP-degrading fraction of the ruminal microbiome, but due to continuous interactions (Puniya et al., 2015), shifts in its fibrolytic part should not be neglected. However, there is a lack of research studying the effects on the rumen microbiome. Experiments providing comprehensive insights into the intra-ruminal N recycling and fibre degradation will be a prerequisite to evaluate the suitability of pre-ensiling treatments for lucerne preservation and therefore allow the optimization of ruminal N utilization from lucerne silages in future.

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CHAPTER 2

Scope of the thesis

The research presented in this thesis is part of a project that is funded by the “Deutsche Forschungsgemeinschaft” (DFG, German Research Foundation) and comprises four manuscripts addressing the questions and problems formulated in the general introduction (Chapter 1). The Chapters 3 and 4 are manuscripts, which are published in scientific journals, whereas the manuscripts in Chapter 5 and 6 have been submitted to or prepared for a scientific journal, but have not yet been published. Consequently, all these Chapters are formatted to the guidelines and layout of the respective journals, but the font has been adjusted to harmonise the appearance of the present thesis.

The first manuscript (Chapter 3) represents a systematic overview on the current state of knowledge regarding microorganisms involved in intra-ruminal nitrogen (N) recycling as well as their interactions and subsequently illustrates dietary options, inclusive of vaccination, that have been applied to manipulate the abundance and activity of nitrogenous compound-degrading rumen microbes. These options are discussed for their effectiveness, but also assessed in terms of their compatibility with other important factors of ruminant nutrition, e.g. feed intake or ruminal fibre degradation. The objective was to outline the very sparse information about how pre-ensiling treatments affect the intra-ruminal N recycling and the nitrogenous compound-degrading rumen microbiome, therefore emphasizing the need for research addressing this topic.

The effects of three pre-ensiling treatments, i.e. DM concentration, wilting intensity and sucrose addition, on the CP fraction and general quality of lucerne silages are subject of the second manuscript (Chapter 4). Thereby, lucerne silage material was comprehensively characterized using wet chemical laboratory methods, including CP fractionation as well as analysis of AA composition and fermentation acids. Additionally, a ruminal fluid-based *in vitro* incubation method was applied to estimate the content of utilizable CP at the duodenum, a precursor to metabolizable protein. High-intensity wilting alone or in combination with further pre-treatments was hypothesized to influence the CP composition in lucerne silages and reduce proteolysis during ensiling. The highest TP preservation was assumed to be present in lucerne silages, which received a high-intensity wilting treatment to high DM concentration combined with a sucrose addition.

After addressing the effects of pre-ensiling treatments on lucerne silage composition, alterations of the *in vitro* ruminal fermentation are presented and discussed in the third

manuscript (Chapter 5). The lucerne silages described in Chapter 4 were incubated in the *in vitro* rumen-simulation technique (Rusitec) and two and seven days after first-time incubating these silages, various characteristics of rumen fermentation were monitored, comprising volatile fatty acids concentrations, pH, fibre degradability as well as variables directly or indirectly related to intra-ruminal N recycling, e.g. concentrations of ammonia-N, AA-N, or branched-chain volatile fatty acids. Thereby, an improved fermentation along with reduced protein degradation as well as increased microbial CP production in high-intensity wilted and sucrose-treated lucerne silages were hypothesized. Secondly, greater fibre degradability in response to a microbial adaptation to the lucerne silages over time was expected.

Chapter 6 comprises a manuscript describing the effects of the pre-ensiling treatments and adaptation time on the microbial community composition in the liquid and solid phase of the Rusitec system. Absolute quantities of the domains bacteria, archaea and anaerobic fungi were determined by quantitative real-time polymerase chain reaction analysis. Moreover, microbial community composition was investigated using 16S rRNA gene amplicon sequencing with subsequent bioinformatic data analysis. Thereby, the hypothesis of contrasting microbiota compositions between the pre-ensiling treatments was set up, with higher microbial abundances and diversities in the microbiota deriving from sucrose-treated lucerne silage incubation.

Lastly, Chapter 7 debates results and discussion key points from Chapters 3 to 6 in an overall context regarding improved protein preservation during ensiling and a subsequently more efficient ruminal N utilization. This general discussion is accompanied by an outlook illustrating potentials and trends, which can relevantly contribute to a resource-efficient and more sustainable beef and milk production from lucerne silages in future.

CHAPTER 3

Does intra-ruminal nitrogen recycling waste valuable resources? A review of major players and their manipulation

T. Hartinger[1], N. Gresner[1], K.-H. Südekum[1]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany

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Abstract

Nitrogenous emissions from ruminant livestock production are of increasing public concern and, together with methane, contribute to environmental pollution. The main cause of nitrogen-(N)-containing emissions is the inadequate provision of N to ruminants, leading to an excess of ammonia in the rumen, which is subsequently excreted. Depending on the size and molecular structure, various bacterial, protozoal and fungal species are involved in the ruminal breakdown of nitrogenous compounds (NC). Decelerating ruminal NC degradation by controlling the abundance and activity of proteolytic and deaminating microorganisms, but without reducing cellulolytic processes, is a promising strategy to decrease N emissions along with increasing N utilization by ruminants. Different dietary options, including among others the treatment of feedstuffs with heat or the application of diverse feed additives, as well as vaccination against rumen microorganisms or their enzymes have been evaluated. Thereby, reduced productions of microbial metabolites, e.g. ammonia, and increased microbial N flows give evidence for an improved N retention. However, linkage between these findings and alterations in the rumen microbiota composition, particularly NC-degrading microbes, remains sparse and contradictory findings confound the exact evaluation of these manipulating strategies, thus emphasizing the need for comprehensive research. The demand for increased sustainability in ruminant livestock production requests to apply attention to microbial N utilization efficiency and this will require a better understanding of underlying metabolic processes as well as composition and interactions of ruminal NC-degrading microorganisms.

Background

Understanding the rumen metabolism is of central importance [1, 2] and a prerequisite to meet the animal's requirements for nutrients and energy. The rumen microbiota constitutes a complex ecosystem, the metabolic activity of which is responsible for rumen metabolism, including intra-ruminal N recycling [3]. Thus, it is a key factor that needs to be taken into consideration when a sustainable and efficient livestock production is pursued. Rumen microbiota-related studies have so far focused on cellulolytic microorganisms, their metabolic pathways and how to optimize ruminal fiber degradation [4, 5]. However, in the rumen, the vast majority of dietary crude protein is microbially degraded to ammonia [6], which constitutes the main and sometimes even sole N source for rumen microorganisms [7]. Excessive ruminal proteolysis and deamination cause inordinate amounts of ammonia, which are absorbed by the ruminant, converted to urea and subsequently predominantly excreted via the urine, leading to increased environmental pollution [8] and poor amino acid (AA) supply to the host. Hence, an efficient utilization of crude protein should be aimed at; also, to ensure the maximum retention of N, knowledge of ruminal NC-degrading microorganisms is indispensable [9].

Research on the abundance, composition and metabolism of NC-degrading microorganisms is particularly needed for developing strategies to cope with the challenge of finding the optimal balance between the inhibition of ruminal NC degradation, without compromising post-ruminal AA absorption, and the simultaneous provision of appropriate amounts of N for the rumen microbiota. Improvements in techniques for studying microbial communities already allow the broad use of culture-independent techniques [10], which enable a more comprehensive characterization of the rumen microbiota compared to cultivation [11]. Omics-based approaches and quantitative real-time polymerase chain reaction (qPCR) assays have markedly enhanced our understanding of the rumen microbiota and are inevitable for the investigation of NC-degrading microorganisms. In particular, when omics methods are combined to analyze not only the abundance and diversity of genes, but also functional compositions as well as protein and metabolite profiles, a deeper knowledge will be obtained [10]. However, as omics approaches are not sufficient to target microorganisms on a species level [12] or to determine absolute abundances [13], qPCR represents an indispensable tool for the investigation of single microbial key species of ruminal NC degradation [8, 14]. The present review represents a starting point and aims to encourage research targeting the lack of knowledge of NC-degrading microorganisms, thereby developing and optimizing strategies for manipulating them. To give a critical status quo on this topic, existing information on the activity and abundance of rumen microorganisms involved in the degradation of proteins,

peptides, AA and urea, as well as their principal interactions, is briefly summarized in the first part of this review. So far, this information is limited and needs expansion by state-of-the-art technologies regarding all aspects of the rumen microbiome, i.e. genome, transcriptome, proteome and metabolome, finally leading to a better understanding of both, its structure and its function. Thereby, functional characterization by omics addressing also uncultivable microorganisms will expand our current knowledge on NC-degrading microbes that was generated predominantly by cultivation and enzymatic activity tests. The second review part will cover options that have been considered so far to influence NC-degrading microorganisms by dietary factors as well as vaccination. Here, we based our review on a systematic literature search, as the high diversity in experimental conditions and applied techniques between the contemplated studies make a meta-analysis inappropriate.

Ruminal microorganisms involved in the degradation of nitrogenous compounds

Rumen microbes are supplied with NC by the diet and with that mainly as proteins, peptides and AA. In addition to the potential provision of urea by feed [6, 15], endogenous urea is supplied to the rumen via the rumino-hepatic circulation [16]. Depending on NC, different ruminal microorganisms are involved in their breakdown (Table 1) and synergistic microbial enzyme activities are often required for the complete degradation of NC to ammonia [17, 18] (Fig. 1). However, one has to emphasize that published studies quantifying the abundances of microorganisms are very heterogenic in their sampling, as well as quantification methods, thus complicating their comparison. High standardization of experimental conditions help to diminish this problem and should be considered in future study designs. As bacterial, protozoal and fungal cells contain different copy numbers of 16S rDNA, 18S rDNA or internal transcribed spacer 1, respectively [19–21], it is particularly difficult to put data from culture-independent approaches in relation to earlier results obtained from cultivation. Moreover, culture-independent techniques allow species-specific identification, but rumen microorganisms are often only characterized on a genus level, e.g. *Prevotella* by Deckardt et al. [22]. Due to the great heterogeneity within one genus [23], the interpretation of such results becomes even more challenging and a considerable part of the potentially acquired information is easily lost.

Table 1. Overview of microorganisms involved in the ruminal degradation of proteins, peptides, AA and urea*.

Group	Microbial species	Proteins	Peptides	AA	Urea	Reference
Bacteria	*Allisonella histaminiformans*			X		[85, 86]
	Butyrivibrio fibrisolvens	X		X		[39, 82]
	Butyrivibrio proteoclasticus	X				[40]
	Clostridium sp.			X		[90]
	Clostridium aminophilum			X		[90]
	Clostridium sticklandii			X		[90]
	Eubacterium sp.	X		X		[60, 82]
	Eubacterium budayi	X				[40]
	Eubacterium pyruvativorans			X		[95]
	Eubacterium ruminantium		X			[66]
	Fibrobacter succinogenes		X			[66]
	Fusobacterium sp.	X				[39]
	Howardella ureilytica				X	[102]
	Klebsiella aerogenes				X	[99]
	Lachnospira multipara	X	X			[62, 66]
	Lactobacillus casei var. *casei*				X	[99]
	Micrococcus varians				X	[100]
	Megasphaera elsdenii		X	X		[66]
	Peptostreptococcus anaerobius			X		[90]
	Prevotella sp.	X	X	X		[67, 83]
	Prevotella albensis	X	X			[52, 74]
	Prevotella brevis	X	X			[65]
	Prevotella bryantii	X	X			[65]
	Prevotella ruminicola	X	X	X		[64, 83]
	Ruminobacter amylophilus	X				[41]
	Selenomonas ruminantium	X		X	X	[15, 55, 82]
	Staphylococcus sp.				X	[99]
	Staphylococcus saprophyticus	X		X	X	[100]
	Streptococcus bovis	X	X	X		[40, 66, 82]
	Streptococcus faecium				X	[99]
Protozoa	*Dasytricha* sp.	X				[107]
	Dasytricha ruminantium	X	X			[105, 114]

Table 1 continued.

Group	Microbial species	Proteins	Peptides	AA	Urea	Reference
	Entodinium spp.	X	X			[113, 114]
	Entodinium caudatum	X		X		[113]
	Entodinium simplex	X				[107, 113]
	Epidinium sp.	X				[107]
	Epidinium caudatum ecaudatum	X				[105, 107, 114]
	Isotricha spp.	X	X			[105, 107]
	Ophryoscolex caudatus	X				[105]
	Polyplastron multivesiculatum	X				[105]
Fungi	*Neocallimastix frontalis*	X	X			[124, 128]
	Neocallimastix patriciarum	X				[126]
	Orpinomyces joyonii	X				[126]
	Piromyces sp.	X	X			[128]
	Piromyces communis	X				[126]

*Without consideration of detection method, quantity of substrate degradation or impact on ruminal N metabolism

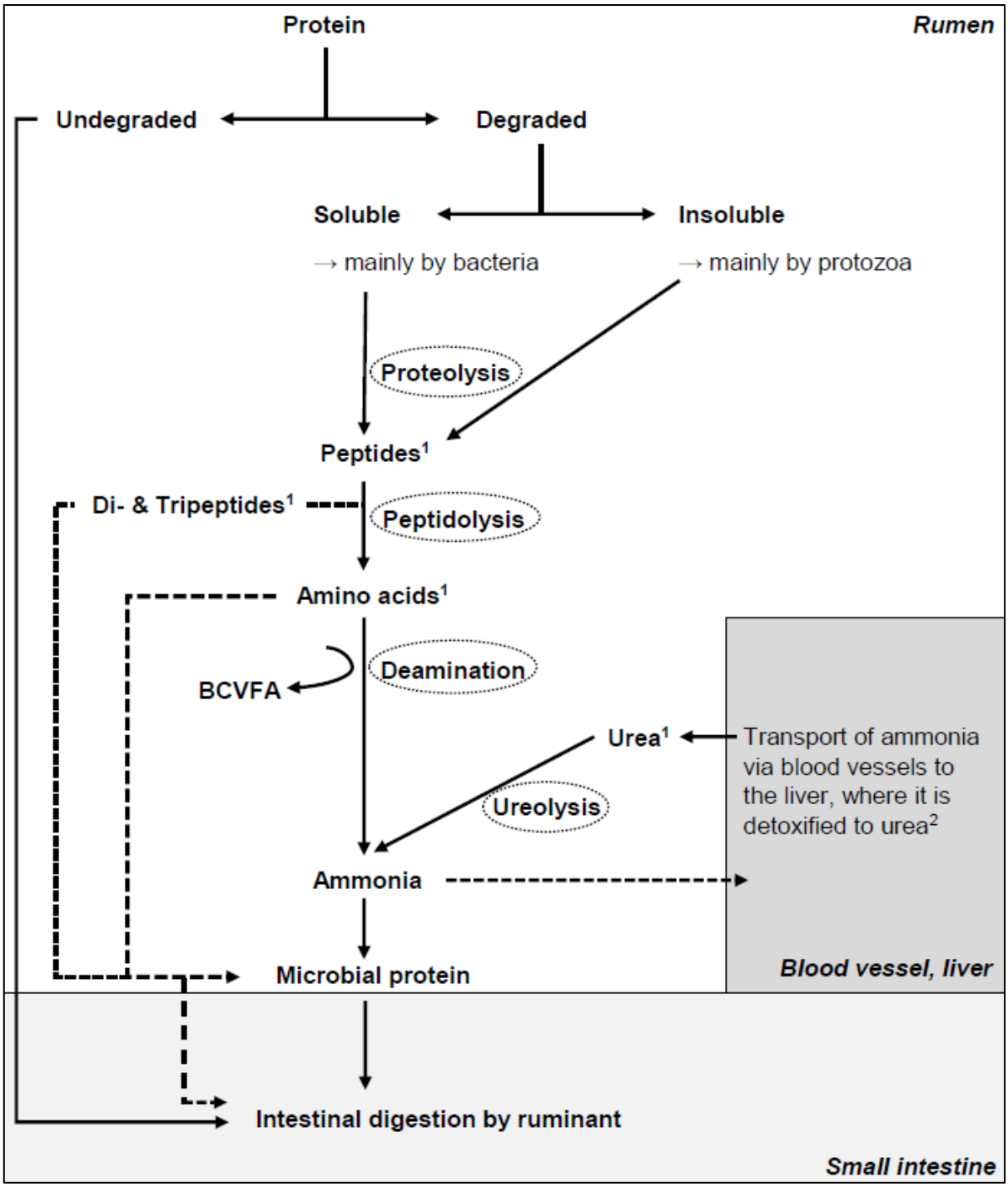

Figure 1. Simplified scheme of intestinal N metabolism and the target sites of manipulation strategies for reducing ruminal NC degradation that have shown effectiveness in vivo or in vitro (according to [6, 22, 86, 87, 102, 138, 151, 155, 156, 160, 166, 168, 202, 208, 218]). [1]This NC can also be supplied with the feed; [2]Urea is partly excreted with urine.

Bacteria

Proteolytic bacteria

Bacteria represent the most abundant domain in the rumen [24] and are present in numbers of 10^{10}–10^{12} cells/g rumen content [2, 11]. In cattle, using rRNA targeted oligonucleotide probes, Lin et al. [25] assigned 60–84% of total rRNA to this domain, while Prins et al. [18] had previously assigned 65% of ruminal proteolytic activity to bacteria, which underlines their involvement in NC degradation.

Two important key species for protein degradation belong to the highly abundant genus *Butyrivibrio* [24], namely *Butyrivibrio fibrisolvens* [26] and *B*. *proteoclasticus* [27]. They are present in ruminant species across all continents [24] and exert high proteolytic activities [27–29]. The increased number of 16S rDNA copies of *B*. *fibrisolvens* when protein supply to dairy cows was increased [30] may confirm its role in protein metabolism, and in sheep *B*. *fibrisolvens* and *B*. *proteoclasticus* accounted for approximately 4.2% and 4.0% of total 16S rDNA copies, respectively [31]. Besides proteolysis, *B*. *fibrisolvens* is also involved in fiber degradation [32]. The results of Vasta et al. [31] regarding the abundance of B. *proteoclasticus* are in accordance with qPCR data from Paillard et al. [14], whereas Reilly et al. [33] observed *B*. *proteoclasticus* to represent 2.01×10^6/mL to 3.12×10^7/mL, which corresponds to only 0.3% of the bacterial population [14]. This could be due to differences in fed diets [24]; however, the application of different primers or DNA extraction procedures can also cause diverse results [34, 35]. In this context, “a universal extraction method with equally efficient lysis of cell walls of all possible microorganisms” [36] is essential to obtain comparable results and calls for mandatory bead beating, particularly as the rumen harbors various hard-to-lyse bacteria [37].

Streptococcus bovis expressed extracellular proteases [26, 27, 29, 38] and high proteolytic activity in the presence of several proteins [38, 39]. According to Attwood et al. [27], *Strep*. *bovis* may be particularly significant for ruminal proteolysis in grazing ruminants due to the semi-continuous grazing pattern and high protein contents of pasture, which would provide unique conditions, enabling this species to become a dominate proteolytic bacterium. Nevertheless, *Strep*. *bovis* can be absent from the rumen [40] or account for only 0.5–1.6% of the ruminal bacterial DNA [30]. However, low abundant microorganisms can also exert high enzymatic activities [41] and are therefore essential for ruminal protein metabolism. Besides protein degradation, *Strep*. *bovis* degrades starch for glucose fermentation and exerted proteolytic activity independent of the available N source, which led to the hypothesis that *Strep*. *bovis* degrades protein not only for subsequent N utilization, but mainly to break down protein matrices, surrounding starch granules [38].

Other bacteria involved in ruminal protein degradation are *Selenomonas ruminantium* [42] and *Ruminobacter amylophilus* [28], although both show low abundance when quantified via quantitative fluorescence *in situ* hybridization in cattle [43] or qPCR in sheep [44] and cattle [45]. However, despite its low abundance, *Rb*. *amylophilus* is assumed to be a highly proteolytic bacterium of the rumen microbiota [46] and showed higher azocasein degradation rates than some *B*. *fibrisolvens* strains [28]. Species of *Eubacterium*, especially *Eubacterium budayi* [27], are further active protein degraders [47] and *Eubacterium* contributed 16% to total proteolytic activity in the rumen [27]. Analyzing ruminal bacteria by competitive PCR in dairy cows, Reilly et al. [47] found that approximately 0.3–0.9% of bacterial cells belonged to *Eubacterium*. Also, *Fusobacteria* have high proteolytic activities [26], but their contribution to ruminal protein degradation in high-yielding dairy cattle may be limited as next-generation sequencing (NGS) obtained no *Fusobacterium* sp. in heifers fed high-grain diets [48].

Several species of *Prevotella* are crucial for hydrolyzing dietary protein in the rumen [49]. For example, *P*. *albensis* exerted proteolytic activity when incubated with varying concentrations of casein [39]. Thereby, it had a lower specific proteolytic activity than *Strep*. *bovis*, but as *Prevotella* is highly abundant in the rumen [24, 50], the contribution of *P*. *albensis* to ruminal proteolysis is substantial [39]. Further studies showed that strains of *P*. *ruminicola* [51], *P*. *brevis* and, to a smaller extent also *P*. *bryantii*, possessed proteolytic activities [28, 52]. However, *Prevotella* is even more important in the subsequent degradation of peptides [53, 54] and will therefore be considered again in the following section.

Finally, there are various rumen bacteria with minor proteolytic activities [26, 42], e.g. *Lachnospira multipara* [49]. However, these microbes may be relevant for overall proteolytic capacity in the rumen, especially as they can have nutritional interdependences with highly proteolytic species [42].

Peptidolytic bacteria

Peptides originating from the diet or ruminal proteolysis are mainly degraded by members of the rumen microbiota. As found for protease activity [18], peptidase activity is also predominantly of bacterial origin [54].

Prevotella represents a highly abundant genus in the rumen [24, 55] and was observed to have a broad peptidolytic activity [53, 54] with high peptidase diversity as recently obtained from metagenomic sequence data [56]. Stevenson et al. [57] observed that *Prevotella* spp. were highly abundant in the rumen of lactating cows and accounted for 42–60% of total eubacterial rDNA copies. Although *Prevotella* primers from [57] were later found to match numerous non-*Prevotella* species [58], the results are in the same range observed by NGS, i.e. 52% of all reads [55]. On a species level, van Gylswyk [49] stated that *P*. *ruminicola*

accounted for up to 60% of total rumen flora when cultivating bacteria from rumen ingesta on several media. Although *P. ruminicola* is a predominant microbe in the rumen [59], this abundance should be overestimated, as cultivation can produce biased results, e.g. due to cells that are in a viable but non-culturable condition [60]. Culture-independent approaches quantified the classical members *P. bryantii*, *P. brevis* and *P. ruminicola* to be 2–5% relative sequence abundance [57]. The exceptionally huge deviation in the abundance of *P. ruminicola* between these studies must be considered critically as it barely represents normal variation between different rumen microbiota. Therefore, the ruminal abundance of specific *Prevotella* species demands further elucidation.

By expressing several peptidases with different substrate specificities [54, 61], *P. albensis* constitutes a central peptidolytic species in the rumen and pure cultures of *P. albensis* and *P. bryantii* expressed peptidase activities higher than or similar to those of rumen fluid when incubated with several peptides [53, 62]. In addition, several *Prevotella* species are potent carbohydrate degraders harboring a variety of CAZymes [63].

Although *Megasphaera elsdenii* lacks peptidase activity [64], this species expresses high dipeptidase activity [53]. Thus, *M. elsdenii* is substantial for the sufficient breakdown of dipeptides to AA in the rumen, especially in the case of protozoa being absent as they suppress the growth of *M. elsdenii* [53]. Concerning the abundance of *M. elsdenii*, there is a high inter-individual variation between ruminants [50], with *M. elsdenii* not being detected by qPCR in steer rumen samples in some studies [40, 65]. However, just as many qPCR-based studies identified this species in the rumen of steers [66], dairy cattle [30, 67] and in vitro systems inoculated with bovine rumen fluid [8, 22, 68]. *Ruminobacter amylophilus* and *Strep. bovis*, as well as *Lachnospira multipara*, *Fibrobacter succinogenes* and *Eu. ruminantium*, express weak peptidolytic activities [53], but their contribution to ruminal peptidolysis appears marginal.

Deaminating bacteria

Only a small amount of AA is directly utilized for microbial protein synthesis. The bulk is deaminated, with volatile fatty acids, ammonia, carbon dioxide and methane being the end-products [6]. Total ruminal deamination is the result of a broad microbial activity, as no microorganism degrades all AA, but each prefers certain ones [69].

In 1961, Bladen et al. [70] stated that predominant bacteria with low ammonia production rates were the main ammonia producers in the rumen. However, as several studies have observed low abundant bacteria with high deaminating capacity [71], it is assumed that ruminal AA deamination is performed by two bacterial fractions: the first one constitutes bacteria present in a high number with low or moderate deaminating activity of about 10–20

nmol ammonia/min/mg protein [41]. This fraction includes *Butyrivibrio fibrisolvens* and *P. ruminicola*, which underlines their central role in ruminal N metabolism, as well as *M. elsdenii* [64, 70]. Although strains of *M. elsdenii* vary significantly in their deamination capacity, some possess ammonia production rates which are comparable to the hyper-ammonia producing bacteria (HAB) [64], belonging to the second fraction of deaminating bacteria in the rumen. Also, *Allisonella histaminiformans* has the ability to decarboxylate histidine, which results in the formation of histamine and small amounts of ammonia [72]. However, it is questionable whether *Allisonella histaminiformans* is part of the native ruminal microbiota as results on its presence are inconsistent [72–75]. The second fraction of deaminating rumen microorganisms are bacteria present in a low number which exert high deaminating activities of more than 300 nmol ammonia/min/mg protein [41]. These microbes are designated as HAB. Due to their high deaminating rates, they are of particular relevance for intra-ruminal N recycling [71]. Although ammonia is an essential N source for cellulolytic rumen microbes [7], an oversupply of ammonia may be the result of the high deamination by HAB, leading to poor ruminal N utilization efficiency and significant N losses [8]. By deaminating AA that could have stimulated the growth and therefore the ammonia utilization of the non-HAB majority [76], HAB might further reduce N utilization efficiency in the rumen.

Paster et al. [77] identified *Clostridium aminophilum*, *Cl. sticklandii* and *Peptostreptococcus anaerobius* as HAB, although they were already isolated earlier as strain F, strain SR [71] and strain C [78], respectively. Each of these three ‘classical’ HAB species had an abundance of 10^7 cells/mL rumen fluid [41] or, by applying 16S rRNA hybridization, made up approximately 1% of total bacteria in the rumen [79]. However, other studies found these HAB to enumerate 5.2% and even 11.6% of total bacterial counts in cows and sheep, respectively [80]. Besides, Rychlik et al. [81] predicted HAB populations to be 4-fold higher in cattle fed hay compared to grain-fed cattle. Aside from their ionophore sensitivity, the ability to use AA as sole carbon, N and energy source is characteristic for HAB. They are unable to hydrolyze proteins and thus rely on peptide- and AA-releasing microorganisms [80]. According to Wallace et al. [82], *Eu. pyruvativorans* can be classified as HAB, although it grew poorly on free AA. In fact, *Eu. pyruvativorans* seems to prefer pyruvate as an energy source, because pyruvate is extracellularly rare in the rumen, AA utilization may be the fermentative niche of this bacterium [82].

So far, it was assumed that HAB were unable to ferment carbohydrates [80], but recent findings provide information on another type of HAB. In the rumen of steers, Bento et al. [83] observed bacterial isolates with ammonia production as high as ‘classical’ HAB [77], but with the ability to ferment carbohydrates, consequently disproving the assumption that carbohydrate-fermenting bacteria would only produce low amounts of ammonia [70, 81].

These ‘new’ HAB isolates [83] might not have been recognized, as HAB were often isolated on selective media in the past, with AA being the sole carbon source [78]. However, this does not mean that they all can only ferment AA, but have a wider metabolic role. Although phylogenetically distinct from the ‘classical’ rumen HAB [77], HAB with carbohydrate-fermenting activity are also present in swine manure and showed significantly less ammonia production when incubated with glucose, thus probably demonstrating a shift in biochemical pathways [84]. It may be possible that ruminal HAB isolates with carbohydrate-fermenting activity [83] can also shift between carbohydrate and AA utilization and would therefore have an advantage at energy generation compared to ‘classical’ HAB [77]. The ‘new’ HAB [83] were mainly assigned to *Clostridiales*, and isolates were closely related to *Cl*. *bifermentans*, *Cl*. *argentinense* or even ‘classical’ HAB [77, 83]. Therefore, the genus *Clostridium* seems to harbor more HAB species that remained unknown to date. Other studies indicate the presence of HAB in the genera *Fusobacterium*, *Eubacterium* [80], *Acidaminococcus* and *Desulfomonas* [85]. To the best of our knowledge, no reports exist on the identification of HAB species in these genera. Research on the isolation and comprehensive metabolic characterization of further HAB is required, in particular studies confirming the existence of carbohydrate-fermenting HAB in the rumen.

Ureolytic bacteria

Urea constitutes a NC that is rapidly degraded by ruminal microorganisms and thus increases the ammonia pool in the rumen [86]. Depending on dietary composition, urea can constitute a part of the diet and therefore enters the rumen by feed intake [6, 15]. Typically, however, urea originates from the rumino-hepatic circulation and is brought into the rumen via saliva or diffusion through the rumen wall [87, 88]. This process may be useful under N-limiting conditions [87, 89], but when ruminants receive diets moderate or high in crude protein, the ammonia originating from ureolysis mainly returns to the blood rather than mixing with the rumen fluid and will be excreted into the environment [90].

Using cultivation-based techniques, several bacterial genera and species exerting ureolytic activity have been identified in the rumen, e.g. different strains of *Staphylococcus* sp., *Lactobacillus casei* var. *casei*, *Klebsiella aerogenes* and *Strep*. *faecium* with urease activities being either intracellular or linked to cell surface. Among these species, *Strep*. *faecium* showed the highest urease activity and was the most abundant of the isolated species [88]. *Howardella ureilytica* hydrolyzing urea for ATP generation was isolated from ovine rumen fluid [91] and strains of *Staph*. *saprophyticus* and *Micrococcus varians* were also isolated from sheep rumen and showed ureolytic activity during incubation with different NC. Besides, the majority of *Staph*. *saprophyticus* strains hydrolyzed casein and deaminated several AA [89], probably contributing to the overall proteolytic and deaminating activity in the rumen.

With an abundance of at least 2 × 10^7/mL, a ureolytic strain of *Sel*. *ruminantium* was isolated from bovine rumen [15]. Despite all these observations, the majority of ureolytic microbes are yet to be identified. Investigating the diversity of urease gene ureC in rumen fluid, digesta and papilla by NGS revealed that on average 55% of total sequences could not be assigned to any phylum. Most of these unknown sequences were found in the rumen papilla samples, where the ureolytic bacterial profile also clearly differed from those in digesta and fluid [92].

Research that considers all sampling sites in the rumen applying culture-independent methods like NGS are demanded as only limited knowledge on ureolytic rumen bacteria exists to date. However, cultivation will still be needed to definitely confirm ureolytic properties predicted with nucleic acid-based approaches [93]. Interestingly, this might be problematic as despite previous isolates being facultative anaerobes and their growth being virtually unaffected by oxygen, a considerable part of them lost the ability to undergo ureolysis during aerobic subculturing [88, 89].

Protozoa

In addition to bacteria, protozoa represent an important part of NC-degrading microorganisms in the rumen [94]. As described by Hungate [3], protozoa are eukaryotes and mainly present in the fluid phase, though they are chemoattracted to released nutrients [95] and thus some species are transiently attached to feed particles. Rumen protozoa enumerate approximately 10^6 protozoal cells/mL rumen fluid [96]. Estimates of protozoal abundances using microscopy can cause misidentification coupled with a low sensitivity [97]. Thus, quantification by molecular techniques targeting the 18S rDNA may be better suited, but variation in 18S rDNA copies between protozoal genera or different growth conditions can interfere [19]. Also, smaller protozoa were underrepresented by 18S rDNA copies when comparing NGS data to protozoal counts [98], which demonstrates that NGS is superior for community structure analysis, but not precise quantification. In this context, an even more severe and general problem may be the procedure for obtaining protozoa samples. It was earlier shown that especially large protozoa are more retained by feed particles when rumen fluid is filtered through cheesecloth [99], which consequently leads to an overestimation of their abundance. Likewise, sedimentation funnels might not only concentrate protozoa, but also cause bias when some species are attracted to the funnel glass or do not sediment well. Thus, hereinafter mentioned abundances should be regarded with some reservations and may better serve as indications.

Protozoa are regarded as detrimental for ruminal N metabolism as they predate bacteria [96] or fungi [100] and large protozoa also engulf smaller protozoa [101], which altogether

reduces ruminal N utilization efficiency. Protozoa also degrade feed protein, particularly insoluble particles [102] and thereby significantly contribute to ruminal dietary protein degradation [94]. Prins et al. [18] emphasized their role in ruminal protein breakdown as almost 20% of the proteolytic activity in rumen fluid is derived from protozoa. In contrast to NC-degrading bacteria, on a species level, little is known about protozoa or their N metabolism, which demands for investigating these microbes in detail. The highest proteolytic activity was found in vitro for *Entodinium caudatum* and *En*. *simplex* [103], with inter-species differences in the expressed protease profiles [94]. Of all rumen protozoa, cellulolytic protozoa, in turn, exerted the lowest proteolytic activity [103]. According to protozoal fractionation and NGS, the genus *Entodinium* is predominant in cows with *En*. *caudatum* and *En*. *simplex*, both accounting for 0.5% of protozoal cells [24, 96].

Also *Dasytricha ruminantium* is highly proteolytic [94] and accounted for up to 34% of protozoal cells obtained from sedimentation funnels. It thus may have a great influence on both dietary protein degradation and the turnover of bacteria [96]. Furthermore, *Polyplastron multivesiculatum* exerted moderate proteolytic activity [94] and can account for 10–20% of the total protozoal population [102]. Members of *Isotricha* as well as *Ophryoscolex caudatus* showed less proteolytic activity, whereas that of *Epidinium caudatum ecaudatum* was higher [94, 102].

Protozoa also degrade dipeptides and *Entodinium* species exerted high dipeptidase activity, followed by *D*. *ruminantium* and members of *Isotricha* [104]. Therefore, protozoa also play a relevant role in the final stages of peptide catabolism. In a more recent study, isotrichids and entodiniomorphids showed chemotaxis towards bacterial, protozoal and soy peptides [105], which thereby indicated protozoal contribution in the ruminal breakdown of polypeptides [106].

Very little is known about deamination by ruminal protozoa [106]. Species of *Entodinium* showed deamination activity, although the quantity is negligible [107]. Hino et al. [16] observed deamination in ruminal protozoa mainly consisting of entodiniomorphids, and Forsberg et al. [108] also described low deaminase activity in protozoa sampled from rumen-cannulated cows. Concerning ureolysis, no urease activity was detected in protozoa [88, 107].

When concentrating on their contribution to ruminal N metabolism, protozoa have an adverse effect and reduce ruminal N utilization efficiency. However, parts of the protozoal population are also important for fiber degradation [109]; due to their ability to temporally incorporate starch granules, which are consequently not metabolized to organic acids, they prevent extensive pH drops and thus support a stable fermentation [110]. Finally, in consideration of

a representative sampling, there is an urge to obtain deeper knowledge on the NC-degrading activity of ruminal protozoa on a species level to be able to diminish detrimental effects on ruminal N turnover.

Fungi

Fungi constitute about 10% of microbial biomass in the rumen [111] and are crucial fiber degraders [112], especially when forages with poor quality are fed to ruminants [100, 112]. Generally, there is only marginal information on metabolic activities of rumen fungi [113] and consequently also on their contribution to NC degradation.

The fungus *Neocallimastix frontalis* PNK2, which was isolated from sheep rumen, showed high extracellular proteolytic activity, probably necessary for degrading structural proteins and for sufficient fiber degradation. Moreover, it was considered possible that proteases could modify activation of other fungus-derived CAZymes [114]. In vitro, proteolytic activity of solid rumen ingesta was considerably increased in the presence of this fungal strain [115]. Thus, the authors assumed proteolytic fungi to play an important role in ruminal protein degradation [114, 115]. Also, the ruminal fungi *Ncm*. *patriciarum*, *Orpinomyces joyonii* and *Piromyces communis* showed proteolytic activities during incubation with different cereal grains. Thereby, protease activity was mainly cell-associated in *Ncm*. *patriciarum* and *Piromyces communis*, whereas it was predominantly extracellular in *Orpinomyces joyonii* [116]. Likewise, Paul et al. [112] observed increased ruminal protease activities in buffaloes supplemented with *Piromyces* FNG5, although it remains unclear whether the increase originated from supplemented fungus or indigenous microbes.

No significant contribution of main ruminal fungi to *in situ* degradation of soybean cake and meat meal or improvements in the proteolytic activity of rumen fluid through fungi were observed by Bonnemoy et al. [117]. Likewise, Michel et al. [118] stated that rumen fungi have limited abilities to degrade proteins; however, several fungal isolates exerted endo- and exopeptidase activities [118] and may therefore promote ruminal peptidolysis. Additionally, a higher fungal diversity during the increment of protein supply to dairy cows [30] might indicate that ruminal fungi partly benefit from high protein provision.

Besides its metabolic activity, knowledge on the abundance of a microorganism can help to evaluate its contribution and meaning to rumen metabolism. Because previous studies only enumerated total abundances [30], studies quantifying ruminal fungi on a species level or with regard to their metabolic activity are needed. In the past, quantification of rumen fungi via microscopy may have led to erroneous results, as flagellate protozoa can be identified as fungal zoospores [100]. Thus, DNA-based methods targeting the 18S rDNA or internal

transcribed spacer 1 [97] represent attractive alternatives for quantification and diversity analysis. Subsequently, fungal DNA amount or copy numbers of internal transcribed spacer 1 can be converted to estimate the fungal biomass in the rumen [119, 120].

Interactions among nitrogenous compound-degrading rumen microorganisms

It is well established that ruminal microorganisms are continuously interacting, but so far these interactions are not fully understood [30]. In the following, relevant interactions between or with the contribution of NC-degrading rumen microorganisms will be reviewed briefly. Basic interactions like interspecies H2 transfer [111] will not be included.

Wallace [42] early showed the existence of reciprocal interactions between NC-degrading rumen microbes, as different proteolytic bacteria grew better in combination than alone, which was ascribed to an increased cooperative hydrolysis or nutritional interdependences. Hyper-ammonia producing bacteria, both 'classical' [77] and 'new' [83] HAB, depend on proteolytic species that supply AA to them [80, 83] as, except one isolate [121], they are unable to hydrolyze proteins to sustain growth. Moreover, *Cl*. *aminophilum* and *Psc*. *anaerobius* produced much higher amounts of ammonia when grown with peptidolytic *P*. *ruminicola* or *P*. *bryantii* [122], thus indicating the dependence on peptidolytics.

Interactions between NC-degrading microorganisms are not always beneficial, but can impair other species, e.g. by bacteriocins [123]. For instance, bovicin HC5, a bacteriocin formed by *Strep*. *bovis* HC5 catalyzing the potassium efflux from cells, inhibited growth and ammonia release of *Cl*. *aminophilum* in pure cultures [124], but also decreased ammonia production of mixed ruminal bacteria in vitro [123]. Strains of *B*. *fibrisolvens* produced a variety of bacteriocins including JL5, which suppressed *Cl*. *sticklandii* as well as cellulolytic *Ruminococcus albus* and *Ruminococcus flavefaciens* [125], which might be explained by the competition for carbohydrates. Generally, the formation of as well as sensitivity against bacteriocins seems to be strain-specific [123, 125].

Although excessive deamination is regarded as detrimental for efficient N utilization [126], there is also a need for deaminating bacteria and linkages between cellulolytic and deaminating rumen microbes. As ammonia represents the sole N source for cellulolytic bacteria [7], deamination is an important process to provide this NC. Branched-chain volatile fatty acids (BCVFA), which are formed during deamination [127], have stimulatory effects on predominant cellulolytic microorganisms and are crucial for their growth [128]. Then again, these deamination processes remove AA that could have promoted growth of cellulolytics [76]. Bacteria like *Strep*. *bovis* exert proteolytic activities to gain access to starch granules

surrounded by protein matrices [38]. Thus, this also affects nutrient provision of other amylolytic microorganisms, which are not capable of degrading such matrices.

On the other hand, proteolytic bacteria can benefit from fiber degraders, as maximal cell wall protein degradation was observed when proteolytic and cellulolytic bacteria were incubated together. It was hypothesized that potentially degradable proteins are protected by structural polysaccharides and become available for proteolytic microorganisms through cellulose degradation [129]. Thereby, rumen fungi may also play a role, as they degrade cell wall structures [100], and can therefore provide access to actually surrounded proteins.

Protozoa predate bacteria [96] and fungi [100], but by degrading insoluble dietary proteins, protozoa promote the growth of peptidolytic and deaminating bacteria, which utilize peptides and AA from protozoal proteolysis. Thereby, protozoa may enhance the deaminating activity of HAB [9] and thus reduce the efficiency of N utilization in the rumen by two modes of action: the predation of bacteria and fungi as well as the release of AA into the rumen. Similar patterns of interaction may occur between peptidolytic or deaminating bacteria and proteolytic fungi that release NC from their protein breakdown [114]. Dehority et al. [130] stated a general negative interaction between fungi and bacteria, as both form inhibitory substances to limit the growth of the other. However, the administration of *Piromyces* FNG5 caused a 2.5-fold increase of bacteria in the rumen of buffaloes [112]. Therefore, the existence, type and extent of interaction between fungi and bacteria may be specific for species or even strains [131] and must be evaluated individually.

Manipulating factors on nitrogenous compound-degrading rumen microorganisms

Several factors influence the rumen microbiota within a ruminant: Age [132], geographical localization, host species [24], breed [133] and diet [24]. Thereby, diet has the strongest influence [24] and is thus of great importance in livestock production.

The meaning of the rumen microbiota with regard to high performance in livestock production was highlighted by the observation that ruminal bacteria communities of steers with higher feed efficiency (defined as “the difference between an animal’s actual feed intake and its expected feed requirements for maintenance and growth over a specific test period” [134]) were more similar between individuals and clearly separated from ruminal bacteria communities of inefficient animals [133]. Therefore, a specific rumen microbiota composition may be significant for satisfying performance of animals. Another study [135] indicated the potential of rumen microbes to influence quality characteristics of milk. The scientists observed high correlations between milkfat yield and the *Firmicutes* to *Bacteroidetes* ratio, which were still present at the genus level [135]. Both studies emphasize the influence of

rumen microorganisms on the host's physiology and thus the meaning of shaping the microbial composition and its activity to improve nutrient and energy use.

Concerning ruminal N utilization, manipulating the number and activity of NC-degrading microorganisms is of particular importance, especially as ammonia release in the rumen often exceeds its efficient utilization [126] and consequently results in high N losses and a waste of resources. Attwood et al. [27] stated the importance of altering the abundance of NC-degrading microorganisms as microbial enzymes would be expressed permanently and thus controlling ruminal NC degradation by affecting enzyme expression could not represent a promising strategy [26]. Bladen et al. [70], in turn, assumed that increased ammonia production was caused by higher deaminase synthesis without alterations in microbial abundances; therefore, influencing enzyme expression would be the better opportunity to control intra-ruminal N recycling. So far, different approaches have been applied to improve ruminal N utilization using one of these two strategies and will be discussed in the following.

Diet composition

Selection of forage species

When preparing ruminant diets, selection of forage species affects the composition of NC supplied to the rumen [136] and thereby also the ruminal microbiome and efficiency of N utilization [127]. In the case of legumes, red clover (*Trifolium pratense*) expresses polyphenol-oxidase (PPO), an enzyme that causes the formation of protein-phenol complexes when plant tissues are damaged [137]. This increases the proportion of ruminally undegraded dietary crude protein (RUP) [138], which is still digestible in the small intestine and thus an available N source for the host. Moreover, red clover phenolic extract inhibited the growth of *C. sticklandii* cultures in vitro [139], which along with PPO, may increase N retention in ruminants. The effect of PPO on ruminal proteolysis was demonstrated in vitro, as the inclusion of red clover to timothy grass-based diets lowered the ammonia to insoluble-N ratio, indicating a limited protein degradation [140]. Likewise, the production of ammonia and i-valerate was lower in fermenters supplied with cocksfoot (*Dactylis glomerata*), a grass species high in PPO, when compared to fermenters incubated with the low PPO grass species tall fescue (*Festuca arundinacea*) [141]. Cocksfoot may hence be an important grass species on permanent pasture used for both grazing and silage production. Likewise, red clover may be an attractive legume for arable pasture areas to improve the RUP supply from forage plants.

Synchronization of dietary energy and nitrogen

Combining different feedstuffs to synchronize the provision of dietary energy and N [142] may also affect certain groups of NC-degrading microorganisms. In diets containing highly degradable NC and less rapidly available energy, e.g. water-soluble carbohydrates (WSC), HAB might increase in activity and abundance [143] as they probably benefit from their ability to utilize AA energetically without being dependent on carbohydrates [71, 78, 80, 83].

The effects of synchronization are not consistent in literature [142, 144], but increased microbial N flow to the duodenum and reduced ruminal ammonia concentration have been reported for the combination of legume silage and grass silage high in WSC [141], thus suggesting less deamination [8] and the direct incorporation of AA into microbial protein [7]. Likewise, microbial N flow at the duodenum tended to increase with a more synchronous supply of dietary energy and N to steers, although ruminal ammonia concentration was not reduced [145]. Studies focusing on whether there are also adaptions in the rumen microbiome are lacking, but could reveal possible linkages that help to reduce N losses from the rumen.

The synchronization of dietary energy and N could reduce detrimental consequences of high deamination activity from HAB as it supplies sufficient energy for microbial utilization of ammonia. Secondly, adequate provision of dietary energy may nullify the advantage of HAB to generate energy from AA. This may reduce their abundance along with long-term alterations of the ruminal microbiota composition and could also explain the predicted 4-fold lower HAB population in cattle fed high-grain diets compared to hay-fed cattle [81]. Moreover, one can speculate whether HAB capable to ferment carbohydrates [83] shift from AA to carbohydrates as the preferred substrate for energy generation and thus still be present in the rumen but exerting a different metabolic pathway with less deamination.

Application of fats

Diets containing higher proportions of fats with unsaturated fatty acids, commonly termed oils, are already used to increase the energy supply to ruminants [146]. Although this does not help to cover the energy needs of rumen microbes [147], fat supplementation may influence ruminal N metabolism [146]. For instance, the addition of 26 mL/d of linseed oil to a basal diet for sheep almost eliminated all rumen protozoa and improved the efficiency of bacterial protein synthesis by more than 50%, whereas increasing linseed oil supplementation to 40 mL/d showed no effect on the efficiency of bacterial protein synthesis [148]. Other studies even reported the increased abundance of proteolytic bacteria and the increased formation of ammonia when feeding linseed or soybean oil to dairy cows [149]. Therefore, the effects of fat supplementation on NC-degrading microorganisms seem to be

variable and difficult to predict. Besides, detrimental impacts like reduced ruminal degradation of organic matter [148] or hemicelluloses [147] and subsequently less availability of dietary energy in the rumen, were repeatedly documented for oil supplementation. This can be explained by the general toxicity of unsaturated fatty acids on rumen microorganisms [147] and might outweigh the positive impact on intra-ruminal N recycling.

Feed treatment

Wilting

Wilting forages is an efficient way to reduce energy losses during ensiling [150], but also influences the composition of NC in silages [151, 152]. Both may affect ruminal NC-degrading microorganisms via variation in the energy supply [152] and differing percentages of true protein in silages [151], thereby influencing the quality of N supply to the rumen. Fast wilted silages have higher true protein contents and lower ruminal ammonia concentrations during in vitro and higher RUP values during *in situ* incubation [151]. Likewise, grass silages with high contents of free AA (> 300 mmol/kg dry matter) and less true protein resulted in higher concentrations of ammonia and BCVFA along with a lower efficiency of N assimilation into microbial protein by liquid-associated bacteria in vitro [143].

In grass silages, wilting also increased WSC [150, 152], which can enhance microbial protein synthesis in the rumen by a higher provision of dietary energy [153]. Interestingly, wilting is assumed to promote PPO activity in red clover [137], thereby increasing RUP by forming phenol-bound proteins [138]. However, no enhanced PPO activity was obtained when wilting cocksfoot [154] suggesting that effects on PPO activity depend on plant species. To the best of the authors' knowledge, there is a lack of research on the effect of differently wilted forages on the ruminal microbiota composition. So far, only microbial metabolite productions have been investigated, but comprehensive experiments analyzing both microbial abundances and metabolites are indispensable to optimize forage conservation in the future.

Organic acid treatment

Influencing the ruminal microbiome by processing feedstuffs with organic acids was found to be an effective option during recent years [22]. Acids alter the solubility and protein structure, thereby affecting the quantity and quality of N supplied to the rumen [155]. Barley treated with lactic acid reduced BCVFA concentration in vitro [22, 68], but did not affect ammonia concentration [22]. Deckardt et al. [22] concluded a decreased AA catabolism, although addition of lactic acid did not affect the abundance of *M. elsdenii* or other NC-degrading microorganisms like *Prevotella* or *Entodinium*. Besides, fiber degradation was enhanced in

acid-treated barley [22], which emphasizes the benefit of processing concentrate with organic acids. Concerning the degradation of soybean meal protein, treatment with 5% propionate reduced proteolysis as well as numerical concentrations of ammonia and i-valerate in vitro [155], indicating reduced metabolic activities of proteolytic and deaminating microorganisms [127, 143]. Manipulating the structure and solubility of proteins through acid treatment seems to be a feasible approach to alter the cascade of NC breakdown in the rumen. Together with the observations for fiber degradation, findings so far give evidence for an improved N retention, which should be pursued in further studies addressing total N flows.

Heat treatment

Heat treatment constitutes a further option to affect ruminal N metabolism. Treating barley grain with 55°C for 48 h decreased protein degradation, the concentration of i-valerate as well as the abundance of *Prevotella* and total protozoa in vitro. However, the degradation of organic matter was also lowered [22]. Extensive heat treatments should be applied carefully, as exposing rapeseed meal to 130°C or 140°C for five minutes led to high RUP contents, but the 140°C treatment also caused poor intestinal protein digestibility [156]. Thus, N becomes unavailable for the rumen microbiota and the host, leading to unnecessary N losses and environmental pollution. Duration and intensity are decisive for the effect of heat treatments on NC and thereby determine whether they improve N utilization in ruminants or actually cause the opposite result.

Feed additives

Plant bioactive lipid compounds

Plant bioactive lipid compounds (PBLC), commonly but misleadingly termed ‘essential oils’ [157], are secondary plant metabolites that are not necessary for plant growth and characterized by a vast diversity [158]. So far, a variety of PBLC has been shown to reduce ruminal methane production [159], but also to affect NC-degrading microorganisms by microbicidal or microbiostatic effects [8].

Reduced deamination as well as concentrations of ammonia [8, 159, 160] and BCVFA were observed with PBLC supplementation in vitro [8, 159]. Application would hence mean an effective dietary strategy to prevent inefficient N utilization by ruminants. However, PBLC do not necessarily decrease ruminal ammonia concentrations or deamination [8, 159, 161], and can even have opposite effects [161].

Plant bioactive lipid compounds cause substantial alterations in the ruminal microbiota, but it is not clear whether they are suited to shape the NC-degrading microbial population in the

rumen. *Streptococcus bovis* is relatively resistant against a multitude of PBLC including thymol [162], clove oil, origanium oil [8] as well as a commercial blend of PBLC [160]. *Ruminobacter amylophilus*, *B. fibrisolvens* and *Sel*. *ruminantium*, as well as *P*. *bryantii* and *P*. *ruminicola*, were highly sensitive against clove and origanium oil [8]. Likewise, protozoa showed high sensitivities against these two substances [8, 159] and also against eucalyptus, garlic and peppermint oil in vitro [159]. Consequently, ruminal peptidolysis and proteolysis may be added to the statement of Calsamiglia et al. [158] that the majority of PBLC could affect ruminal deamination.

Abundances of deaminating bacteria, particularly HAB, were often reduced by PBLC supplementation and may explain reduced ammonia concentrations [160]. Origanium oil decreased 16S rDNA copies of *M*. *elsdenii*, *Cl*. *aminophilum* and *Cl*. *sticklandii* in a bovine rumen fluid-based in vitro system [8] and the PBLC blend of McIntosh et al. [160] inhibited the growth of *Cl*. *sticklandii* and *Psc*. *anaerobius* pure cultures. However, in contrast, *Cl*. *aminophilum* was not affected by different PBLC blends [160, 163].

The aforementioned results were all obtained during in vitro experiments and thus must be evaluated under in vivo conditions. Thereby, the supplementation form needs to be considered as well, as it can influence the effect on ruminal microorganisms. For instance, 16S rDNA copies of *Prevotella* spp. and *Cl*. *aminophilum* declined in sheep fed pelleted rosemary leaves (*Rosmarinus officinalis* L.), whereas 16S rDNA copies of the same species were not affected when sheep received the same dosage as pure ‘rosemary essential oil’. The authors suggest that differences in chemical composition between the supplementation forms are responsible for the deviating effects [164]. Also, ‘rosemary essential oil’ might not have emulsified properly with the rumen fluid and lacked effectiveness as it floated on top.

Generally, the effects on ruminal NC-degrading microorganisms seem to be specific for the applied PBLC. However, contradictory findings between studies investigating the same PBLC [159, 161, 163] clearly illustrate that the underlying modes of action are poorly understood and a definite statement in this regard is impossible. Consequently, as claimed previously [8], there is an urgent need for systematic studies on PBLC using standardized conditions to obtain reliable knowledge on the effects on ruminal N metabolism and microorganisms. Hereby, PBLC should also be critically evaluated for their effect on feed digestion, as several PBLC combinations reduced dry matter digestibility in vitro [163].

Condensed tannins

As Patra et al. [165] have summarized, supplementing ruminant diets with tannins can influence ruminal metabolism and consequently also intra-ruminal N recycling. In fact, condensed tannins (CT) have protein binding effects at pH 3.5–7.0 [166] leading to reduced

proportions of soluble protein in the rumen and probably increased RUP values [106]. In the abomasum and the proximal duodenum, dietary protein would be available for the host due to low pH values causing the breakdown of these complexes. However, the risk of repeated formation of protein-tannin-complexes or tannin-binding to the host's enzymes in lower gut sections remains present [166].

In pure cultures, bacteria were unable to degrade proteins when incubated with calliandra CT (*Calliandra calothyrsus*) [121], and a protein-preserving effect of sainfoin CT on ruminal proteolysis was also evident using ovine rumen fluid, as the ammonia to insoluble-N ratio was lowered [140]. Likewise, quebracho CT reduced ammonia concentration in vitro [167]. All these observations may be explained by two factors: the formation of protein-tannin-complexes [121, 166] and morphological alterations of bacterial cell walls [168] suppressing the growth and proteolytic activity of NC-degrading bacteria [121, 168, 169].

On the other hand, as ammonia constitutes the main N source for cellulolytic bacteria, an excessive tannin-induced protein protection bears the risk of ruminal ammonia concentrations below the critical level for sufficient forage digestion [170] and would have adverse effects on the host's supply with dietary energy and nutrients. Besides, CT can directly inhibit ruminal cellulolytic species [170], their cellulase activity [171] or form complexes with lignocellulose [170].

Feeding CT-rich plants often reduces feed intake [31] due to the reduced palatability and lower degradation rates [172]. Thus, by directly feeding CT-rich plants, it may be difficult to achieve tannin concentrations causing adequate protection of dietary protein in the rumen; however, supplementing purified CT extracts to ruminants should be an option to affect ruminal degradation of NC, particularly of proteins. These positive effects must then be weighed carefully against the potential detrimental impact on cellulolytic rumen species.

Saponins

Besides PBLC and tannins, saponins are a third group of secondary plant metabolites with bioactive functions [158]. Several saponins were evaluated as feed additives in animal nutrition [173–175], but as with PBLC, the results are not consistent. Quillaja saponins (*Quillaja saponaria* Molina) lowered ammonia concentration by 20% in vitro, which was probably due to a reduction of protozoal 18S rDNA copies [8]. It is assumed that saponins form complexes with sterols in the protozoal membrane surface, which thereby becomes disrupted [176] and leads to cell death. Similarly, *Yucca schidigera* saponins inhibited protozoal predation and reduced ammonia concentrations in vitro [177]. In contrast to the in vitro data [177], protozoal counts in rumen fluid were not altered, when *Yucca schidigera*

saponin extract was supplemented to dairy cows [173]. Tea saponins, however, reduced ruminal ammonia concentration and protozoal 18S rDNA copies when fed to sheep [174].

The effects of saponins on several NC-degrading bacteria are even less clear. Quillaja saponins increased the 16S rDNA copies of *Rb*. *amylophilus*, *Sel*. *ruminantium*, *P*. *ruminicola* and *P*. *bryantii* [8], what was also observed for *Prevotella* [175] and *P*. *bryantii* [177] during in vitro application of *Yucca schidigera* saponins. In contrast to quillaja saponins [8], *Yucca schidigera* saponins suppressed the growth of pure cultures of *Rb*. *amylophilus* [178] and also that of *B*. *fibrisolvens* [177]. 16S rDNA copies of *M*. *elsdenii* and 'classical' HAB [77] were not affected by quillaja saponins, which seems contradictory in view of the reduced ammonia concentration [8]. The authors suggested the decrease of protozoa to be responsible. However, reductions of proteolytic and deaminating bacteria that were not targeted by qPCR, e.g. 'new' HAB [83], could also be causative [8]. Additionally, one can speculate whether saponins have suppressing effects on metabolic activities but not abundances of NC-degrading bacteria.

So far, the use of saponins may be an option to modulate ruminal NC degradation in a beneficial way, but inhibitory effects on protozoa or bacteria seem to depend on dosage as well as saponin type and remain poorly understood [8]. Under practical conditions, however, the ability of saponins to reduce feed intake [173] clearly limits their application to dairy diets.

Anacardic acids

As summarized by Kobayashi et al. [179], anacardic acids predominantly present in the by-products of cashew (*Anacardium occidentale*) and ginkgo (*Ginkgo biloba*) nut production, are discussed as modifiers of rumen fermentation. Anacardic acids are characterized as a group of few closely related organic compounds, differing in saturation and side chain length [180]. A first in vitro study investigating the effect of anacardic acid containing ginkgo by-products found decreased concentrations of ammonia, which may be caused by high sensitivities of 'classical' HAB [77] against anacardic acids [181]. However, the effects on other ruminal NC-degrading microbes are less clear and even contradictory in parts. For example, 16S rDNA copies of *M*. *elsdenii* and *Sel*. *ruminantium* increased in vitro when fermenters were supplied with either ginkgo extract [181] or cashew nut shell liquid [182]. 16S rDNA copies of *P*. *ruminicola* increased with ginkgo extract [181], but declined when cashew nut shell liquid was added [182]. This inconsistency may be explained by differences in the structure of anacardic acids contained in ginkgo and cashew byproducts [181] or also by the presence of other antimicrobial compounds [179]. As both studies used similar diets for the in vitro incubation as well as bead beating-based DNA extractions and identical primers for qPCR analysis, laboratory procedures may not have been a contributing factor for the deviating

microbial abundances. Therefore, further studies are indispensable to evaluate whether anacardic acids are an option to shape the ruminal NC-degrading microbiota and which structural form is most effective.

Bitter substances

Although bitter substances were early found to have antimicrobial properties [183], their consideration as modulators of the rumen microbiota is new and scarcely explored. First in vitro investigations by Flythe [184] observed hops flowers (*Humulus lupulus* L.) and hops extract to inhibit ammonia production in mixed rumen fluid, as well as growth and ammonia production in pure cultures of 'classical' HAB [77]. These suppressing effects are likely caused by humulone and lupulone, which are the main bitter substances in hops, and are also known as α-acid and β-acid, respectively [184]. Additionally, growth of *Strep*. *bovis* was inhibited by lupulone when cultivated in pure culture [185]. Likewise, ammonia production in rumen fluid incubated with spent craft brewer's yeast was lower than that with baker's yeast. This supports the assumption that hops bitter substances can decrease ruminal ammonia production and indicate reduced deamination [186].

The target site of humulone and lupulone is the cell membrane's lipophilic region, where they cause membrane leakage and consequently cell death [183]. However, except for 'classical' HAB [77] and *Strep*. *bovis*, no information is available regarding their effect on other rumen microorganisms. As the supplementation with two hops cultivars not only decreased in vitro degradability of crude protein by up to 36%, but also degradability of dry matter by up to 33% [187], inhibiting effects on other parts of the rumen microbiome are likely. Thus, bitter substances like humulone and lupulone could provide an opportunity to affect ruminal N metabolism in the future; however, sparse knowledge bases on in vitro trials and hitherto an assessment cannot be made.

Ionophores

Since their ban as feed additives in the EU in 2006 [188], ionophores only represent an option in other parts of the world, e.g. North America where ionophores, predominantly monensin, are widely applied in beef production [189]. Ionophores are described as a heterogeneous group of membrane-active molecules impairing transmembrane concentration gradients [190]. Concerning their effect on protozoa, it must be distinguished between short-term and long-term effects. Although naïve protozoal populations were nearly completely eliminated in vitro [16], repeated application of monensin did not have an effect on protozoal cultures [191]. These adaption patterns do also apply for in vivo long-term monensin application [192]. Thus, also decreased ammonia concentrations observed shortly after a monensin-induced protozoa reduction [16] may not last and return to pre-treatment

level. This might even be the case if protozoa would stay absent as bacteria proliferating in the absence of protozoa could replace protozoal activity [104].

Although there is no clear-cut difference in the susceptibility against monensin between gram-negative and gram-positive bacteria [193], gram-negative bacteria are generally more resistant [192, 194] due to their outer membrane structure and cell wall constitution [194]. However, also cell flocculation or synthesis of protective extracellular polysaccharides affect the effectiveness of monensin and can occur in both gram-positive and gram-negative bacteria [193]. Thus, future studies should address microbial taxa [195] to provide a superior picture about the monensin susceptibility of NC-degrading microorganisms in the rumen.

The gram-positive 'classical' and 'new' HAB [77, 83] are monensin-sensitive [71, 78, 83] and monensin is undoubtedly effective at reducing deamination [196], ammonia concentration and abundances of 'classical' HAB [77] in vitro [16, 160, 196] and in vivo [79, 197].

In vitro application of the ionophore hainamycin decreased 16S rDNA copies of *B*. *fibrisolvens*, *Cl*. *sticklandii*, *Cl*. *aminophilum* and *Psc*. *anaerobius*, whereas *M*. *elsdenii* was unaffected and *P*. *ruminicola* increased [198]. Actually, these shifts can be considered beneficial as they were accompanied by decreased ammonia concentration, deaminase activity and proportions of BCVFA in total volatile fatty acids.

Altogether, the aforementioned findings suggest a general ability of ionophores to influence NC-degrading bacteria and underline their potential to reduce ruminal N wastage. Nonetheless, research on long-term application of ionophores should confirm lasting alterations in abundance and activity of NC-degrading rumen microbes.

Probiotics

Probiotics are defined as live microorganisms that confer a health benefit on the host by improving its intestinal microbial balance [199] and are widely used in animal nutrition [200]. Applying qPCR, 16S rDNA copies of *Rb*. *amylophilus* and *Strep*. *bovis* decreased in steers supplied daily with viable *Saccharomyces cerevisiae* I-1077 through rumen cannulas. 18S or 16S rDNA copies of total protozoa and *Sel*. *ruminantium*, in turn, increased due to the supplementation [201]. Concerning N metabolism, proteolytic and peptidolytic activities of *P*. *albensis*, *B*. *fibrisolvens* and *Strep*. *bovis* decreased during in vitro incubation with viable *S*. *cerevisiae* I-1077 [202]. However, despite various alterations on a microbiological level, other studies reported no improvements of the amount and AA composition of microbial N reaching the duodenum in dairy cows supplemented with live yeast culture of *S*. *cerevisiae* [203]. Therefore, comprehensive studies evaluating the effects of *S*. *cerevisiae* on ruminal NC-degrading microorganisms are encouraged as probiotic effects seem to be strain-specific

[204]. Additionally, the combination of probiotics either with prebiotic substances, leading to so-called synbiotics [200], or with plant extracts (e.g. tannins) could enhance the effects in ruminants [205].

A chance to inhibit AA catabolism through probiotics was indicated by Callaway et al. [196], who found that the *L*. *lactis*-derived bacteriocin nisin [206] suppressed deamination and the growth of *Cl*. *aminophilum* in vitro. Thus, supplying ruminants with bacteriocin-producing probiotics might particularly alter deamination, but studies on the identification of strains that inhibit growth [205] and activity of HAB are still pending. Because existing data [123–125, 196] were only obtained from in vitro experiments, more efforts must be made to evaluate such mechanisms in vivo. However, various interactions with other microbes and the host as well as the general high complexity of the rumen microbiome could make it hardly possible to relate any measured effect to bacteriocins.

To expand the field of probiotic candidates, the transfer of microorganisms from one ruminant species to another might constitute a further option to improve ruminal N metabolism. Administering a fungal strain from wild blue bull (*Boselaphus tragocamelus*) to buffaloes (*Bubalus bubalis*) increased N retention along with higher protease activity, but equal ammonia concentration [112], indicating a higher breakdown of dietary crude protein but concomitantly enhanced ruminal N utilization. Besides, the increase of cellulolytic and hemicellulolytic bacteria [112] may have contributed to higher N retention, as it could lead to a more synchronized fermentation of carbohydrates and NC. It is noteworthy that microbes showing effectiveness in one ruminant species can fail to colonize the rumen of other species as the establishment of exogenously added microorganisms is difficult [67].

Metals

A variety of metal compounds is added to ruminant diets to meet mineral element requirements [207]. Besides, metal ions have antimicrobial effects and in case of NC-degrading microorganisms iron, copper, tin and chromium decreased bacterial dipeptidase activity by interacting with sulfhydryl groups or by displacing the metal ion from the enzyme [208]. Although iron effectively reduced dipeptidase activity in pure cultures of *P*. *albensis* [208], the effect may be nullified in rumen fluid as lactobacilli can take up high amounts of iron [209]. Besides the effects on rumen bacteria, Brade et al. [210] described defaunating effects of zinc-enriched diets in the rumen of dairy cows. Likewise, Mihaliková et al. [211] found high sensitivities of *En*. *caudatum* cultures against copper and chromium.

Lead, cadmium and mercury also inhibit microbial dipeptidase activity [208], but are irrelevant for controlling ruminal N metabolism due to the tremendous toxicity to humans and animals [212].

Metal ions are probably not specific to peptide metabolism [208] and therefore impair other ruminal microorganisms as well. Besides, some metals are also not suitable for every host; for example, several sheep breeds have low tolerance levels for copper [213]. Therefore, extensive metal utilization to improve N utilization in ruminant production is unlikely. This is particularly true as the majority of metals will be excreted via feces when fed in higher concentrations [214], thus only shifting but not mitigating the problem of environmental pollution.

Vaccination

Vaccination against protozoa has been examined to reduce bacterial predation thereby improving the efficiency of N utilization [215]. Although applied vaccines increased specific immunoglobulins (Ig)G titers in plasma and saliva, they failed to decrease the ruminal ammonia concentration and protozoal counts in vivo. This failure might also be caused by Ig breakdown in the rumen; although IgG were resistant against degradation for eight hours in vitro [215], the in vivo situation may be different. Analogous to the secretory component of IgA, which conserves this Ig from proteolysis in the gut [216], a protective component may also improve the effect of vaccination against protozoa in the rumen. Though, it should be noted that a general absence of protozoa does not necessarily mean an improvement of ruminal metabolism as, besides their role in bacteria, fungi and NC breakdown, protozoa are also important for sufficient ruminal fiber degradation [109]. Thus, as already claimed [96, 106], it is desirable to inhibit specific protozoal species to improve N utilization and simultaneously maintain fiber degradation. Future approaches should also concentrate on long-term bacterial-protozoal interactions as bacteria may replace protozoal dipeptidase activity in the rumen [104].

A recent vaccination strategy targets the ruminal breakdown of urea and showed reduced ureolysis and ammonia concentration in vitro when incubating rumen fluid of cows that had been vaccinated against urease. Accordingly, ruminal urease activity decreased by 17% after vaccination in vivo [86] leading to the assumption that immunization against urease has the potential to control ruminal ureolysis. In this study [86], samples were taken from rumen fluid, but provided that anti-urease Ig are not only present in saliva, but also diffuse through the rumen wall, it may be interesting to investigate effects on the epimural ureolytic bacteria, which therefore should be strongly affected. Nevertheless, for evaluating the overall benefit of urea vaccination, effects on total N utilization must be analyzed, too.

Future perspectives

This review discussed dietary factors by which ruminal NC degradation processes and related microorganisms can be influenced in adult animals. As a future perspective, manipulation of the rumen microbiome in young pre-ruminants may become an opportunity to shape ruminal metabolism and microbiota in adult animals. As a specific rumen microbiota composition is associated with improved animal performance, corresponding microbial compositions could be used as inoculum or feed additive in young ruminants to establish a favorable microbiome. Consequently, the adult ruminant may achieve an enhanced performance, not only with regard to energy utilization but also in terms of ruminal N utilization.

Inhibiting the establishment of only specific microorganisms in the evolving rumen could be a second approach for shaping the rumen microbiota in a more permanent manner. Thereby, other rumen microbes could occupy the niche of inhibited species consequently excluding them from the adult microbiota. Suppressing the colonization of HAB already in the developing rumen may become a beneficial strategy regarding intra-ruminal N recycling.

To investigate such options in the future and for further research on the rumen ecosystem in general, studies employing large sample numbers will be required to overcome the confounding effect of natural animal-to-animal variation and enhance the statistical power of rumen microbiota-related studies. However, the need for cannulated animals considerably limits the broad examination of the rumen microbiome and stomach tubing, the main source for rumen microbiome in many studies, is misleading because it under-represents particle-associated microorganisms. Recent findings indicate that non-invasive regurgitated ingesta samples are suitable for the precise prediction of rumen microbiota compositions [217]. Therefore, this can constitute an appropriate sampling method for determining rumen microbial communities on a large scale. However, phylogenetic information is unable to fully explain all underlying mechanisms or relevant activities of ruminal NC-degrading microorganisms. Thus, besides capturing the microbial composition in the rumen, the concurrent pursuit of a functional classification may be decisive in future to further improve our understanding of intra-ruminal N recycling and consequently how to manipulate it beneficially.

Conclusions

In summary, ruminal NC degradation is not a wasteful process per se, but as ruminal ammonia concentration often exceeds the microorganisms' capacity to incorporate, high N losses are the consequence. Controlling intra-ruminal N recycling may not only help

improving N utilization and optimizing ruminant livestock production, but also contributes to a more sustainable agriculture due to less N-containing emissions and lowered resource input. Thereby, the superordinate aim is the sufficient suppression of ruminal NC degradation, particularly deamination, along with maintaining an adequate N provision for the rumen microbiota to ensure high fiber degradation and host supply with microbial protein. The information on NC-degrading rumen microbes is still very limited and investigations on their phylogenic and functional characterization are far from complete. It is known that ruminal NC-degrading microorganisms mainly belong to bacteria, but also to protozoa and fungi. Bacteria are present at all stages of ruminal NC breakdown. Because of high deamination rates, HAB particularly contribute to excessive ruminal ammonia release and subsequent poor N utilization. So far, these bacteria were assumed to show low abundance in the rumen and to use AA as energy and N sources, but not carbohydrates. However, recent findings indicate the presence of HAB able to ferment both AA and carbohydrates. Protozoa are particularly active at degrading insoluble proteins, dipeptides and are responsible for bacterial and fungal predation. The contribution of fungi to ruminal NC degradation is smaller and concentrates on proteo- and peptidolysis. Due to nutritional interdependences and competing demands, NC-degrading microorganisms are continuously interacting with each other and further members of the rumen microbiota. By using different dietary strategies or vaccination, the composition as well as the metabolic activity of NC-degrading microorganisms can be manipulated. Thereby, the targeted composition as well as the treatment of feedstuffs provide promising approaches. Besides, a variety of feed additives including probiotics, condensed tannins or PBLC may constitute effective tools for controlling ruminal N metabolism. The limited existence and partial inconsistency of results confound the exact evaluation of so far investigated ways to manipulate NC-degrading microorganisms. Beyond that, too many approaches being effective in vitro were not followed up in vivo but this should be undertaken in the future. Thus, systematic and comprehensive studies investigating the composition and metabolism of the rumen microbiome are crucial to obtain a deeper knowledge that will subsequently allow a targeted manipulation. Thereby, omics and qPCR will play a leading role, supported by new developments in sampling techniques.

Abbreviations

AA: Amino acid; *B.*: *Butyrivibrio*; BCVFA: Branched-chain volatile fatty acids; *Cl.*: *Clostridium*; CT: Condensed tannin; *En.*: *Entodinium*; *Eu.*: *Eubacterium*; HAB: Hyper-ammonia producing bacteria; Ig: Immunoglobulin; *L.*: *Lactobacillus*; *M.*: *Megasphaera*; N: Nitrogen; NC: Nitrogenous compound; *Ncm.*: *Neocallimastix*; NGS: Next-generation sequencing; *P.*:

Prevotella; PBLC: Plant bioactive lipid compound; PPO: Polyphenol oxidase; *Psc.*: *Peptostreptococcus*; qPCR: quantitative real-time polymerase chain reaction; *Rb.*: *Ruminobacter*; RUP: Rumen-undegraded dietary crude protein; *S.*: *Saccharomyces*; *Sel.*: *Selenomonas*; *Staph.*: *Staphylococcus*; *Strep.*: *Streptococcus*; WSC: Water-soluble carbohydrates

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CHAPTER 4

Effect of wilting intensity, dry matter content and sugar addition on nitrogen fractions in lucerne silages

T. Hartinger[1], N. Gresner[1], K.-H. Südekum[1]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany

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Abstract

Pre-ensiling treatments can significantly influence the composition of lucerne (*Medicago sativa* L.) silages (LS). Besides dry matter (DM) content and availability of water-soluble carbohydrates (WSC), wilting intensity may exert a strong impact on the crude protein (CP; nitrogen [N] × 6.25) fractions. The present study aimed to evaluate the effects of DM level, wilting intensity, and sucrose addition on N compounds and fermentation products in LS. Pure lucerne stand (cultivar Plato) was wilted with either high or low intensity to DM contents of 250 and 350 g kg^{-1}, respectively, and ensiled with or without the addition of sucrose. Non-protein-N (NPN) concentration in LS was affected by all pre-ensiling treatments and with 699 g kg^{-1} CP, NPN was lowest in high-intensity wilted high-DM LS with sucrose addition. No effects were observed on in vitro-estimated concentrations of utilizable CP at the duodenum, a precursor to metabolizable protein. Sucrose addition and higher DM level decreased acetic acid and ammonia-N concentration in the silages. Therefore, the present study demonstrated the beneficial manipulation of CP fractions in LS by high-intensity wilting to higher DM contents and that the provision of WSC may be necessary for sufficient silage fermentation and protein preservation.

Introduction

Compared with other forage species, lucerne (*Medicago sativa* L.) has a high crude protein (CP; Nitrogen [N] × 6.25) content and depending on its degradability in the rumen, a considerable part of the ruminant's demand for amino acids (AA) can be supplied by feeding lucerne [1]. Preserved as lucerne silage (LS), this forage is continuously available as a component for dairy and beef cattle diets, independently from vegetative growth periods. However, the vast majority of CP in LS is ruminally readily-degradable non-protein-N (NPN), i.e., from 50 up to 87% of total CP [2–4], which can be ascribed to proteolytic activities of lucerne-derived proteases before ensiling, and microbial enzymes during the ensiling process [5]. Legumes are also characterized by low proportions of water-soluble carbohydrates (WSC) [6], which firstly make them difficult to ensile, and secondly also result in silages with minimal concentrations of rapidly fermentable carbohydrates. Solely feeding LS leads to an inefficient microbial N fixation in the rumen [4,7] and consequently high N excretion causing increased environmental pollution. However, substantial N excretion may still occur in mixed LS-based diets because the provision of rapidly fermentable carbohydrates by concentrate is limited due to the risk of rumen acidosis [8]. Therefore, adequately meeting the microbial energy demand for fixing the N arising from the rapid degradation of high NPN amounts in LS is hardly feasible. Consequently, manipulating the CP fractions in LS should be targeted, and in order to improve this fraction, meaning by increasing true protein (TP) concentrations and decreasing low-molecular-weight CP, high-intensity wilting, i.e., with high solar radiation, may be an effective option that to date has not received much attention. Rapid drying should inactivate plant-derived proteases, whose functions rely on water, and consequently stabilize TP content of lucerne plants. Likewise, a previous study by Edmunds et al. [9] already showed that high-intensity wilting results in higher TP percentages in grass silages. Thus, we hypothesized that high-intensity wilting alone or in combination with further treatments may influence the CP composition in LS and decrease proteolysis during ensiling. Because lucerne contains limited amounts of WSC [6], the effect of sucrose addition before ensiling on the N fractions in LS was further tested, particularly as there is clear evidence for decreased ammonia-N concentration in glucose- and fructose-added LS [10] and a more stable silage fermentation in general [11]. Therefore, the objective of the present study was to evaluate N fractions in LS wilted with different intensities to DM contents of 250 or 350 g kg^{-1} and with or without the addition of sucrose. The hypothesis was that the highest TP preservation would occur in those LS, which received high-intensity wilting to 350 g kg^{-1} and with sucrose addition.

Materials and methods

Preparation of silages

The procedure for the preparation of the LS was adopted from Edmunds et al. [9] and partly modified as described in the following. On the 19th of July 2016, the third cut of a one hectare pure lucerne stand (cultivar Plato) at the early bud stage of maturity was harvested using a disc mower without a mechanical conditioner at 10 cm stubble height at the Educational and Research Centre Frankenforst of the Faculty of Agriculture, University of Bonn (Königswinter, Germany, 7° 12' 22'' E; 50° 42' 49'' N). The harvested material was immediately collected from the field and equally spread on either black plastic in the sun (high-intensity wilting; HI) or on white plastic in the shade (low-intensity wilting; LI). The lucerne layers on each plastic had a thickness of approximately 10 cm to ensure sufficient and consistent exposure of the entire plant material to the solar radiation. Immediately, a composite sample was taken and stored at -20°C for later analysis. This composite sample consisted of 20 single samples that were taken from different places of the lucerne layers on the white and the black plastic, respectively. During silage preparation, the sky was clear, and the weather conditions were sunny with a relative humidity of 59%, a maximum temperature of 32°C and 15 h of sunshine during the day and a minimum temperature of -20°C during the night. The plant material was wilted to DM levels (DML) of 250 and 350 g kg^{-1}, respectively, and ensiled either without or with sucrose addition (SU) of 125 g kg^{-1} DM. The amount of added sucrose was chosen as it constitutes the difference between the average WSC content of lucerne with 65 g kg^{-1} DM and perennial ryegrass (*Lolium perenne* L.) with 190 g kg^{-1} DM [12], which is good to ensile [13]. The compaction of the lucerne at ensiling was calculated according to the recommendations of the Federal Working Group for Forage Preservation (Bundesarbeitskreis Futterkonservierung; [12]) in Germany with 190.4 (±2.3) kg DM m^{-3} for low-DM LS and 215.8 (±4.6) kg DM m^{-3} for high-DM LS. The lucerne was ensiled in duplicate in 60 l plastic containers and stored for 120 days. Thus, eight different silage treatments were finally prepared, which are referred to as: 250HISU, 250HI, 250LISU, 250LI, 350HISU, 350HI, 350LISU, and 350LI. The required wilting durations were 2.5 h for 250HISU and 250HI, 4.0 h for 250LISU and 250LI, 7 h for 350HISU and 350HI and 22 h for 350LISU and 350LI.

Basic Analysis

After 120 days, the two plastic containers of each LS were pooled and three composite samples, each comprising 20 single samples from different spots of the silage heap, were taken and checked for the presence of mould or any other signs of spoilage. All composite samples were thoroughly mixed and 800 g fresh matter of each were freeze-dried and

ground successively using 3 mm and then 1 mm sieves (SM 100, Retsch, Haan, Germany). These samples were used for the following analyses, except fermentation pattern analysis, which was conducted with two subsamples (50 g) of each LS that were immediately taken after silo opening and stored at -20°C.

The proximate analyses were conducted in accordance with the Association of German Agricultural Analytic and Research Institutes (VDLUFA; [14]). The DM content was determined by drying the fresh silages overnight at 60°C and subsequently at 105°C for at least 3 h (method 3.1). Using the equation from Weissbach and Kuhla [15], DM was corrected for the loss of volatile compounds that occur during drying. Crude protein was determined by the Kjeldahl method (method 4.1.1) using a Vapodest 50s carousel (Gerhardt, Königswinter, Germany) and multiplying N by 6.25. Proportions of neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom), acid detergent fibre expressed exclusive of residual ash (ADFom), and acid detergent lignin (ADL) were determined in accordance with methods 6.5.1, 6.5.2, and 6.5.3, respectively.

Crude Protein Fractionation and Amino Acid Analysis

The CP fractionation was performed according to the Cornell Net Carbohydrate and Protein System [16], following recommendations and standardizations of Licitra et al. [17]. Briefly, five fractions (all expressed as g kg^{-1} CP; A, B1, B2, B3, and C) were obtained; fraction A represents NPN, fraction B1 represents rapidly ruminally degradable TP, fraction B2 represents moderately ruminally degradable TP, fraction B3 represents slowly ruminally degradable TP and fraction C represents indigestible TP. Fraction A is the difference between total CP and TP, which precipitates in tungstic acid. Fraction B1 is the difference between total TP and borate-phosphate-buffer-insoluble TP. Fraction B2 is borate-phosphate-buffer-insoluble TP minus neutral-detergent-insoluble TP and fraction B3 is the difference between neutral-detergent-insoluble TP and acid detergent-insoluble TP. Fraction C is acid-detergent-insoluble TP. Subsequently, total TP concentrations (g kg^{-1} CP) of samples were calculated by 1000 minus fraction A. The contents of free AA and total AA (sum of peptide-bound and free AA), including gamma-aminobutyric acid (GABA), were determined by ion-exchange chromatography according to the Commission Regulation (EC) No. 152/2009 of the European Communities [18]. This method is not valid for the determination of tryptophan and cannot differentiate between D and L forms of AA. Briefly, free AA were extracted with diluted hydrochloric acid and co-extracted nitrogenous macromolecules were precipitated with sulfosalicylic acid and removed by filtration before the free AA determination by ninhydrin reaction with spectrophotometric detection at 570 nm. The procedure for total AA determination depended on AA under investigation. Prior to hydrolysis, Cys and Met were oxidized with a performic acid-phenol mixture to cysteic acid

and methionine sulphone, respectively, whereas Tyr was determined in unoxidized samples only. All remaining AA were determined in either the oxidized or unoxidized sample. Samples were then hydrolyzed with hydrochloric acid and determined by ninhydrin reaction using spectrophotometric detection at 570 nm or 440 nm for Pro.

Modified Hohenheim Gas Test

In order to estimate utilizable CP at the duodenum (uCP), the modified Hohenheim gas test [19,20] was conducted as outlined in detail by Edmunds et al. [21]. Briefly, ruminal fluid was collected before morning feeding from two rumen-fistulated sheep receiving a 1:1 grass hay-pelleted compound maintenance ration twice daily. An amount corresponding to 200 mg DM of each sample was incubated in duplicate in each of two runs in 30mL of ruminal fluid-buffer solution for 8 and 48 h, as recommended for forages [22]. At the end of these incubation periods, syringe contents were analyzed for ammonia-N applying a Vapodest 50s carousel and uCP was calculated using the following equation:

$$\text{uCP (g kg}^{-1}\text{ DM)} = ((\text{ammonia-N}_{\text{blank}} + \text{N}_{\text{sample}} - \text{ammonia-N}_{\text{sample}})/\text{sample weight (mg DM)}) \times 6.25 \times 1000,$$

where ammonia-N is in mg 30 mL^{-1}, blank refers to the ruminal fluid-buffer solution without sample substrate, sample refers to the ruminal fluid-buffer solution with sample substrate, N_{sample} is N added to the syringe through the sample substrate (mg), and sample weight is the amount of sample substrate (mg DM) weighed into the syringe. When using a live product such as ruminal fluid, small biological fluctuations among runs are inevitable. To correct for this a protein standard provided by the University of Hohenheim was analyzed with every run. The standard was a concentrate mixture of (kg^{-1} DM) 450 g rapeseed meal, 300 g faba beans, and 250 g molasses sugar beet pulp, and had a CP content of 254 g kg^{-1} DM. The correction follows the same method as for gas production [23] whereby the mean uCP value for the standard, provided by the University of Hohenheim for 8 or 48 h, is divided by the recorded value of the standard for that run and all other samples are multiplied by the resulting correction factor. Whole runs were repeated if the correction factor, for either incubation time, lay outside the range of 0.9–1.1. The hay and concentrate standards typically used for correcting gas production were also included in the incubation, not only to correct gas production values, but to ensure the ruminal fluid solution followed typical fermentation. After the correction of obtained uCP, values from the incubation times were plotted against a log ((ln) time) scale and the resulting regression equation was used to calculate the effective uCP at passage rates of 0.02, 0.05, and 0.08 hr^{-1}, which are referred to as uCP2, uCP5, and uCP8, respectively. These passage rates represent the ruminal

digesta flow, including the solid and liquid phase, in animals with different production levels [24].

Fermentation Pattern Analysis

Subsamples (50 g) of all silages were used for fermentation pattern analysis. Procedures, as well as detection limits, are described in detail by Brüning et al. [25]. Briefly, a cold-water extract was prepared from all samples by blending the frozen substrate with 200 mL distilled water and 1 mL toluene and refrigerated overnight at 4°C. Extracts were then filtered using MN 615 filter paper (Macherey-Nagel, Düren, Germany) and subsequently microfiltered (Minisart RC, 0.45 µm pore size; Sartorius, Göttingen, Germany). Ammonia-N concentration was analyzed colorimetrically based on the Berthelot reaction [26]. The pH of the extracts was determined potentiometrically and lactic acid concentration was analyzed by high-performance liquid chromatography with refractive index detection in accordance with Weiß and Kaiser [27]. Volatile fatty acids, alcohols (methanol, ethanol, propanol, butanol, 2,3-butanediol), ethyl lactate, ethyl acetate, propyl acetate, and acetone were determined by gas chromatography with flame ionization detection [28,29]. The concentrations of WSC were determined using the anthrone method [30].

Statistical Analysis

Statistical analysis was performed with the GLM procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) using the following model: $Y = \mu + a_i + b_j + c_k + e_{ejk}$ where μ is the mean, a_i is the effect of the SU, b_j is the effect of the wilting intensity (WI), c_k is the effect of the DML and e_{ijk} is the residual error. The significance level was set at $\alpha = 0.05$. In order to test for interactions, field replicates would have been necessary [31], which were not available in the present study. As a consequence, silos were pooled to avoid an artificially created variation and only the main effects were tested. Particularly due to the limited extent of the present study, we preferred to cautiously draw conditional conclusions from a smaller data set as recommended by Lowry [32].

Results

General Chemical composition

As shown in Table 1, DM content was affected by SU and was slightly higher in SU LS. Concerning the CP content, effects of all three pre-ensiling treatments could be observed, whereby CP proportions ranged from 188 to 219 g kg^{-1} DM and were higher in LS without SU, LI, and 250DML, respectively. The SU treatment also affected the fibre fractions aNDFom and ADFom, which were lower in SU LS. No treatment factor had an effect on ADL.

Table 1. Effect of sucrose addition (SU), wilting intensity (WI), and dry matter (DM) level (DML) on DM content (g kg^{-1}), crude protein content (g kg^{-1} DM), and fibre fractions (g kg^{-1} DM) in lucerne silages (fresh lucerne values are provided as ease for comparison).

Silage	DM	CP	aNDFom	ADFom	ADL
Fresh lucerne	213.1	213	431	340	91
250HISU	254.8	195	458	322	88
250HI	240.5	215	463	364	88
250LISU	255.0	198	422	325	87
250LI	246.8	219	429	355	86
350HISU	344.5	188	416	325	88
350HI	340.0	211	446	338	90
350LISU	346.8	195	390	312	96
350LI	339.0	213	421	336	95
Results of statistical analyses					
SEM	18	4	9	6	1
SU	**	**	*	*	NS
WI	NS	*	NS	NS	NS
DML	**	*	NS	NS	NS

250HISU = 250 g kg^{-1}, high-intensity wilting and sucrose addition; 250HI = 250 g kg^{-1}, high-intensity wilting and no sucrose addition; 250LISU = 250 g kg^{-1}, low-intensity wilting and sucrose addition; 250LI = 250 g kg^{-1}, low-intensity wilting and no sucrose addition; 350HISU = 350 g kg^{-1}, high-intensity wilting and sucrose addition; 350HI = 350 g kg^{-1}, high-intensity wilting and no sucrose addition; 350LISU = 350 g kg^{-1}, low-intensity wilting and sucrose addition; 350LI = 350 g kg^{-1}, low-intensity wilting and no sucrose addition; DM = Dry matter; CP = Crude protein; aNDFom = Neutral detergent fibre after incineration and amylase treatment; ADFom = Acid detergent fibre after incineration; ADL = Acid detergent lignin; SEM = Standard error of the mean (without consideration of fresh lucerne); NS = not significant; * = $p < 0.05$; ** = $p < 0.01$.

Crude Protein Fractions and Amino Acids

The CP fractionation revealed various differences between the eight silage treatments (Table 2). Non-protein N was the largest CP fraction in all LS but was more than 110 g kg CP^{-1} higher for 250LI when compared to 350HISU. Likewise, NPN (fraction A) was affected by all three factors, i.e., SU, WI, and DML, with increased proportions in 250DML silages. Both HI and SU decreased the NPN proportion in LS. Moderately ruminally degradable TP (fraction B2) was the second largest fraction and highest in silages with 350DML and SU. As with NPN, the largest difference for moderately ruminally degradable TP was found between 250LI and 350HISU. Rapidly (fraction B1) and slowly ruminally degradable TP (fraction B3), as well as indigestible TP (fraction C), were present in small proportions of total CP and slowly ruminally degradable TP was partly not quantifiable. Thus, the effects of SU and DML on these fractions are negligible. Total TP was calculated by subtracting NPN (fraction A) from total CP. Consequently, 250LI had the lowest TP content and, except for 350LI, was clearly separated from 350DML silages.

Both SU and DML affected several AA concentrations determined as peptide-bound and free AA, whereas only a few were influenced by WI (Table 3). Concentrations of Thr, Arg, Ser, Asp, and Glu were increased by SU, whereas it decreased Ile, Leu, Val, and Ala. Besides, a strong tendency ($p = 0.06$) for increased Lys concentrations in SU LS were observed. The HI treatment decreased the concentrations of Ile, and Val. The 350DML treatment increased the concentrations of Cys, Lys, Thr, Arg, His, Ser, Pro, Asp, and Glu. In contrast, concentrations of Ile, Leu, Val, and Ala were decreased in high-DM LS. The DML treatment affected free AA more than SU or WI (Table 4) and HI tended to decrease Ile concentrations ($p = 0.09$). The SU treatment increased the concentration of free Thr and tended to increase free Glu ($p = 0.07$), whereas it decreased free Ile, Leu, Val, and Ala. The 350DML LS showed higher concentrations of free Lys, Thr, Pro, Asp, Glu as well as free His that was not detectable in 250DML LS. Free Ile, Leu, Val, and Ala were reduced in 350DML LS. Regarding the amount of total free AA, SU decreased total free AA, whereas no influence of other pre-ensiling treatments was observed. Moreover, 350DML and SU reduced the concentrations of free and total GABA (Tables 3 and 4).

Table 2. Effect of sucrose addition (SU), wilting intensity (WI), and dry matter level (DML) on crude protein (CP) fractions (g kg^{-1} CP) and true protein content (g kg^{-1} CP) in lucerne silages (fresh lucerne values are provided as ease for comparison).

	Crude protein fraction†					
Silage	A	B1	B2	B3	C	TP
Fresh lucerne	259	289	383	27	42	741
250HISU	772	13	174	0	54	228
250HI	799	6	154	0	53	201
250LISU	782	11	16	0	47	218
250LI	812	11	139	0	58	188
350HISU	699	6	251	2	49	301
350HI	744	6	206	0	49	256
350LISU	718	3	253	2	47	282
350LI	779	7	182	0	46	221
Results of statistical analyses						
SEM	14	1	27	0	1	14
SU	**	NS	**	*	NS	**
WI	*	NS	NS	NS	NS	*
DML	**	NS	**	*	#	**

† According to the Cornell Net Carbohydrate and Protein system [16]; 250HISU = 250 g kg^{-1}, high-intensity wilting and sucrose addition; 250HI = 250 g kg^{-1}, high-intensity wilting and no sucrose addition; 250LISU = 250 g kg^{-1}, low-intensity wilting and sucrose addition; 250LI = 250 g kg^{-1}, low-intensity wilting and no sucrose addition; 350HISU = 350 g kg^{-1}, high-intensity wilting and sucrose addition; 350HI = 350 g kg^{-1}, high-intensity wilting and no sucrose addition; 350LISU = 350 g kg^{-1}, low-intensity wilting and sucrose addition; 350LI = 350 g kg^{-1}, low-intensity wilting and no sucrose addition; TP = True protein; SEM = Standard error of the mean (without consideration of fresh lucerne); NS = not significant; # = $0.05 < p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$.

Table 3. Effect of sucrose addition (SU), wilting intensity (WI), and dry matter (DM) level (DML) on contents (g kg^{-1} DM) of total amino acids (AA; the sum of peptide-bound and free AA) and gamma-aminobutyric acid (GABA) in lucerne silages.

AA	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Val	GABA
250HISU	21.1	2.3	11.3	1.2	10.2	9.6	1.9	9.5	14.8	3.2	3.2	9.3	6.2	3.2	3.6	11.8	10.3
250HI	29.4	1.7	4.2	0.7	5	3.5	1.3	10.3	16.5	2.5	2.2	8.5	2.1	2.2	2	13.3	16.7
250LISU	22.3	2.1	10.6	1.3	10.3	9.7	2	9.7	15	3.1	3.2	9.5	6	2.9	3	12	10.7
250LI	28.8	1.7	4.4	0.9	5.5	6	1.6	10.6	16.3	2.6	2.7	8.4	2.3	2.2	2	13.4	16.5
350HISU	14.7	3.1	17.6	0.13	12.5	8.9	3.3	8.9	14.4	7.6	3	9.1	8.7	4.7	6.4	11	8
350HI	19.1	2.3	13	1.4	9.7	10	3.6	10	16	7.2	3.2	9.4	8.4	3	4	12.6	12.5
350LISU	14.4	3.2	19.9	1.4	12.5	9.2	3.5	9.1	14.5	8.6	3.1	9.4	10.3	5.1	6.9	11.7	7.4
350LI	18.1	2.3	14.4	1.4	10.9	10.3	4.4	10.3	16.3	7.8	3.4	10.2	10.9	3.4	4.4	13.2	10.9
SEM	2.02	0.2	1.99	0.16	1.01	0.84	0.4	0.21	0.31	0.95	0.13	0.2	1.18	0.38	0.65	0.31	1.23
Results of statistical analyses																	
SU	**	**	**	NS	**	NS	NS	**	**	#	NS	NS	NS	**	**	**	**
WI	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	*	NS
DML	**	**	**	#	**	NS	**	**	*	**	NS	NS	**	**	**	*	**

250HISU = 250 g kg^{-1}, high-intensity wilting and sucrose addition; 250HI = 250 g kg^{-1}, high-intensity wilting and no sucrose addition; 250LISU = 250 g kg^{-1}, low-intensity wilting and sucrose addition; 250LI = 250 g kg^{-1}, low-intensity wilting and no sucrose addition; 350HISU = 350 g kg^{-1}, high-intensity wilting and sucrose addition; 350HI = 350 g kg^{-1}, high-intensity wilting and no sucrose addition; 350LISU = 350 g kg^{-1}, low-intensity wilting and sucrose addition; 350LI = 350 g kg^{-1}, low-intensity wilting and no sucrose addition; SEM = Standard error of the mean; NS = not significant; # = $0.05 < p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$.

Table 4. Effect of sucrose addition (SU), wilting intensity (WI), and dry matter (DM) level (DML) on contents (g kg^{-1} DM) of free amino acids (AA) and gamma-aminobutyric acid (GABA) in lucerne silages.

AA	Ala	Arg	Asp	GABA	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Thr	Val
250HISU	21.9	0	0.6	9.7	3	6	0	6	10.4	0	2.1	5.9	2.9	1.4	7.8
250HI	32.5	0	0	12.5	0	0.8	0	8	12.8	0	1.2	5.3	0	0.2	10.6
250LISU	23.5	0	0.4	10.1	3.1	6.2	0	6.3	10.7	0	2.2	6.1	2.8	0.9	8.3
250LI	32	0	0.3	12.4	0.5	3.1	0	8.3	12.7	0	1.7	5.3	0.3	0.3	10.6
350HISU	13.4	0	5.4	7.6	3.9	4.7	1	4.8	8.8	3.6	1.5	4.9	4.6	3.7	6.1
350HI	19.8	0	6	9.3	3	6.6	1.7	7.1	11.4	4.1	1.7	5.8	6	2.1	9.1
350LISU	12.7	0	7.4	7.1	4.1	4.6	1.5	4.9	8.7	4.4	1.5	5.1	6.1	4	6.4
350LI	18.4	0	7.5	8.2	4.3	6.5	2.2	7.3	11.5	4.5	1.7	6.5	8.3	2.4	9.4
SEM	2.64	0	1.21	0.72	0.57	0.71	0.32	0.47	0.55	0.79	0.11	0.19	1.03	0.51	0.61
Results of statistical analyses															
SU	**	NS	NS	**	#	NS	NS	**	**	NS	NS	NS	NS	**	**
WI	NS	NS	NS	NS	NS	NS	NS	#	NS	NS	NS	NS	NS	NS	NS
DML	**	NS	**	**	*	NS	**	**	**	**	NS	NS	*	**	**

250HISU = 250 g kg^{-1}, high-intensity wilting and sucrose addition; 250HI = 250 g kg^{-1}, high-intensity wilting and no sucrose addition; 250LISU = 250 g kg^{-1}, low-intensity wilting and sucrose addition; 250LI = 250 g kg^{-1}, low-intensity wilting and no sucrose addition; 350HISU = 350 g kg^{-1}, high-intensity wilting and sucrose addition; 350HI = 350 g kg^{-1}, high-intensity wilting and no sucrose addition; 350LISU = 350 g kg^{-1}, low-intensity wilting and sucrose addition; 350LI = 350 g kg^{-1}, low-intensity wilting and no sucrose addition; SEM = Standard error of the mean; NS = not significant; # = $0.05 < p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$.

Modified Hohenheim Gas Test

Irrespective of calculated passage rate, pre-ensiling treatments had no effect on effective uCP values of LS (Table 5). Only uCP8 values tended to be higher for 250DML LS ($p = 0.08$).

Fermentation Pattern

Acetone, 2,3-butandiol, i-valeric acid, n-valeric acid and propyl acetate were not detected in any sample during fermentation pattern analysis. The SU treatment decreased silage pH and ammonia-N concentration, but increased lactic acid concentration as well as ethyl acetate and ethyl lactate (Table 6). Besides, LS without SU tended to have higher concentrations of acetic acid ($p = 0.09$), WSC ($p = 0.09$), and ethanol ($p = 0.06$). In contrast, WI had no effect on response variables. The 350DML reduced acetic acid as well as methanol concentration and tended to decrease ammonia-N ($p = 0.06$) and propanol ($p = 0.09$) in LS compared to 250 DML (Table 6).

Table 5. Effect of sucrose addition (SU), wilting intensity (WI), and dry matter (DM) level (DML) on effective utilizable crude protein at the duodenum (g kg^{-1} DM).

Silage	uCP2	uCP5	uCP8
250HISU	72	109	127
250HI	82	114	131
250LISU	74	112	131
250LI	76	110	128
350HISU	74	105	121
350HI	80	107	121
350LISU	75	108	124
350LI	74	103	118
SEM	1.2	1.3	1.7
Results of statistical analyses			
SU	NS	NS	NS
WI	NS	NS	NS
DML	NS	NS	#

250HISU = 250 g kg^{-1}, high-intensity wilting and sucrose addition; 250HI = 250 g kg^{-1}, high-intensity wilting and no sucrose addition; 250LISU = 250 g kg^{-1}, low-intensity wilting and sucrose addition; 250LI = 250 g kg^{-1}, low-intensity wilting and no sucrose addition; 350HISU = 350 g kg^{-1}, high-intensity wilting and sucrose addition; 350HI = 350 g kg^{-1}, high-intensity wilting and no sucrose addition; 350LISU = 350 g kg^{-1}, low-intensity wilting and sucrose addition; 350LI = 350 g kg^{-1}, low-intensity wilting and no sucrose addition; uCP2 = effective utilizable crude protein at the duodenum to passage rate of 0.02 hr^{-1}; uCP5 = effective utilizable crude protein at the duodenum to passage rate of 0.05 hr^{-1}; uCP8 = effective utilizable crude protein at the duodenum to passage rate of 0.08 hr^{-1}; SEM = Standard error of the mean; NS = not significant; # = $0.05 < p < 0.1$.

Table 6. Effect of sucrose addition (SU), wilting intensity (WI), and DM level (DML) on lactic acid, volatile fatty acids, ester compounds, alcohols, water-soluble carbohydrates (g kg^{-1} DM), and ammonia-nitrogen (N; g kg^{-1} N) in lucerne silages.

Silage	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Caproic acid	Ethyl acetate	Ethyl lactate	Methanol	Ethanol	Butanol	Propanol	WSC	Ammonia-N
250HISU	4.58	50.6	38.2	0.8	0.7	0.0	0.2	0.1	2.1	7.9	0.1	1.9	10.1	175
250HI	6.12	5.4	42.8	2.1	21.9	0.8	0.1	0.0	2.5	6.2	0.1	0.3	2.3	276
250LISU	4.61	52.4	38.2	1.5	1.5	0.0	0.2	0.1	2.6	8.7	0.1	1.7	10.6	157
250LI	5.85	15.3	48.4	2.0	7.2	0.8	0.1	0.0	3.0	5.4	0.1	0.3	3.1	221
350HISU	4.77	39.7	31.1	1.0	0.6	0.0	0.2	0.1	1.7	6.5	0.0	0.2	17.6	145
350HI	5.81	21.6	34.0	0.3	0.5	0.0	0.1	0.0	2.2	6.3	0.1	0.2	5.0	217
350LISU	4.65	36.2	31.2	0.8	0.3	0.0	0.2	0.1	1.2	5.8	0.0	0.1	46.0	149
350LI	5.73	38.4	31.4	1.3	0.3	0.0	0.1	0.0	1.8	4.3	0.1	0.2	4.8	191
Results of statistical analyses														
SEM	0.24	5.94	2.22	0.2	2.7	0.13	0.02	0.02	0.20	0.49	0.02	0.26	5.12	15.9
SU	**	*	#	NS	NS	NS	**	**	NS	#	NS	NS	#	**
WI	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DML	NS	NS	**	NS	NS	NS	NS	NS	*	NS	NS	#	NS	#

WSC = Water-soluble carbohydrates; 250HISU = 250 g kg^{-1}, high-intensity wilting and sucrose addition; 250HI = 250 g kg^{-1}, high-intensity wilting and no sucrose addition; 250LISU = 250 g kg^{-1}, low-intensity wilting and sucrose addition; 250LI = 250 g kg^{-1}, low-intensity wilting and no sucrose addition; 350HISU = 350 g kg^{-1}, high-intensity wilting and sucrose addition; 350HI = 350 g kg^{-1}, high-intensity wilting and no sucrose addition; 350LISU = 350 g kg^{-1}, low-intensity wilting and sucrose addition; 350LI = 350 g kg^{-1}, low-intensity wilting and no sucrose addition; SEM = Standard error of the mean; NS = not significant; # = $0.05 < p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$.

Discussion

General Chemical Composition

Crude protein contents were lower in SU LS, which likely reflects a dilution caused by the SU. The same may apply to aNDFom and ADFom concentrations. Besides a dilution effect, a stronger acidic hydrolysis of hemicelluloses by acids [33] originating from microbial sucrose metabolism may have occurred during ensiling, consequently causing the lower aNDFom concentrations in SU LS. Nonetheless, proportions of aNDFom and ADFom were in a similar range of other LS [34,35]. Likewise, proportions of fibre fractions in fresh lucerne material were similar to previous findings [3,34,35].

The DML also affected CP content of LS, which was higher in 250 DML LS. This slight difference could have been caused by mechanical losses during harvest and consequently a lower leaf proportion in the ensiled plant material. Therefore, LS with higher DM contents seem to be favorable with regards to CP composition, but should not exceed a certain level to preserve the leaf fraction that dries faster than the stem part and thus is more prone to field losses [36]. The WI treatment also effected CP content, but the effect seems negligible as mean CP difference between LS with HI and LI was only 4 g kg^{-1} DM.

Crude Protein Fractions

Crude protein fractions in fresh lucerne material were in a typical range for this forage legume, although the present proportion of NPN compounds of 259 g kg^{-1} CP was substantially higher than literature data, i.e., 150 g kg^{-1} CP [3], 170-183 g kg^{-1} CP [37] and 180–190 g kg^{-1} CP [35]. It may be noted that the highest discrepancy in NPN proportion, found between the results of the present study and of Guo et al. [3], might partly also derive from different methodologies. In the present study, tungstic acid was used to precipitate TP, which cuts off peptides of an approximate chain length of more than three AA [17]. Guo et al. [3], however, used trichloroacetic acid to precipitate TP, which cuts off at about 10 AA [17].

The pattern of CP fractions in LS with NPN being the largest, and moderate ruminally degradable TP being the second largest proportion of total CP corresponded to the literature [3,35]. It is notable that NPN contents of present LS were similar to those from Broderick [2] and Seale et al. [10], but higher than values reported by others, for instance, 684 g kg CP^{-1} [3] or 599 g kg CP^{-1} [38] in untreated LS. However, these NPN values were determined after only 35 or 30 days of ensiling, respectively, probably underestimating NPN in LS as intrinsic protease and carboxypeptidase were recently shown to remain largely active for more than 30 days after ensiling [38]. Therefore, NPN values of the present LS, which were stored for

120 days, might provide a more realistic insight and should be considered when comparing different results or designing experiments for silage additive evaluation in LS. In this context, it may be noted that the Federal Working Group for Forage Preservation (Bundesarbeitskreis Futterkonservierung; [12]) recommends at least 90 days of ensiling for any silage-related experiment, e.g., when evaluating the effect of silage additives. Besides, the influence of the cut number should also be taken into account as NPN was 10% higher in third-cut LS when compared to NPN proportion of first-cut LS from the same sward [35]. Likewise, present LS was produced from a third cut, which thus may have been a contributing factor and should be investigated in future studies.

The SU reduced NPN along with increasing moderately ruminally degradable TP, which was likely caused by faster and stronger acidification, consequently suppressing proteolytic microorganisms in the silos [11]. These observations were in accordance with Seale et al. [10] who analyzed the effect of glucose and fructose addition with or without microbial inoculants on LS. However, in Italian ryegrass (*Lolium multiflorum* LAM.), Heron et al. [39] found that plant-derived proteases remained active over a wide pH range, which is also true for lucerne with major endopeptidases having optimum activities at pH 4 [40]. Thus, despite SU treatment and probably rapid acidification, the relevant plant-derived proteolytic activity may have taken place, particularly in 250DML LS.

An effect of WI was found for NPN concentration, which was higher in LI LS. Likewise, high-intensity wilted grass silages had approximately 100 g kg^{-1} CP lower NPN proportions compared to low intensively wilted grass silages [9] and, together with the present observations, demonstrate the TP stabilizing effect of HI treatments. The rapid inhibition of plant-derived proteases, which depend on sufficient water availability [5], may be causative. In this context, Owens et al. [41] produced LS with a DM of 350 g kg^{-1} and observed reduced NPN amounts of approximately 50 g kg^{-1} total N in those LS that needed shorter wilting periods to reach the desired DM, which thus can be ascribed to a higher WI. Likewise, when wilting times were different due to varying levels of shade during wilting, they also observed an increase of NPN with shade, thus substantiating the TP stabilizing effect of an HI treatment. However, it must be considered that although there is evidence for a reduction of plant-derived protease activity by HI treatment, it is very arguable whether plant enzymes were completely deactivated as the moisture loss was only until a DM content of maximal 350 g kg^{-1}. Thus, plant-derived proteases may still have contributed to overall proteolytic processes resulting in the still substantial conversion of TP to NPN in high-intensity wilted LS.

The DML treatment also effected NPN proportion, which was lower in 350DML LS. This confirmed previous findings [9,42] and may be explained by a lower water activity in the silos,

consequently reducing microbial metabolism [34]. However, this mechanism should be even more pronounced at DM contents above 500 g kg^{-1} [9]. The effect of DML on slowly ruminally degradable TP may be of marginal importance as this fraction could not be determined in six of eight LS. The higher contents of moderately ruminally degradable TP in 350DML LS, however, may be beneficial regarding the quality of CP that is provided to the animal, meaning a decelerated ruminal CP degradation and therefore potentially improved N utilization by rumen microorganisms. As obtained for the TP proportion of 350HISU, the combination of HI, elevated DML and SU should have limited both plant-derived and microbial CP degradation and thus most effectively stabilized the TP content in the present study.

Amino Acids

A variety of factors influence the AA composition in silages, including wilting rate, acidification, and the microbial activity in the silo, but also plant-associated factors like tannin concentration or activity of plant proteases [5]. To the authors' knowledge, information about the effects of WI, DML, or SU on AA composition of LS is rare [3,43]. However, as the vast majority of AA is degraded in the rumen, knowledge on AA profiles seems to be more important for feedstuffs with high ruminally undegradable CP [9], which does not apply to the present LS. Though, it is worthy of remark that pre-ensiling treatments clearly effected the AA composition of LS. For instance, the higher proportions of free His, Asp, Lys, Thr, Glu, and Pro in 350DML LS should be the result of reduced microbial activity [5]. Likewise, a similar pattern was observed for total AA. The reducing effect of SU on total free AA content supported the TP preserving effect that was also observed for the distribution of CP fractions and should be caused by rapid acidification [5].

Biogenic amines are predominantly formed during proteolysis in silages [44] and Ohshima and McDonald [45] described the decarboxylation of Glu to GABA during lucerne ensiling, which is reflected by the lower Glu concentrations in the present LS without SU. As summarized by Scherer et al. [44], biogenic amines are associated with lower feed intake and potential impairments to animal health. Thereby, GABA is an important biogenic amine and known to act as a neurotransmitter. It is also involved in the sensation of pain and anxiety as well as neurological diseases [46]. Although there is no clear trend for the effect of GABA on feed intake [44], a negative correlation between feed intake and total amine concentration has been observed [47] and the reduced GABA concentration in LS with 350DML or SU may, therefore, be interpreted as beneficial; particularly also because of potential health risks when biogenic amines would be absorbed by host animals, who, however, are more susceptible under acidotic conditions [48].

Modified Hohenheim Gas Test

None of the pre-ensiling treatments had an effect on effective uCP values at any calculated passage rate. As Edmunds et al. [9] observed higher uCP values for fast wilted grass silages, the absence of any effect was not expected. Although artificially dried lucerne (90°C for 3 min) showed reduced effective N degradability and degradation rates in the rumen [49], the WI achieved in the present study may not have been high enough to cause a similar impact. Moreover, the generally limited availability of WSC in all present LS may have prevented an effect on uCP as CP was degraded to ammonia-N, but not reused for microbial protein synthesis in the syringes.

Edmunds et al. [9] found that varying CP contents in grass silages from the same sward can confound the detection of possible effects on uCP as uCP values are calculated from the difference between N content in the syringe, which is determined by the CP content of the sample, and ammonia-N in the syringe. Therefore, these authors recalculated the effective uCP values with an average CP concentration. Thus, as the CP concentrations for the present LS also showed a variance, the effective uCP values were recalculated using the average CP content of LS with and without sucrose, respectively. Thereby, no effects of pre-ensiling treatments on uCP were obtained (data not shown). Moreover, a greater standard deviation might further impair the determination of clear effects. However, the inclusion of additional runs did not reduce standard deviation in the present study and thus were not included in the calculation of effective uCP.

Fermentation Pattern

The pH values were lower for SU LS, which was reflected by higher lactic acid concentration in these silages. Without the SU treatment, the high buffering capacity of lucerne [7] may have hindered rapid and strong acidification and consequently resulted in higher pH. In this context, the higher ammonia-N contents should also be considered, which can limit the pH drop in silages, as well [50]. Besides, low lactic acid concentration may be caused by metabolic activities of lactate-utilizing lactobacilli [51]. Owens et al. [7] stated a pH below 5.0 as a threshold to maintain forage quality and limit protein degradation in the silo, which thus was only met by SU LS. Likewise, the pH of these silages was within the common range for legume silages at this DM content [50]. Water-soluble carbohydrates [52] as well as total non-structural carbohydrates [7] decrease during wilting of lucerne due to plant enzyme activity and respiration, which are both reduced by moisture loss [53]. Therefore, there should have been less WSC degradation in the plant material undergoing HI treatment and consequently, a stronger pH drop along with increased lactic acid concentrations in HI LS was initially expected. However, the lack of a wilting effect may be explained by the overall

very low WSC concentration of lucerne [6], which further was cut in the morning when WSC concentrations are again lower compared to the afternoon [7].

An impact of DM content on silage pH was often described in the literature [34,50]. Thereby, silages with DM contents below 300 g kg^{-1} are extremely susceptible to clostridial fermentation [50], which results in elevated pH values as well as high butyric acid concentrations. In the present study, however, DM content had neither an effect on silage pH nor on butyric acid concentration. Possibly the SU treatment superimposed a potential effect of DML, which is indicated by closer examination of butyric acid concentrations, which were numerically but not statistically significantly higher in 250DML LS. Moreover, the influence of DM content on clostridial fermentation and thus silage pH is more pronounced at DM contents of 400 g kg^{-1} or more [11], which is confirmed by the findings of Santos and Kung [34].

The lower ammonia-N concentration in SU LS further strengthens the assumption that addition of rapidly fermentable carbohydrates better inhibited degradation of nitrogenous compounds in these LS compared to LS without SU, and similar trends have been observed previously [10]. Regarding the impact of DML, ammonia-N concentration is generally higher in wet silages, which corresponds to present findings and is often ascribed to clostridial fermentation [50]. Likewise, reduced ammonia-N contents in LS with high DM contents were also observed by Santos and Kung [34]. Thus, a greater WI seemed to preserve CP from degradation in the silo. However, according to Wyss et al. [35], ammonia-N proportions lower than 100 g kg^{-1} N is preferable for LS. This threshold was not met in the present study, even for 35HISU. In comparison to fresh-cut lucerne [35], plant material of the present study already showed a higher NPN proportion before ensiling, and may explain the high ammonia-N concentration in LS, irrespectively of applied pre-ensiling treatment. However, the increase of NPN from fresh-cut material to silage material were on the same level in the study by Wyss et al. [35] and the present study. Regarding the high ammonia-N concentration in the present LS, the variation of CP composition between different lucerne cultivars [54] should be considered, as well. Moreover, it can be speculated whether chopping of plant material subsequently supporting silo compression would have increased TP proportions as it was described earlier [50,55]. However, as LS was prepared according to recommended guidelines [12], the latter point may be of minor importance. Besides, higher ammonia-N concentration is assumed to be associated with undesirable metabolites like biogenic amines [50], which is in line with the present observations for higher GABA concentration in 250DML LS.

High concentration of acetic acid is associated with high DM and energy losses [50] as well as considerably reduced ad libitum feed intakes [56]. Compared to the literature [34,35], the

acetic acid contents of LS in the present study can be classified as slightly high for 250DML LS and thus would negatively impact their nutritive value. Increased activity of Enterobacteriaceae [57], as well as increased deamination [5], could be causative for acetic acid formation, which is further favoured by high moisture contents [50] and in accordance with the higher acetic acid concentration in 250DML LS. However, the presence of acetic acid is not a disadvantage per se. The average 32 g kg^{-1} DM acetic acid in the 350DML LS, however, might be regarded beneficial as such concentrations have a positive effect on aerobic stability of silages [58].

A butyric acid concentration higher than 5 g kg^{-1} DM indicate elevated clostridial activity and due to high losses of energy, this means diminished energy supply to the animal and, consequently, performance may suffer [50]. This threshold was not exceeded for 350DML LS and only applies to 250HI and 250LI. Likewise, these two treatments also had the highest ammonia-N concentration, which further points to clostridial fermentation [50]. Together with the observation that 250HI and 250LI did also not meet the pH threshold for maintaining forage quality in the silo [7], these two LS should, therefore, be classified as poor-quality silages and potentially spoiled material.

Concerning ethanol, SU tended to increase this alcohol in the silages. Though, ethanol concentration was low for all LS and thus does not indicate elevated yeast metabolism [50,59]. Minor amounts of ethanol can also originate from heterofermentative lactic acid fermentation [60], which can never be fully prevented during ensiling. Weiß and Kalzendorf [52] observed higher concentrations of ethanol and ester compounds in LS with low DM contents and further postulated a positive correlation between ethanol and ester concentration in silages, which is both confirmed by the present findings for ethanol and ethyl lactate as well as ethyl acetate. The effect of esters in silages is not fully clear [47,61], but negative correlations to short-term DM intake were observed earlier [61]. Thus, despite lower NPN proportions in SU LS, the effect of SU on ester occurrence could be regarded as critical.

General Considerations

Up to now, WI has not received much attention in silage preparation and studies investigating the effect of different WI on silage characteristics are rare. It has been reported that wilting per se effects CP composition, for instance by reducing ammonia-N contents in LS [54]. Thus, applying HI treatments may even be more effective for stabilizing TP content in LS, which is underlined by the present findings that confirm our hypothesis of a TP preserving effect by the HI treatment. A variety of silage additives exists that limit proteolysis in LS [38]. However, they cause costs for acquisition, and in case of organic acids, also for

maintenance of corroded machinery and concrete [62]. In contrast, HI treatment does not require additional application systems or further technical equipment and in this regard is an easy to apply tool for improving the quality of on-farm produced protein, and consequently might help to reduce costs for ruminant diets and increase sustainability. Additionally, feed intake of wilted grass silage was increased when compared to non-wilted [63]. In case this also applies to LS, a superior energy and nutrient provision to the animal may be achieved by intensively wilting lucerne to higher DML; provided that mechanical losses during harvest do not exceed the benefits of HI. Regarding the practicality of HI treatment, if possible high solar radiation along with high wind speed should be present during lucerne harvesting. However, this cannot always be guaranteed, which restricts the practicability of HI treatments. To support the effect of rapid dehydration, maceration can be an effective addition to further increase the wilting rate [64] or to compensate weather conditions that may not be as ideal for HI as described in the present study. However, the risk of mechanical losses and thereby associated nutrient changes can be higher when using maceration [64], which needs to be taken into account. Besides, artificial drying treatments are surely a more weather independent option to obtain similar TP preservation [65] as here found for HI in the sun. However, increased production costs due to high energy demands may outweigh the beneficial effects [36] of artificial drying on CP composition.

Concerning the effects of SU on fermentation and CP quality, provision of rapidly fermentable carbohydrates is recommended. Particularly because an inoculation with lactic acid producing bacteria alone may not improve the situation as long as there is not enough easily accessible substrate for lactic acid fermentation [10]. Thus, relating to large scale on-farm conditions, mixing lucerne crop with molasses, crushed cereals, or high WSC forage species before ensiling may constitute a method for equivalently substituting SU treatment in LS. A delayed cutting of lucerne in order to increase non-structural carbohydrates, particularly starch, may not be appropriate as Owens et al. [7] did not find a protein preserving effect in LS differing in WSC content due to different cutting times during the harvest. However, present results revealed concerns about promoted ester and ethanol formation in SU LS that should be kept in mind and require further investigation.

Finally, the pre-ensiling treatment combination of all three factors, i.e., HI treatment to high DML with SU, has the strongest potential to reduce the extent of CP degradation during ensiling, thus improving the protein value and potentially increasing ruminal N retention, particularly when combined with an appropriate carbohydrate source.

Conclusions

The effect of WI in silage preparation has not received much attention thus far. However, the present findings underline the importance of HI to limit CP degradation in LS. Therefore, if possible, at harvest, HI should be considered during silage production with lucerne. Regarding the observed effects of SU, providing an additional carbohydrate source to lucerne crop before ensiling is effective to minimize TP degradation and improves silage fermentation quality. However, caution should be paid to volatile organic compounds when operating with SU. Combining an HI treatment to DML of 350 g kg^{-1} with the provision of rapidly fermentable carbohydrates will maintain higher TP proportions along with improving fermentation quality in LS. Otherwise, there is a high chance for poor quality LS that in consequence cannot be fed without the risk of impairing animal performance and health. In order to underpin the present findings and to expand the sparse knowledge on WI, it is necessary to investigate such pre-ensiling treatments over several growth cycles and to further examine if the beneficial effects observed at silage stage can be transferred to rumen fermentation and animals.

Author Contributions

Conceptualization, N.G. and K.-H.S.; formal analysis, T.H.; investigation, T.H.; validation, T.H., N.G. and K.-H.S.; writing—original draft preparation, T.H.; writing—review and editing, T.H., N.G. and K.-H.S.; visualization, T.H.; supervision, K.-H.S.; project administration, K.-H.S.; funding acquisition, K.-H.S.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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CHAPTER 5

In vitro ruminal fermentation characteristics of alfalfa silages in response to different pre-ensiling treatments

T. Hartinger[1], N. Gresner[1], K.-H. Südekum[1]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany

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Abstract

The present study investigated the *in vitro* ruminal fermentation of alfalfa silages (AS) that had been produced using different pre-ensiling treatments, *i.e.*, by changing the wilting intensity and dry matter concentration, and adding sucrose, and therefore differed in silage quality and their N fractions. The data were obtained using an *in vitro* rumen-simulation technique (Rusitec) system, in which the AS were incubated isonitrogenously in quadruplicate. Samples were taken after 2 days (first time point) and 7 days (second time point) of AS incubation, and a variety of fermentation characteristics as well as the degradability of fiber fractions and organic matter were determined. Sucrose addition substantially raised the propionate concentration during both sampling time points from an average of 17.8 to 29.7 mmol/L ($P < 0.001$), which might be explained by microbial utilization of residual sugars and lactate from the AS. The extraordinary high concentrations of isovalerate and ammonia-N with all AS point to enhanced deamination activity. At the second time point, the n-butyrate concentration increased during the incubation of high-intensity wilted AS ($P = 0.007$), which might have been caused by the higher hemicellulose degradability that was also observed for these silages ($P = 0.002$). However, the organic matter degradability decreased ($P= 0.035$), indicating a lower degradability of other feed fractions. The gas production ($P<0.001$) and degradability of organic matter ($P = 0.002$) and fiber fractions ($P < 0.001$) decreased from first to second time point, whereas the concentrations of ammonia-N ($P = 0.004$), acetate ($P < 0.001$), and isovalerate ($P < 0.001$) increased. Thus, it seemed that alterations in the Rusitec system and the microbial community occurred, yet it is unclear why the acetate concentration increased, whereas the fiber degradability decreased. The beneficial effects of combining all three pre-ensiling treatments on silage quality, *i.e.*, higher acidification and increased true protein preservation, were not fully transferred to the *in vitro* ruminal fermentation system, and comprehensive research on pre-ensiling treatments will pave the way for an optimized ruminal N utilization from AS in the future.

Introduction

Alfalfa (*Medicago sativa* L.) silages (AS) are common forage ingredients in dairy cow rations since they represent an on-farm produced protein source with a high acceptance by ruminants and simultaneously providing fiber (Dewhurst et al., 2003; Coblentz and Grabber, 2013). However, the vast majority of the crude protein (CP) fraction in AS consists of non-protein N (NPN; Coblentz and Grabber, 2013) and together with the low concentration of water-soluble carbohydrates (WSC; Luscher et al., 2014), this can lead to poor ruminal N fixation, which increases N excretion into the environment and reduces the sustainability and feed protein utilization efficiency of dairy farming (Coblentz and Grabber, 2013). This scenario might be particularly true when feeding low-concentrate dairy cow rations. In addition to environmental concerns, the metabolic costs associated with removing excess N through urea synthesis also pose a metabolic burden to the animal, which can eventually result in reduced performance (Reed et al., 2017). Thus, there is strong interest in identifying options to improve the N retention from AS in ruminants and efforts have focused on the stabilization of true protein during the ensiling of alfalfa. These efforts include the investigation of different alfalfa cultivars (Zheng et al., 2017), the application of various silage additives (Yuan et al., 2017), and the addition of WSC to alfalfa before ensiling (Seale et al., 1986), alone or in combination with varying wilting regimes (Hartinger et al., 2019) that were not considered so far during AS preparation.

Since silage additives incur costs for their acquisition, and in the case of organic acids, also for the maintenance of corroded machinery and concrete (Lorenzo and O'Kiely, 2008), high-intensity wilting to approximately 350 g/kg dry matter (DM) concentration, alone or in combination with WSC addition, has recently been shown to be an appropriate tool for improving the quality of on-farm produced CP from AS (Hartinger et al., 2019). The true protein was affected by all three factors with higher true protein concentration for AS produced with a high-intensity wilting treatment, 350 g/kg DM, or sucrose addition. Likewise, the highest true protein concentration was present when high-intensity wilting to 350 g/kg DM was combined with sucrose addition before ensiling (Hartinger et al., 2019). However, it remains unclear how these silages will be fermented in the rumen and whether the beneficial effects of the pre-ensiling treatments found during ensiling can be transferred to the rumen. Thus, the aim of this study was to evaluate *in vitro* ruminal fermentation characteristics of differently produced AS in a rumen-simulation technique (Rusitec) system by taking samples 2 and 7 days after starting to incubate the AS. Hence, the adaptation process can be followed by investigating fermentation characteristics during the adaptation, *i.e.* the first time point (FIR) after 2 days, and after the adaptation is finished, *i.e.* the second time point (SEC)

after 7 days. It was hypothesized that there would be varying *in vitro* fermentation patterns between the different AS and an improved fermentation along with reduced protein and AA degradation, as well as increased microbial CP in AS subjected to high-intensity wilting and sucrose addition before ensiling. Secondly, a higher fiber degradability at the second time point than the first due to an adaptation of the microorganisms to the AS was hypothesized.

Materials and methods

The animals used for obtaining liquid and solid ruminal content were kept according to the German Animal Welfare legislation at the Educational and Research Center Frankenforst of the Faculty of Agriculture, University of Bonn in Germany. All experimental procedures were conducted in accordance with the German guidelines for animal welfare and were approved (file number 84-02.04.2017.A247) by the Animal Care Committee of the state of North Rhine-Westphalia in Germany.

Experimental diets

The chemical composition of the 8 AS used in this study is presented in Table 1. The present article provides the *in vitro* ruminal fermentation data of these AS, for which the production, analysis, and chemical composition has been described previously and can be obtained in detail from Hartinger et al. (2019). Briefly, a third-cut pure alfalfa stand (cultivar Plato) was harvested at the early bud stage of maturity and equally spread on either black plastic in the sun, *i.e.*, the high-intensity wilting treatment (HI), or on white plastic in the shade, *i.e.*, the low-intensity wilting treatment (LI) until DM concentrations of 250 g/kg and 350 g/kg, were reached, respectively. The alfalfa was then ensiled in duplicate in 60 L plastic containers either without or with (SA) sucrose addition of 125 g/kg DM to reach the average WSC content of perennial ryegrass (*Lolium perenne* L.; Bundesarbeitskreis Futterkonservierung, 2011). Therefore, 8 different AS were produced, which are referred to as: 25HI, 25HISA, 25LI, 25LISA, 35HI, 35HISA, 35LI, and 35LISA. The silages were stored for 120 days and subsequently cut by hand to simulate the exposure to chewing by the ruminant, resulting in particle lengths of no more than 5 cm. Daily portions of each silage that were scheduled to be incubated in the Rusitec system were then weighed into poly-ethylene vacuum bags (300 × 200 mm, 160 µm, Frey Serviceverpackungen, Dombuhl, Germany) and stored anaerobically at 7°C until *in vitro* incubation.

Table 1. Chemical composition of alfalfa silages.

	Treatment[1]								
DM[2] concentration	250 g/kg				350 g/kg				
Wilting intensity	High		Low		High		Low		
Sucrose addition	+	-	+	-	+	-	+	-	
Item	25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM
DM, g/kg	255	241	255	247	345	340	347	339	18
Ash, g/kg DM	111	127	110	125	106	119	108	118	3
aNDFom[3], g/kg DM	458	463	422	429	416	446	390	421	9
ADFom[4], g/kg DM	322	364	325	355	325	338	312	336	6
ADL[5], g/kg DM	88	88	87	86	88	90	96	95	1
CP[6], g/kg DM	195	215	198	219	188	211	195	213	4
Ammonia-N, g/kg N	175	276	157	221	145	217	149	191	16
Lactic acid, g/kg DM	50.6	5.4	52.4	15.3	39.7	21.6	36.2	38.4	5.94
WSC, g/kg DM	10.1	2.3	10.6	3.1	17.6	5.0	46.0	4.8	5.12
CP fractions[7], g/kg CP									
A	772	799	782	812	699	744	718	779	14
B1	13	6	11	11	6	6	3	7	1
B2	174	154	166	139	251	206	253	182	27
B3	0	0	0	0	2	0	2	0	0
C	54	53	47	58	49	49	47	46	1

[1]Treatments include different: DM concentrations, i.e. 250 g/kg DM (25) or 350 g/kg DM (35); wilting intensities, i.e. low (LI) or high (HI); and sucrose addition (SA).

[2]DM = Dry matter.

[3]aNDFom = NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

[4]ADFom = ADF expressed exclusive of residual ash.

[5]ADL = Acid detergent lignin.

[6]CP = Crude protein.

[7]According to the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992).

Experimental procedure

A Rusitec system as described by Czerkawski and Breckenridge (1977) consisting of 6 vessels, which formed the experimental units, each with a volume of 800 mL, was used in the present experiment. Therefore, the 8 AS were tested in an 8 × 8 Latin square with 2 columns and 2 rows missing, *i.e.*, an incomplete Latin square design. All treatments were repeated in quadruplicate in a balanced 2 × 2 × 2 factorial arrangement, and each replicate was allocated to a different experimental run, resulting in six experimental runs and 4 independent measurements per silage.

Each experimental run lasted 17 days, the first 8 days of which were used for equilibration of the system followed by 9 days of the experimental phase. An adaptation ration consisting of hay and concentrate (70:30 on a DM basis; DM: 903 g/kg; on a DM basis: CP 205 g/kg DM, ash 96 g/kg DM, crude fat 36 g/kg DM, NDF assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom) 432 g/kg DM, ADF expressed exclusive of residual ash (ADFom) 259 g/kg DM, acid detergent lignin (ADL) 77 g/kg DM, and starch 83 g/kg DM) was incubated in the Rusitec vessels during the first 8 days to ensure a stable fermentation in the Rusitec system prior to the AS incubation. During the subsequent experimental phase, the AS were incubated isonitrogenously, which corresponded to incubated quantities of 14-16 g DM.

On the first day of each run, ruminal fluid and solid rumen digesta were obtained before the morning feeding from 3 rumen-cannulated Holstein steers that were fed a ration covering the maintenance requirements consisting of hay, a mixed concentrate (wheat 253 g/kg, sunflower meal 200 g/kg, palm kernel expeller 180 g/kg, barley 150 g/kg, rapeseed meal 86 g/kg, sugar beet vinasse 50 g/kg, wheat bran 49 g/kg, CaCO3 19 g/kg, beet molasses 10 g/kg, and NaCl 3 g/kg), and rapeseed meal (70:20:10 on a DM basis; DM: 862 g/kg; CP 154 g/kg DM, ash 76 g/kg DM, crude fat 38 g/kg DM, aNDFom 477 g/kg DM, ADFom 276 g/kg DM, ADL 46 g/kg DM, and starch 54 g/kg DM) twice daily. Before use, equal volumes of ruminal fluid from all steers were pooled and filtered through 2 layers of cheesecloth. Each Rusitec vessel was filled with 500 mL ruminal fluid, 200 mL buffer solution (McDougall, 1948), and 100 mL deionized water. Afterwards, two nylon bags (140 × 80 mm, 1000 µm pore size, Klein & Wieler oHG, Königswinter, Germany) were incubated in each vessel, one filled with the adaptation ration and the other filled with 80 g of pooled solid ruminal digesta. On the second day, the nylon bag with the solid rumen digesta was removed from the vessel and replaced by another nylon bag containing the adaptation ration. On each of the following days, the nylon bag that had remained for 48 h in the vessels was replaced by a new bag containing the respective ration, *i.e.*, the adaptation ration or AS. The vacuum bags containing the AS were opened just before filling in the nylon bags for Rusitec incubation in

order to minimize the effects of aerobic exposure on the silages. The removed 48 h bag was washed twice with a total of 50 mL pre-warmed buffer solution and gently squeezed to separate the loosely attached microorganisms from the feed particles. The emerging liquid was transferred into the respective vessels, which were then tightly closed and flushed with carbon dioxide for 1 min in order to reestablish anaerobic conditions.

The effluent fluids of the vessels were collected in bottles that were placed in ice-filled polystyrene boxes and connected to airtight gas collection bags (Cali-5-Bond™, Ritter Apparatebau GmbH & Co KG, Bochum, Germany). The buffer solution was continuously infused at a rate of 450 mL/day using an 8-channel peristaltic pump (MV-CA/04, Ismatec SA Labortechnik, Glattbrugg, Switzerland). The correct infusion of the buffer solution was monitored by the daily volumes of effluent fluids.

Daily samples

The gas volume, effluent volume, and pH were measured daily immediately before the feed bag exchange. 10 mL of fluid was taken directly from each vessel *via* a 3-way-valve immediately before feed bag exchange and the pH was subsequently determined by potentiometry (BlueLine 14 pH, SI Analytics, Mainz, Germany, and pH 315i, WTW, Weilheim, Germany). The gas bags were detached from the Rusitec system just before vessel opening and the volumes were determined using the water displacement technique (Soliva and Hess, 2007). The daily effluent volumes were determined using a measuring cylinder.

The feedstuff residues from days 11 and 12 as well as days 16 and 17 were stored in two aliquots at -20°C for the determination of organic matter (OM), aNDFom, and ADFom, as well as the microbial CP attached to the feed residues (MCPF).

For OM and fiber fraction analyses, feed residues from days 11, 12, 16, and 17 were thawed on ice, samples from days 11 and 12 and samples from days 16 and 17 were pooled, respectively, and then lyophilized. In addition, aliquots of the feed residues were analyzed for their DM concentration according to method 3.1 of the Association of German Agricultural Analytical and Research Institutes (VDLUFA, 2012), *i.e.*, drying overnight at 60°C then at 105°C for at least 3 h. Since volatiles constitute a substantial proportion of rumen digesta for which the analyzed DM concentration should be corrected (Shiels et al., 1999), the equation for moist forages from Weissbach and Kuhla (1995), $DM_{corrected}$ (%) = 2.08 + 0.975 × $DM_{uncorrected}$ (%), was used to correct for the loss of volatile compounds that occurs during drying. The ash concentration was determined according to method 8.1 and the concentrations of aNDFom and ADFom were analyzed according to methods 6.5.1 and 6.5.2. For the calculation of OM, the ash content was subtracted from the DM of the feed residues. The apparent disappearance of aNDFom, ADFom, and OM was calculated from

the difference between the amount of aNDFom, ADFom, and OM in the AS before the Rusitec incubation and the amount recovered in the feed residue after the Rusitec incubation, and denoted aNDFom, ADFom, and OM degradability.

The first step of the MCPF estimation was detachment of the microbial biomass in accordance with Boguhn et al. (2013). Briefly, the feed residue was thawed on ice and incubated with 160 mL pre-warmed methylcellulose solution consisting of 0.1% methylcellulose (w/v; Carl Roth GmbH+Co KG, Karlsruhe, Germany) and 0.9% NaCl (w/v; VWR Chemicals, Leuven, Belgium) at 39°C for 45 min. Then, 200 mL of cold methylcellulose solution was added to the mixture and incubated at 4°C for 4 h. Afterwards, the feed residues were squeezed and the detached microbes were separated from the remaining suspension by a 2-step centrifugation. First, the mixture was centrifuged at 500 × *g* at 4°C for 10 min to separate the residual feed particles from the microbial cells. The supernatant was then transferred to a new tube and centrifuged again at 20,000 × *g* at 4°C for 10 min to obtain a microbial pellet. This pellet was resuspended in NaCl solution (0.9%; w/v) and the centrifugation step was repeated twice. The final pellet was lyophilized, ground with a pestle and mortar, and analyzed for its N concentration according to method 4.1.2 using Dumas combustion (rapid N cube, Elementar Analysensysteme GmbH, Hanau, Germany). The amount of microbial CP was calculated by multiplying the obtained N concentration by 6.25. To calculate the daily production of MCPF, the microbial CP was compared to the amount of feed residue that was recovered after 48 h of Rusitec incubation and then halved to obtain the MCPF production per 24 h of incubation.

Hourly measurements

In order to test for alterations in the diurnal fermentation pattern and to obtain robust mean values, AA-N, ammonia-N, and volatile fatty acids (VFA; acetate, propionate, butyrate, valerate, isobutyrate, isovalerate, and caproate) were determined 2, 4, 12, and 23 h after feedbag exchange on days 10 and 15. For AA-N and VFA, 2 mL of fluid was taken directly *via* a 3-way-valve from each vessel and subsequently stored at -20°C until further analysis. Similarly, 10 mL was sampled and stored at -20°C for ammonia-N determination.

The analysis of VFA was performed by GC (GC Autosystem, Perkin Elmer Inc., Waltham, MA) equipped with a flame-ionization detector and a 25 m × 0.32 mm capillary column (Macherey & Nagel GmbH & Co KG, Duren, Germany). The temperature of the injector and detector was 250°C and 260°C, respectively. Helium was used as the carrier gas with a flow rate of 31 mL/min. Before VFA analysis, the samples were thawed on ice and centrifuged at 20,000 × *g* for 15 min. The supernatant (1 mL) was transferred into a new tube, mixed with 100 µL of formic acid, and centrifuged again. Then, the VFA concentration of the clear

supernatant was analyzed. The ammonia-N concentration was determined by automated distillation (Vapodest 50 s carousel, Gerhardt, Königswinter, Germany) according to Keay and Menage (1969).

Amino acid-N was analyzed using the Primary Amino Nitrogen Assay Kit (Megazyme u.c., Wicklow, Ireland), which spectrophotometrically determines isoindole derivatives originating from the reaction of free AA-N with N-acetyl-L-cysteine and orthophthaldialdehyde. Before analysis, the samples were thawed on ice, centrifuged at 18,400 × *g* at 4°C for 15 min, and 1.4 mL supernatant was transferred into a new tube. In order to precipitate the residual protein, 420 µL of 10% trichloroacetic acid (w/v; Carl Roth GmbH+Co KG, Karlsruhe, Germany) was added, the mixture was gently vortexed and centrifuged again. The supernatant was transferred to a new tube and analyzed for AA-N using a multi-mode microplate reader (Synergy HTX, BioTek Instruments Inc., Winooski, VT,USA) according to the manufacturer's protocol for microplate assays with an increase in the sample volume to 300 µL. Briefly, 300 µL of N-acetyl-L-cysteine-containing solution was mixed with 50 µL of blank, sample, or standard, and absorbance at 340 nm was measured after 2 min of incubation. Subsequently, 10 µL of ortho-phthaldialdehyde-containing solution was added, mixed, and incubated for 15 min. Then, the absorbance at 340 nm was read again. The concentration of AA-N was calculated according to the equation AA-N (g/L) = ($\Delta absorbance_{Sample}/\Delta absorbance_{Standard}$) × S, where $\Delta absorbance_{Sample}$ is the difference in absorbance between the first and the second reading of the sample, $\Delta absorbance_{Standard}$ is the difference in absorbance between the first and the second reading of the standard, and S is the concentration of the isoleucine standard.

Statistical analyses

Assuming a type I error of 0.05, an average statistical power of 80% ±10% for the pairwise comparisons within each variable was post-hoc confirmed for the present study design.

The diurnal measurements were performed on days 10 and 15 of each experimental run, constituting a sampling at FIR and SEC, *i.e.*, after 2 and 7 days of the AS incubation in the Rusitec system, respectively. To obtain robust mean values for the ammonia-N, AA-N, and VFA concentrations, means of the 4 hourly time points, *i.e.*, 2, 4, 12, and 23 h after feedbag exchange, were calculated for each vessel and run. Thus, the mean daily pH and gas production measurements of days 10 and 11 as well as days 15 and 16 were calculated and, together with the means of the hourly measurements, were used for statistical analyses. All statistical analyses were performed using the repeated-measurements MIXED model procedure using SAS version 9.4 (SAS Institute Inc., Cary, NC). To test for the effects of DM

concentration, wilting intensity, sucrose addition, and their interactions during FIR and SEC, respectively, the model was:

$$Y_{ijklm} = \mu + D_i + W_j + S_k + D_i*W_j + D_i*S_k + W_j*S_k + D_i*W_j*S_k + V_l + R_m + e_{ijklm}$$

where Y_{ijklm} is the observed response, μ is the overall mean, D_i is the fixed effect of DM concentration, W_j is the fixed effect of wilting intensity, S_k is the fixed effect of sucrose addition, V_l is the random effect of vessel, R_m is the random effect of experimental run, and e_{ijklm} is the residual error. In addition, the effect of the sampling time point on the analyzed variables was tested. Due to the experimental design, interference by run regarding the comparison among the treatments could not be excluded.

Significance was defined at $P < 0.05$ and a trend at $0.05 \leq P < 0.1$. When interactions were not significant, differences between the least squares means were tested using a Tukey's test. The UNIVARIATE procedure was applied to test assumptions of the model by analysis of the residuals. If model assumptions were not met, log-transformation of the data was tested.

Results

From the FIR to SEC, an overall decrease in fiber degradability of 6.0 and 9.7 percentage units for aNDFom ($P < 0.001$) and ADFom ($P < 0.001$), respectively, was observed (Table 2). Likewise, the degradability of OM decreased by 1.8 percentage units between the 2 sampling time points ($P = 0.002$). The total VFA concentrations were not affected ($P > 0.05$), however, the acetate ($P < 0.001$) and isovalerate ($P < 0.001$) concentrations increased, whereas the concentrations of n-butyrate ($P < 0.001$), n-caproate ($P < 0.001$), and to a slight extent also n-valerate ($P = 0.028$) decreased between FIR and SEC. No effect of time point were observed on the concentrations of isobutyrate and propionate ($P > 0.05$). Whilst the daily gas production decreased from FIR to SEC ($P < 0.001$), the ammonia-N concentration increased by 1.4 mmol/L ($P = 0.004$), and the pH ($P = 0.058$) and AA-N ($P = 0.083$) tended to slightly increase, whereas the daily amount of MCPF decreased by 26% from FIR to SEC ($P = 0.002$; Table 2).

Table 2. Effect of sampling time point on *in vitro* rumen fermentation characteristics and fiber degradability of alfalfa silages.

Item	First	Second	SEM	*P*-value
pH	6.72	6.74	0.01	0.058
Gas volume, mL/day	1229	1155	37.3	<0.001
Ammonia-N, mmol/L	30.8	32.2	0.74	0.004
AA-N[1], mg/L	129	133	2.01	0.083
MCPF[2], mg/day	46.1	34.3	5.91	0.002
Volatile fatty acid concentration, mmol/L				
Total	138	139	0.42	0.704
Acetate	78.9	81.9	1.48	<0.001
Propionate	23.4	23.8	0.21	0.833
n-Butyrate	18.2	15.3	1.42	<0.001
n-Valerate	4.76	4.58	0.09	0.028
n-Caproate	3.28	2.59	0.34	<0.001
Isobutyrate	1.95	1.97	0.01	0.781
Isovalerate	7.07	9.24	1.08	<0.001
Degradability, g/kg DM				
Organic matter	550	532	6.36	0.002
aNDFom[3]	349	289	21.21	<0.001
ADFom[4]	344	247	34.29	<0.001

[1]AA-N = Amino acid-N

[2]MCPF = Microbial CP attached to feed residues

[3]aNDFom = NDF assayed with a heat stable amylase and expressed exclusive of residual ash

[4]ADFom = ADF expressed exclusive of residual ash

As shown in Table 3, at FIR, sucrose-treated AS reduced the pH ($P < 0.001$). The daily gas production was affected by sucrose addition ($P < 0.001$), which increased the gas volumes and a higher DM concentration tended to elevate the daily gas production values ($P = 0.053$). When compared to 25HI, the incubation of 35HI silages resulted in 95 mL/day higher gas volumes ($P = 0.013$). Addition of sucrose led to lower ammonia-N concentrations than non-treated AS ($P = 0.006$) and 35HI produced lower ammonia-N concentrations than 35LI and 25HI ($P = 0.018$). The incubation of high DM AS led to lower AA-N concentrations compared to low DM silages ($P = 0.009$). The degradability of aNDFom increased by both low DM ($P < 0.001$) and high-intensity wilting treatments ($P < 0.001$), such that 25HI had a higher degradability of aNDFom and ADF at 412 g/kg DM and 379 g/kg DM, respectively, than 25LI or 350 g/kg DM AS ($P < 0.001$). The 25SA silages also showed a higher aNDFom degradability than low DM AS without sucrose addition or high DM with or without sucrose addition ($P < 0.001$). Sucrose addition prior to ensiling reduced the ruminal ADFom degradability of AS ($P = 0.021$), whereas low DM increased its degradability ($P < 0.001$), and a trend for a three-way interaction for ADFom was observed ($P = 0.059$). The OM degradability was higher during the incubation of sucrose-treated AS ($P < 0.001$) and low DM silages tended to have a higher OM degradability than high DM silages ($P = 0.091$). Consequently, the 25SA silages had higher OM degradability compared with low DM AS without sucrose addition or high DM silages in general ($P = 0.045$). More OM was degraded during fermentation of the 25HI silages than the 35HI AS ($P = 0.019$). In addition, high-intensity wilted AS tended to increase the daily MCPF production ($P = 0.073$).

The fermentation of sucrose-treated AS produced 18.4 mmol/L higher total VFA concentrations ($P < 0.001$) than non-treated AS. High DM levels increased the concentrations of propionate ($P = 0.049$) and isobutyrate ($P = 0.010$), and reduced the concentration of isovalerate ($P < 0.001$). Compared to non-treated AS, sucrose treatment increased the concentrations of propionate ($P < 0.001$), n-butyrate ($P = 0.004$), n-valerate ($P = 0.001$), and isovalerate ($P = 0.034$). The wilting intensity also affected n-valerate levels, with higher concentrations produced during the incubation of high-intensity wilted AS ($P = 0.047$). No effects were observed on the acetate or n-caproate concentration ($P > 0.05$), however, among the branched-chain VFA, DM × wilting intensity interaction showed the lowest isobutyrate concentrations for 25HI ($P = 0.004$), whereas 35LI had the lowest concentrations of isovalerate ($P = 0.004$). Three-way interactions were observed for both branched-chain VFA, *i.e.*, isobutyrate ($P = 0.006$) and isovalerate ($P = 0.041$).

Table 3. Effects of dry matter (DM) concentration, wilting intensity (WI) and sucrose addition (SA) on *in vitro* rumen fermentation characteristics and fiber degradability of alfalfa silages at the first time point (2 days after first-time alfalfa silage incubation).

	Treatment[1]															
DM concentration	250 g/ kg				350 g/kg											
Wilting intensity	High		Low		High		Low									
Sucrose addition	+	-	+	-	+	-	+	-		*P*-value						
Item	25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM	DM	WI	SA	DM×WI	DM×SA	WI×SA	DM×WI×SA
pH	6.68	6.76	6.67	6.76	6.67	6.77	6.66	6.79	0.02	0.405	0.332	<0.001	0.986	0.022	0.677	0.753
Gas volume, mL/day	1433	956	1470	1044	1533	1045	1460	1025	87.4	0.053	0.681	<0.001	0.013	0.786	0.194	0.965
Ammonia-N, mmol/L	30.0	31.1	29.4	31.7	29.8	30.1	32.1	31.7	0.36	0.186	0.357	0.006	0.018	0.196	0.735	0.751
AA-N[2], mg/L	132	132	138	127	124	124	122	130	1.93	0.009	0.594	0.955	0.266	0.170	0.864	0.626
MCPF[3], mg/day	50.4	38.4	43.3	36.2	53.2	71.3	40.0	44.9	4.00	0.121	0.073	0.873	0.239	0.115	0.742	0.472
Volatile fatty acid concentration, mmol/L																
Acetate	80.4	76.8	78.8	80.5	79.8	78.1	80.5	76.8	0.57	0.324	0.296	0.329	0.497	0.309	0.741	0.191
Propionate	26.9	17.5	25.6	19.8	31.6	19.0	27.6	20.9	1.77	0.049	0.655	<0.001	0.681	0.369	0.167	0.450
n-Butyrate	18.6	18.9	18.1	18.1	22.0	14.8	19.1	15.6	0.78	0.386	0.574	0.004	0.994	<0.001	0.589	0.094
n-Valerate	4.80	4.21	5.19	4.63	4.76	4.78	5.24	4.46	0.12	0.101	0.047	0.001	0.659	0.321	0.259	0.164
n-Caproate	3.23	3.23	3.83	3.18	3.14	3.50	3.20	2.91	0.10	0.549	0.717	0.176	0.209	0.211	0.247	0.901
Isobutyrate	1.98	1.73	1.77	2.13	2.17	2.07	1.93	1.85	0.06	0.010	0.658	0.227	0.004	0.060	0.075	0.006
Isovalerate	7.73	6.81	7.69	7.51	7.06	6.93	6.77	6.05	0.20	<0.001	0.812	0.034	0.004	0.311	0.987	0.041

Table 3 continued.

	Treatment[1]															
DM concentration	250 g/kg				350 g/kg											
Wilting intensity	High		Low		High		Low									
Sucrose addition	+	-	+	-	+	-	+	-								
Item	25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM	*P*-value DM	WI	SA	DM×WI	DM×SA	WI×SA	DM×WI×SA
Degradability, g/kg DM																
Organic matter	585	535	558	531	550	528	562	549	2.36	0.091	0.944	<0.001	0.019	0.045	0.325	0.317
aNDFom[4]	437	387	345	323	326	356	299	323	5.52	<0.001	<0.001	0.403	<0.001	<0.001	0.291	0.123
ADFom[5]	361	396	358	361	309	312	318	340	3.78	<0.001	0.984	0.021	0.007	0.587	0.606	0.059

[1]Treatments include different: DM concentrations, i.e. 250 g/kg DM (25) or 350 g/kg DM (35); wilting intensities, i.e. low (LI) or high (HI); and sucrose addition (SA).

[2]AA-N = Amino acid-N.

[3]MCPF = Microbial CP attached to feed residues.

[4]aNDFom = NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

[5]ADFom = ADF expressed exclusive of residual ash.

Table 4 illustrates the *in vitro* rumen fermentation characteristics and degradability of the fiber fractions and OM at the SEC. The pH was reduced by 0.17 units during sucrose-treated AS incubation ($P < 0.001$). No effects were observed on AA-N ($P > 0.05$). High DM AS resulted in lower ammonia-N concentrations ($P = 0.012$). The DM concentration and wilting intensity affected aNDFom degradability with higher values for silages that received a high-intensity wilting treatment ($P = 0.002$) and lower values for high DM AS ($P < 0.001$). Similarly, ADFom was less degraded in high DM AS ($P = 0.003$) or silages with sucrose addition ($P = 0.016$), and the degradability of OM was decreased by high-intensity wilting ($P = 0.035$). The daily gas production was increased by a high DM concentration ($P < 0.001$) and sucrose addition ($P < 0.001$), such that incubation of 35SA silages tended to result in the highest gas volumes ($P = 0.053$). Furthermore, a three-way interaction was observed for daily gas production ($P < 0.001$). No effects were observed on the daily MCPF at SEC ($P > 0.05$).

The pattern of VFA concentrations at SEC were similar to FIR. Similarly, sucrose treatment increased the total VFA concentration by approximately 25 mmol/L ($P < 0.001$). High-intensity wilting raised the concentrations of n-butyrate ($P = 0.007$) and n-caproate ($P = 0.001$), but decreased the concentrations of n-valerate ($P = 0.035$). The high DM concentration increased the propionate concentrations ($P = 0.004$), but reduced the concentrations of isovalerate ($P < 0.001$) and n-caproate ($P < 0.001$). The incubation of sucrose-treated AS increased the concentrations of acetate ($P = 0.005$), propionate ($P < 0.001$), n-butyrate ($P < 0.001$), isovalerate ($P < 0.001$), and n-valerate ($P < 0.001$), but decreased the concentration of n-caproate ($P < 0.001$). Similarly, incubating low DM AS prepared without sucrose addition resulted in higher n-caproate concentrations (3.30 mmol/L) than the other treatments ($P < 0.001$), which had concentrations of between 2.24 and 2.45 mmol/L. In contrast, 25SA fermentation produced higher isovalerate concentrations ($P < 0.001$) and 35SA produced higher n-valerate ($P = 0.012$) and n-butyrate concentrations ($P < 0.001$), although there was no difference in the n-butyrate concentrations between the low DM AS with or without sucrose addition. Additionally, high-intensity wilted AS without sucrose addition resulted in higher n-butyrate concentrations than low-intensity wilted silages without sucrose addition ($P = 0.032$). Acetate ($P = 0.011$), n-butyrate ($P = 0.015$), and n-caproate ($P = 0.002$) were affected by the interaction of all three pre-ensiling treatments. The concentration of isobutyrate was not affected by any treatment at SEC ($P > 0.05$).

The diurnal concentrations of isovalerate for 35HISA and 25LI AS showed an overall increase with higher concentrations for all hourly time points ($P < 0.001$) at SEC (Fig. 1). The isovalerate concentration peak shifted from 12 h at FIR to 4 h at SEC, whereas the

ammonia-N concentration pattern did not change from FIR to SEC for both silages. No alterations in the diurnal concentration pattern were found for the other variables ($P > 0.05$).

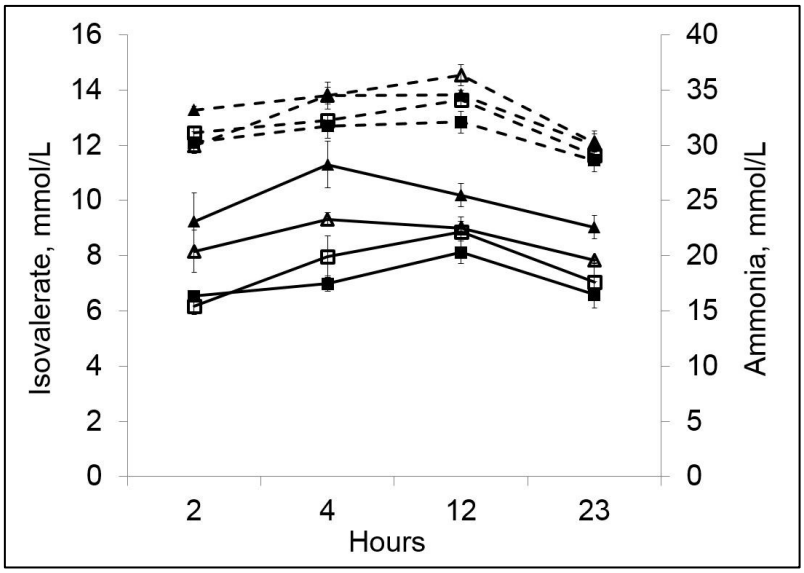

Figure 1. Hourly concentrations of isovalerate for 35HISA (350 g/kg dry matter, high-intensity wilting, and sucrose addition) at the first time point (–■–), isovalerate for 35HISA at the second time point (–▲–), isovalerate for 25LI (250 g/kg dry matter, low-intensity wilting, and no sucrose addition) at the first time point (–□–), isovalerate for 25LI at the second time point (–Δ–), ammonia-N for 35HISA at the first time point (-■-), ammonia-N for 35HISA at the second time point (-▲-), ammonia-N for 25LI at the first time point (-□-), and ammonia-N for 25LI at the second time point (-Δ-).

Table 4. Effects of dry matter (DM) concentration, wilting intensity (WI) and sucrose addition (SA) on *in vitro* rumen fermentation characteristics and fiber degradability of alfalfa silages at the second time point (7 days after first-time alfalfa silage incubation).

	Treatment[1]															
DM concentration	250 g/kg				350 g/kg											
Wilting intensity	High		Low		High		Low									
Sucrose addition	+	-	+	-	+	-	+	-								
Item	25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM	*P*-value DM	WI	SA	DM×WI	DM×SA	WI×SA	DM×WI×SA
pH	6.64	6.81	6.65	6.80	6.66	6.83	6.66	6.83	0.03	0.102	0.587	<0.001	0.889	0.580	0.622	0.836
Gas volume, mL/day	1407	913	1343	938	1453	970	1490	945	92.4	<0.001	0.436	<0.001	0.997	0.053	0.424	<0.001
Ammonia-N, mmol/L	32.5	33.9	34.5	32.8	31.5	30.6	31.3	30.8	0.50	0.012	0.878	0.560	0.756	0.572	0.388	0.207
AA-N[2], mg/L	133	138	133	134	129	127	126	142	1.95	0.275	0.833	0.581	0.932	0.722	0.203	0.639
MCPF[3], mg/day	33.1	38.1	37.5	31.4	34.6	44.4	26.3	37.3	2.06	0.799	0.599	0.168	0.806	0.164	0.369	0.813
Volatile fatty acid concentration, mmol/L																
Acetate	82.2	80.6	86.3	80.8	84.3	79.6	82.2	79.4	0.84	0.455	0.756	0.005	0.299	0.873	0.897	0.011
Propionate	30.1	13.8	30.9	16.0	33.1	18.0	31.4	17.1	2.91	0.004	0.822	<0.001	0.065	0.494	0.455	0.919
n-Butyrate	15.8	17.2	15.7	13.5	19.6	11.4	18.8	10.5	1.17	0.333	0.007	<0.001	0.067	<0.001	0.032	0.015
n-Valerate	4.92	3.88	5.27	4.04	4.87	4.24	5.09	4.37	0.18	0.131	0.035	<0.001	0.865	0.012	0.682	0.631
n-Caproate	2.33	3.65	2.57	3.01	2.28	2.48	2.19	2.23	0.18	<0.001	0.001	<0.001	0.492	<0.001	0.001	0.002
Isobutyrate	1.99	1.99	2.05	2.02	1.99	2.04	1.84	1.85	0.03	0.647	0.733	0.946	0.267	0.719	0.957	0.723
Isovalerate	11.3	8.49	11.9	8.58	9.59	8.23	8.56	7.29	0.56	<0.001	0.192	<0.001	0.038	<0.001	0.916	0.177

Table 4 continued.

	Treatment[1]															
DM concentration	250 g/kg				350 g/kg											
Wilting intensity	High		Low		High		Low									
Sucrose addition	+	-	+	-	+	-	+	-								
Item	25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM	*P*-value DM	WI	SA	DM×WI	DM×SA	WI×SA	DM×WI×SA
Degradability, g/kg DM																
Organic matter	530	536	521	545	521	525	540	538	1.14	0.608	0.035	0.150	0.142	0.175	0.352	0.808
aNDFom[4]	325	342	297	267	270	278	237	264	4.31	<0.001	0.002	0.564	0.155	0.204	0.492	0.086
ADFom[5]	234	285	269	275	204	235	223	247	3.49	0.003	0.157	0.016	0.884	0.973	0.191	0.311

[1]Treatments include different: DM concentrations, i.e. 250 g/kg DM (25) or 350 g/kg DM (35); wilting intensities, i.e. low (LI) or high (HI); and sucrose addition (SA).

[2]AA-N = Amino acid-N.

[3]MCPF = Microbial CP attached to feed residues.

[4]aNDFom = NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

[5]ADFom = ADF expressed exclusive of residual ash.

Discussion

Gas production, VFA, NH_3-N, and AA-N concentrations

The total and individual VFA concentrations reflected a typical magnitude found *in vivo* in the rumen (Puniya et al., 2015) and in the Rusitec or other *in vitro* systems (Jaurena et al., 2005; Copani et al., 2015). However, with 7.07 mmol/L and 9.24 mmol/L for FIR and SEC, respectively, the isovalerate concentrations were higher than the 2-5 mmol/L stated as typical ruminal concentrations (Puniya et al., 2015). Since isovalerate is mainly formed during deamination (Carro and Miller, 1999), its high concentration in the present study indicates an elevated level of deamination during AS incubation. This assumption is further supported by the present ammonia-N concentrations, which were substantially higher than those reported for other *in vitro* experiments (Jaurena et al., 2005; Boguhn et al., 2013; Copani et al., 2015) and can probably be explained by the overall high NPN concentrations in all 8 AS, irrespectively of applied pre-ensiling treatment (Hartinger et al., 2019).

Since the concentration of isovalerate increased from FIR to SEC, this might also indicate an adaptation of the microorganisms to the AS, therefore causing a change in the microbiota composition towards protein and AA degrading microorganisms. This change also became apparent from the diurnal patterns of ammonia-N and isovalerate during the incubation of 35HISA and 25LI, which had the highest and lowest true protein concentrations, respectively (Hartinger et al., 2019). For both of these AS, the isovalerate concentration peak shifted from 12 h at FIR to 4 h at SEC and thus indicated a microbial adaptation to the higher NPN level. The difference in the true protein concentrations of the AS did not affect the ammonia-N concentrations and therefore disproved the hypothesis of reduced ruminal CP degradation during the Rusitec incubation of AS that had received a high-intensity wilting treatment and the addition of sucrose before ensiling. Consequently, it appears that the beneficial effects of the applied pre-ensiling treatments on the CP composition of the silages (Hartinger et al., 2019) cannot be transferred and expressed during ruminal fermentation.

Although relatively higher propionate proportions are correlated with lower gas productions (Blümmel and Ørskov, 1993), also propionate concentrations were higher for sucrose-treated AS at both sampling time points. Thus, propionate production might not have been a substantial hydrogen sink during *in vitro* fermentation or, more likely, the overall fermentation activity was enhanced to an extent that outweighed the reducing effect of propionate formation on ruminal gas production. The lower pH of sucrose-treated AS incubations was in line with the higher VFA concentrations and since the Rusitec system represents a highly buffered *in vitro* model, the differences for pH would be even more pronounced *in vivo*.

Propionate is produced at proportionally higher levels during the fermentation of rapidly fermentable carbohydrates (Balch and Rowland, 1957), therefore, the higher propionate concentrations during sucrose-treated AS incubation might partly derive from microorganisms fermenting the residual sucrose in the silages. However, since the WSC concentration was also low for sucrose-treated AS, the high concentrations of propionate were also likely to have been caused by the utilization of lactate (Counotte et al., 1981), which was strongly formed during ensiling (Hartinger et al., 2019). Likewise, previous studies have already shown a positive relationship between the lactate concentration in silages and the propionate concentration in ruminal fluid (Jalč et al., 2009). Together with the overall high ammonia-N concentrations, the present VFA pattern might suggest a high presence and activity of lactate-utilizers such as *Megasphaera elsdenii*, which is a major ruminal lactate-fermenting bacterium (Counotte et al., 1981) with a high deamination capacity (Rychlik et al., 2002). Similarly, *M. elsdenii* produces isovalerate and n-butyrate during serine degradation (Wallace, 1986) and since the concentration of this AA in AS was increased by the addition of sucrose (Hartinger et al., 2019), it might explain the increased concentrations of these two individual VFA in sucrose-treated AS; particularly since *M. elsdenii* cultures showed higher branched-chain VFA production under glucose-limited conditions (Allison, 1978). Additionally, also other lactate-utilizers (Eaton and Gabelman, 1992) or microorganisms metabolizing AA into propionate (Sirotnak et al., 1953) should have contributed to the increase in propionate.

It is noteworthy that *M. elsdenii* is also one of only few known caproate producers in the rumen, mainly forming this VFA during carbohydrate fermentation (Marounek et al., 1989). Since fiber degradability declined from FIR to SEC, the reduced caproate concentrations were in line with this. Thereby, the lower caproate but increased propionate and isovalerate concentrations could be explained by an adapted metabolism, resulting in less C6 and more C3 and C5 fatty acids. However, it should be considered that despite the observed decline, the caproate concentrations were similar to *in vivo* data (Puniya et al., 2015) during both time points.

Concerning AA-N, to the best of the authors' knowledge, reports of AA concentrations in ruminal fluid in forage-based *in vitro* studies are rare and the present results showed only few inconsistent effects of the pre-ensiling treatments on AA-N concentration. An increased incorporation of AA-N into microbial protein might be causative for the reduced AA-N by high DM concentration at FIR. However, no treatment effects on AA-N were observed for SEC, indicating a general adaptation of the microbial community, which should be pursued by further nucleic acid-based analysis. Since the daily MCPF formation was lower at SEC, the

increased AA-N concentration from FIR to SEC might therefore be a partial consequence of less AA incorporation into microbial biomass.

MCPF production and methodical consideration

The higher MCPF production during the incubation of high-intensity wilted AS supported the hypothesis of an increased microbial protein synthesis with those silages, although this effect was only present at FIR. The overall daily MCPF production was considerably lower and only a fractional amount of the other silage-based Rusitec studies (Jaurena et al., 2005; Boguhn et al., 2013), which therefore cannot be explained by the limited availability of dietary energy (Satter and Slyter, 1974). Apart from nutritional causes, there might be valuable concerns regarding the effectiveness of the methylcellulose treatment to detach the microorganisms from the feed particles, particularly as an additional Stomacher treatment (Jaurena et al., 2005) was not included. The attempt to quantitatively capture microorganisms seemed inferior to marker-based approaches (Jaurena et al., 2005; Boguhn et al., 2013). Thus, the present MCPF values might not be comparable to literature, but only within the study.

Degradability of fiber and OM

The overall degradability of OM, aNDFom, and ADFom were 6.0%, 8.8%, and 6.7% higher, respectively, than those observed for ground (1 mm sieve mesh size) grass silages after 7 days of adaptation in the system and a 48 h incubation (Boguhn et al., 2013). Jaurena et al. (2005) incubated red clover silages in the Rusitec system and detected considerably higher OM and fiber degradability after 8 days, i.e., 790 g/kg DM for OM, 600 g/kg DM for NDF, and 580 g/kg DM for ADF. However, Jaurena et al. (2005) determined the DM of feed residues without correcting for losses of volatile compound during drying, which constitute a substantial proportion of the ruminal digesta (Shiels et al., 1999). Consequently, the degradability of both OM and fiber was likely to be overestimated since the residual proportions were related to an apparently lower DM. Secondly, NDF and ADF values were expressed inclusive of residual ash (Jaurena et al., 2005), which further increases the fiber fraction values.

Since high-intensity wilting increased the degradability of aNDFom, but not of ADFom, it is apparent that the hemicelluloses were mostly affected by wilting. Similarly, the concentrations of n-butyrate, a predominant end-product of ruminal fiber degradation (Balch and Rowland, 1957), were higher during the incubation of high-intensity wilted AS, which corresponds to a higher hemicellulose degradability. In contrast to aNDFom, the OM degradability was 1.4 percentage units lower for the high-intensity wilted AS at SEC. Therefore, the degradability of components other than hemicelluloses must also have been affected by the wilting intensity. The lower proportions of n-valerate during high-intensity

wilted AS incubation could be the consequence of a reduced deamination of specific AA such as arginine, ornithine, proline, or lysine (El-Shazly, 1952), and suggests a reduced degradability of N compounds in those silages. However, the concentration of AA-N was not affected by the wilting intensity, although it was numerically lower in high-intensity wilted AS. Thus, the interpretation of a potentially lower degradability of nitrogenous compounds in high-intensity wilted AS causing the observed lower OM degradability must be regarded with caution and needs further investigation in future studies.

A stimulation of cellulolytic rumen microbes by isovalerate, as has often been monitored *in vivo* (Liu et al., 2009, 2014) and *in vitro* (Allison et al., 1962), was not observed in this study since isovalerate concentration was higher, whereas ADFom degradability was lower for sucrose-treated AS. Likewise, although the isovalerate concentration increased, the fiber degradability markedly decreased from FIR to SEC, which clearly contradicted an isovalerate-induced stimulation of fibrolytic activity. Similarly, these fiber degradability patterns do not support the second hypothesis of a better microbial adaptation to AS over time. It is possible that a decrease of anaerobic fungi and protozoa in the vessels (Wallace and Newbold, 1991; Martínez et al., 2010) contributed to the reduced fiber degradability, since they considerably participate in the initial colonization and degradation of fiber (Gordon and Phillips, 1998; Newbold et al., 2015), particularly hemicelluloses (Williams and Coleman, 1985).

Since the aNDFom degradability was higher in 25SA silages at FIR, there might have been a partial acidic hydrolytic cleavage of hemicelluloses (Dewar et al., 1963) originating from microbial sucrose metabolism during ensiling and consequently causing facilitated access for rumen microbes to these structural carbohydrates. However, this degradability pattern was not observed at SEC, which might indicate a microbial adaptation to the non-sucrose-treated AS that compensated for the initially facilitated degradation of aNDFom in 25SA.

Since high DM AS quality was superior than low DM AS, i.e., it had a reduced concentration of acetic acid and less NPN (Hartinger et al., 2019), an improved or similar fiber fermentation of high DM AS was expected at first. An elevated microbial activity due to higher water availability in the silo (Santos and Kung, 2016), not only in terms of proteolysis (Fijałkowska et al., 2015), but also fiber breakdown (Dewar et al., 1963; Ren et al., 2007), might have increased the cleavage of fibrous structures during ensiling, thus causing the higher degradability of aNDFom and ADFom in low DM AS.

The overall decrease in fiber degradability from FIR to SEC was consistently accompanied by a reduced daily gas production, which has already been postulated by Satter and Slyter (1974) and is in accordance with Blümmel and Ørskov (1993), who stated “the gas volume

reflected substrate fermentation to VFA and thus appears to be an estimate of rumen apparent digestibility". However, since the total VFA concentration did not decrease and the acetate concentration even increased, the VFA profile contrasted the assumption of Blümmel and Ørskov (1993) and the present observations of fiber degradability and daily gas production, which needs further clarification.

Conclusions

The pre-ensiling treatments DM concentration, wilting intensity, and sucrose addition affected the AS composition and thereby the *in vitro* ruminal fermentation. High DM and sucrose addition were beneficial for both VFA formation and gas production, but negatively impacted ruminal fiber degradation and therefore the dietary energy available from fiber. A reduced fiber degradability was particularly true for sucrose-treated high DM silages. Wilting intensity as a pre-ensiling treatment affected several fermentation characteristics in different ways, since a higher degradability of aNDFom but a lower OM degradability in high-intensity wilted AS was observed. It can therefore be concluded that not only the final DM concentration of the silage is important, but also under which conditions this DM is achieved, since this will eventually impact ruminal fermentation. However, the beneficial effects of combining all three pre-ensiling treatments on silage quality were not fully transferred to rumen fermentation. The underlying mechanisms are unclear and future studies including analyses of the microbiome will help to elucidate the modes of action. Efforts should be intensified to explore how the wilting regime, alone or in combination with other treatments, can be designed for realizing the most powerful manipulation of rumen fermentation in terms of an increased microbial N fixation and fiber degradability.

Declaration of Competing Interests

The authors have no conflict of interest.

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CHAPTER 6

Differently pre-treated alfalfa silages affect the *in vitro* ruminal microbiota composition

T. Hartinger[1], J. E. Edwards[2], R. Gómez Expósito[2], H. Smidt[2], C. J. F. ter Braak[3], N. Gresner[1], K.-H. Südekum[1]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany

[2]Laboratory of Microbiology, Wageningen University and Research, Wageningen, Netherlands

[3]Biometrics, Wageningen University and Research, Wageningen, Netherlands

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Abstract

Alfalfa (*Medicago sativa* L.) silage (AS) is an important feedstuff in ruminant nutrition. However, its high non-protein nitrogen content often leads to poor ruminal nitrogen retention. Various pre-ensiling treatments differing with respect to dry matter concentrations, wilting intensities and sucrose addition have been previously shown to improve the quality and true protein preservation of AS, and have substantial effects on *in vitro* ruminal fermentation of the resulting silages. However, it is unknown how these pre-ensiling treatments affect the ruminal microbiota composition, and whether alterations in the microbiota explain previously observed differences in ruminal fermentation. Therefore, during AS incubation in a rumen simulation system, liquid and solid phases were sampled 2 and 7 days after first incubating AS, representing an early (ET) and late (LT) time point, respectively. Subsequently, DNA was extracted and qPCR (bacteria, archaea, and anaerobic fungi) and prokaryotic 16S rRNA gene amplicon sequence analyses were performed. At the ET, high dry matter concentration and sucrose addition increased concentrations of archaea in the liquid phase ($P = 0.001$) and anaerobic fungi in the solid phase ($P < 0.001$). At the LT, only sucrose addition increased archaeal concentration in the liquid phase ($P = 0.014$) and anaerobic fungal concentration in the solid phase ($P < 0.001$). Bacterial concentrations were not affected by pre-ensiling treatments. The prokaryotic phylogenetic diversity index decreased in the liquid phase from ET to LT ($P = 0.034$), whereas the solid phase was not affected ($P = 0.060$). This is suggestive of a general adaption of the microbiota to the soluble metabolites released from the incubated AS, particularly regarding the sucrose-treated AS. Redundancy analysis of the sequence data at the genus level indicated that sucrose addition ($P = 0.001$), time point ($P = 0.001$), and their interaction ($P = 0.001$) affected microbial community composition in both phases. In summary, of the pre-ensiling treatments tested sucrose addition had the largest effect on the microbiota, and together with sampling time point affected microbiota composition in both phases of the rumen simulation system. Thus, microbiota composition analysis helped to understand the ruminal fermentation patterns, but could not fully explain them.

Introduction

Reducing the import of protein-rich feedstuffs, such as soybean meal, in favor of increasing the production of inexpensive on-farm produced protein, such as forage legumes, supports the sustainability of ruminant livestock production (Lüscher et al., 2014), provided that the dietary nitrogen (N) is efficiently utilized in the rumen. Alfalfa (*Medicago sativa* L.) silages (AS) represent an important on-farm grown protein feedstuff for dairy cow rations and are well accepted by ruminants as a dietary component (Dewhurst et al., 2003). Therefore, AS feeding can provide the animals with sufficient amounts of crude protein and dietary fiber (Dewhurst et al., 2003; Coblentz and Grabber, 2013) to maintain rumination activity and microbial protein production in the rumen. However, the bulk of crude protein in AS is in the form of non-protein N (NPN), which is rapidly metabolized to ammonia in the rumen. Excessive ruminal ammonia production results in its absorption by the host, before it is excreted as urea into the environment (Coblentz and Grabber, 2013). In order to improve the ruminal fermentation and N retention, the effects of different alfalfa pre-ensiling treatments, i.e., dry matter (DM) concentration, wilting intensity and sucrose addition on the resulting AS crude protein fractions was investigated using eight different AS (Hartinger et al., 2019a). It was observed that wilting alfalfa with high intensity to 35% DM concentration in combination with sucrose addition at ensiling lead to highest true protein preservation, as well as a generally improved silage quality.

Incubation of the same eight AS in an *in vitro* rumen-simulation system (Rusitec) revealed substantial effects on the ruminal fermentation and fiber degradation as comprehensively discussed in Hartinger et al. (2019b). Mainly, for sucrose-treated AS, increased daily gas production and concentrations of total volatile fatty acids and in specific propionate, n-butyrate, and isovalerate were observed, along with decreased acid detergent fiber degradation (Supplementary Table S1). The DM concentration of the AS showed substantial effects on the ruminal fermentation pattern, with higher gas production and increased concentrations of propionate and isovalerate, as well as lower degradability of neutral and acid detergent fiber. In contrast, high-intensity wilting only enhanced degradation of neutral detergent fiber. These effects of pre-ensiling treatments on ruminal fermentation patterns and fiber degradation of the AS indicate that the microbiota in the Rusitec may have been affected. However, the effect of the different AS on the microbiota has not been investigated so far, but should be since the microbiota can substantially affect animal performance (Guan et al., 2008); an aspect of animal nutrition that has gained more attention during the last decades. Among multiple influencing factors, diet is considered to have the strongest impact on the ruminal microbiota (Henderson et al., 2015). Despite this awareness, microbiota-based research has only focused on the effects of different ensiled forages on the ruminal

microbiota (Zhang et al., 2014; Liu et al., 2016), but not on the effect of different pre-ensiling treatments that are commonly applied during forage conservation.

Due to the reduced acid detergent fiber degradability as well as higher gas and volatile fatty acid concentrations during incubation of sucrose-treated AS, we hypothesized contrasting microbiota compositions with higher microbial abundances and diversity in the communities deriving from sucrose-treated AS incubation. Limited influence of the other pre-ensiling treatments on microbiota composition was expected. Secondly, we hypothesized that the microbial community composition changed with prolonged AS incubation, as several fermentation characteristics were previously shown to be altered between early (2 days) and late (7 days) sampling time points, e.g., rise of pH, ammonia-N and isovalerate concentrations. Therefore, the aim of the present study was to assess the effect of the pre-ensiling treatments and incubation time on the microbial community composition and concentrations using liquid and solid phase samples preserved from the previously reported Rusitec study (Hartinger et al., 2019b). This analysis was done using barcoded amplicon sequencing of the prokaryotic 16S rRNA gene, and qPCR analyses of bacteria, archaea, and anaerobic fungi.

Materials and methods

Experimental design and sample collection

An *in vitro* study was previously performed where eight varyingly pre-treated AS differing in terms of crude protein composition and silage fermentation quality (Hartinger et al., 2019a) were incubated in an *in vitro* Rusitec system (Hartinger et al., 2019b). The preparation and chemical composition of the eight AS have been described in detail and can be obtained from Hartinger et al. (2019a). Briefly, pure alfalfa sward was harvested and similarly spread on either black plastic in the sun, i.e., high-intensity wilting treatment (HI), or on white plastic in the shade, i.e., low-intensity wilting treatment (LI). Each of these wilting treatments was used to generate material with 25 or 35% DM concentration (i.e., 25 or 35). These four different sets of treated alfalfa were then ensiled with or without the addition of 125 g/kg DM sucrose (SA), generating a total of eight different AS, which are referred to as: 25HISA, 25HI, 25LISA, 25LI, 35HISA, 35HI, 35LISA, and 35LI.

The eight different AS were subsequently incubated in a Rusitec system for 9 days (Czerkawski and Breckenridge, 1977), the procedure of which has been previously described in detail (Hartinger et al., 2019b). Briefly, each experimental run lasted 17 days, and prior to the incubation of the AS, the Rusitec system was equilibrated by supplementation of a hay and concentrate diet (70:30) for the first 8 days. Subsequently, all silages were incubated

isonitrogenously for 9 days. During the AS incubation, two time points were selected, an early time point (ET), i.e., 2 days after the start of the AS incubation, and a late time point (LT), i.e., 7 days after the start of the AS incubation. To obtain the liquid-associated microorganisms, for each time point, vessel fluid (2 ml) was directly collected 2, 4, 12, and 23 h after feed bag exchange. For the solid-associated microorganisms, 48 h feed residues were collected from the vessels 3 and 8 days after the start of AS incubation period as these feed residues were left from the AS fermentation of the prior day, when the liquid phase samples were collected. Subsequently, DM concentration was determined in additional feed residue aliquots and all samples were stored at −80°C until DNA extraction.

DNA extraction

Prior to DNA extraction, the four frozen liquid phase samples (i.e., sampled at 2, 4, 12, and 23 h) from each vessel at each time point (i.e., ET or LT) were thawed at 4°C, pooled, and mixed well. An aliquot of the pooled sample (2 ml) was centrifuged at 800 × *g* and 4°C for 15 min to remove feed particles. The supernatant was transferred to a clean tube, and then centrifuged at 21,000 × *g* and 4°C for 40 min to obtain a microbial pellet. For the solid phase samples, a subsample of the feed residues (approximately 5 g wet weight) from each vessel at each time point (i.e., ET or LT) were ground in liquid nitrogen using a mortar and pestle. DNA extraction of liquid and solid phase samples was performed from the obtained microbial pellets and 250 mg (wet weight) of ground feed residue, respectively, using the First-DNA all-tissue Kit (Gen-IAL GmbH, Troisdorf, Germany) according to the manufacturer's protocol with some minor adjustments. In brief, a bead-beating step with a Precellys® 24 tissue homogenizer (bertin Instruments, Montigny-le-Bretonneux, France) was used to enhance DNA recovery, and an RNase A treatment (VWR International GmbH, Darmstadt, Germany) was performed in order to remove RNA. The DNA yield and purity was determined using a NanoDrop 8000 spectrophotometer (NanoDrop® Technologies, Thermo Fisher Scientific, Waltham, MA, United States), and DNA integrity was checked using agarose gel electrophoresis. Subsequently, DNA extracts were stored at -20°C until further use.

qPCR

The absolute quantification of the bacterial and archaeal 16S rRNA genes as well as the anaerobic fungal 5.8S rRNA gene was performed on a CFX384 Real-Time PCR Detection System (Bio-Rad Laboratories, Veenendaal, Netherlands). Details of the amplification conditions and reaction mixtures have been previously described elsewhere (van Lingen et al., 2017). Briefly, all qPCR reactions were performed in triplicate using a 10 µl final reaction volume. The forward and reverse primers 1369F/1492R (Suzuki et al., 2000) and 787F/1059R (Yu et al., 2005) were used in SYBR green-based qPCR assays to quantify

bacterial and archaeal 16S rRNA genes, respectively. The Neo qPCR For and Neo qPCR Rev primers were applied in a TaqMan probe-based assay to quantify anaerobic fungi (Edwards et al., 2008). Standard curves (10^2–10^8 copies/µl) were prepared from custom synthesized DNA prepared from known sequences of *Ruminococcus albus* (sequence data in ENA under accession number: CP002403.1; bacterial qPCR standard), *Methanobrevibacter millerae* (sequence data in ENA under accession number: CP011266.1; archaeal qPCR standard), and *Neocallimastix* sp. (sequence data in ENA under accession number: GU055516.1; fungal qPCR standard). The copy number of each microbial group was calculated per ml vessel fluid and per g DM feed residue for liquid and solid phase, respectively.

Prokaryotic 16S rRNA gene barcoded amplicon sequencing

For microbial composition analysis, barcoded amplicons of the V4 region of the prokaryotic 16S rRNA gene were generated using the modified F515-806R primer set (Walters et al., 2016). The PCRs were performed in triplicate with a SensoQuest Labcycler (SensoQuest, Göttingen, Germany) in 35 µl reactions containing 7 µl of 5x HF buffer (Finnzymes, Vantaa, Finland), 0.7 µl of dNTPs (10 mM each; Promega, Leiden, Netherlands), 0.35 µl of Phusion Hot start II DNA polymerase (2 U/µl; Finnzymes, Vantaa, Finland), 0.7 µl of the barcoded primer mix (100 µM each), 0.7 µl of template DNA (20 ng/µl) and 25.5 µl of PCR-grade water. The cycling conditions consisted of an initial denaturation at 98°C for 30 s, followed by 25 cycles of: 98°C for 10 s, 50°C for 10 s, 72°C for 10 s, and a final extension step at 72°C for 7 min. The size of the PCR products was confirmed by agarose gel electrophoresis and PCR products were then purified with HighPrep™ (MagBio Europe Ltd., Kent, United Kingdom). Concentrations of the purified PCR products were fluorometrically determined using a Qubit in combination with the dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, United States). The purified PCR products were then mixed in equimolar amounts into pools including synthetic mock communities, i.e., MC3 and MC4 from Ramiro-Garcia et al. (2016), to control for potential technical biases (Ramiro-Garcia et al., 2016). Samples were then sequenced on the Illumina HiSeq platform (GATC-Biotech, Konstanz, Germany, now part of Eurofins Genomics Germany GmbH) and sequencing data was analyzed using the NG-Tax pipeline version 1.0 (Ramiro-Garcia et al., 2016). Operational taxonomic units (OTU) were defined with an open reference approach and taxonomy was assigned using the SILVA 16S rRNA gene reference database version 128 (Quast et al., 2013).

Statistical analysis and visualization

Data of liquid- and solid-associated microorganisms were analyzed separately. Statistical analysis of the Log_{10} transformed qPCR data was performed with MIXED procedure of SAS

version 9.4 (SAS Institute Inc., NC, United States) and repeated measures ANOVA due to the repeated sampling from the same vessel (i.e., ET and LT). To test for the effects of DM concentration, wilting intensity, sucrose addition and their interactions during ET and LT, respectively, the mixed model used was:

$$Y_{ijklm} = \mu + D_i + W_j + S_k + D_i*W_j + D_i*S_k + W_j*S_k + D_i*W_j*S_k + V_l + R_m + e_{ijklm}$$

where Y_{ijklm} is the observed response, μ is the overall mean, D_i is the fixed effect of DM concentration, W_j is the fixed effect of wilting intensity, S_k is the fixed effect of sucrose addition, V_l is the random effect of vessel, R_m is the random effect of experimental run, and e_{ijklm} is the residual error. Furthermore, the effect of the time point on overall quantities of bacteria, archaea, and anaerobic fungi in the liquid and solid phase was analyzed. When interactions were not significant, differences between least squares means were tested with Tukey's test. The UNIVARIATE procedure was applied to test assumptions of the model by analysis of the residuals.

The prokaryotic 16S rRNA gene sequence data was analyzed in Rstudio 3.5.3 using the packages phyloseq, microbiome, ape, vegan, picante, and ggplot2 (Dixon, 2003; Paradis et al., 2004; McMurdie and Holmes, 2013; Lahti and Shetty, 2017). For estimating the alpha diversity, phylogenetic diversity (PD) index described by Faith (1994) was calculated and checked using the Shapira-Wilk's normality method with a *P*-value of > 0.05 confirming normal distribution. Subsequently, the PD data was analyzed using a Kruskal-Wallis test. To determine the effect of pre-ensiling treatments as well as incubation time on beta diversity, unweighted and weighted UniFrac distances were used to perform principal co-ordinate analysis (PCoA). Sample groupings in the PCoA were tested for significance by adonis (Anderson, 2001). Constrained partial redundancy analysis (RDA) of the prokaryotic 16S rRNA sequence data was used to assess the relationship between genus-level phylogenetic groupings of the OTUs and incubation time or pre-ensiling treatments. Liquid and solid phase samples were analyzed separately, and the analysis was performed using Canoco 5.11 (Šmilauer and Lepš, 2014). The data was transformed [log(fraction + 0.0001)] and significance of explanatory variables was tested using a Monte Carlo permutation test with a total of 999 permutations using the factors vessel and experimental run as covariates. The significance level was defined at $P < 0.05$ and trends were declared at $0.05 < P < 0.10$ for all statistical analyses. Regarding the multivariate data, RDA and PCoA analyses each used a single test statistic based on all genera and OTUs, respectively (a pseudo F-value), the significance of which is tested by Monte Carlo simulation and therefore did not need a *P*-value adjustment due to multiple testing.

Results

qPCR

In both phases, bacteria were the most abundant group being in general at least 100-fold higher in concentration than archaea and anaerobic fungi (Tables 1, 2). Anaerobic fungi were less than archaea in the solid phase, with only traces of anaerobic fungi detected in the liquid phase as the low quantities were below the detection limit of the assay. The effects of pre-ensiling treatments on microbial concentrations at the ET were assessed (Table 1). In the liquid phase, archaeal concentration was increased by higher DM concentration (P = 0.001) and were highest for high DM sucrose-treated AS (P = 0.044). High DM concentration and sucrose addition tended to increase the concentration of bacteria (P = 0.057) and archaea (P = 0.061), respectively, in the liquid phase. For the solid phase, sucrose addition increased the concentration of anaerobic fungi (P < 0.001), while DM concentration tended to increase the anaerobic fungal concentration (P = 0.057). Wilting intensity had no effect on the concentration of any microbial group (Table 1).

The effects of pre-ensiling treatments on microbial concentrations at the LT were also assessed (Table 2). Addition of sucrose increased the archaeal concentration in the liquid phase (P = 0.014), but tended to decrease archaeal concentration in the solid phase (P = 0.050). The anaerobic fungal concentration was increased by sucrose addition (P < 0.001), and also tended to be higher during incubation of high-intensity wilted AS (P = 0.055). The concentration of bacteria, however, was not affected by any pre-ensiling treatment (Table 2).

The average concentrations of bacteria, archaea, and anaerobic fungi for the eight AS for both phases and time points are summarized in Table 3. Overall, during the transition from the early to the late time point, the concentration of archaea increased for both phases (P < 0.001), whereas the bacterial concentration increased only in the solid phase (P <0.001). From the early to the late time point, anaerobic fungi decreased in the solid phase (P = 0.036; Table 3).

Table 1. Effect of pre-ensiling treatments[a] on concentrations of liquid- and solid-associated microorganisms at the early time point. Values are provided as Log_{10} transformed rRNA gene copies per mL vessel fluid and g dry matter of feed residue for liquid- and solid-associated microorganisms, respectively.

		Treatment									*P*-value						
Phase	Group	25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM[b]	DM	WI	SA	DM×WI	DM×SA	WI×SA	DM×WI×SA
Liquid	Bacteria	9.19	9.33	9.33	9.23	9.36	9.22	9.41	9.38	0.03	0.057	0.362	0.448	0.727	0.173	0.801	0.136
Liquid	Archaea	7.13	7.14	7.18	7.10	7.42	7.12	7.38	7.28	0.04	0.001	0.305	0.061	0.401	0.044	0.798	0.038
Liquid	An. fungi[c]	–[d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Solid	Bacteria	11.41	11.48	11.45	11.38	11.52	11.37	11.40	11.52	0.02	0.658	0.900	0.918	0.691	0.900	0.564	0.070
Solid	Archaea	9.69	9.73	9.68	9.56	9.73	9.58	9.60	9.80	0.03	0.861	0.727	0.861	0.279	0.616	0.477	0.059
Solid	An. fungi	9.07	8.82	9.21	8.56	9.47	8.84	9.25	8.83	0.10	0.057	0.374	<0.001	0.963	0.629	0.526	0.066

[a]Treatments include different: dry matter (DM) concentrations, i.e. 25 or 35; wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA)

[b]Standard error of the mean

[c]Anerobic fungi

[d]Below the detection limit of the method

Table 2. Effect of pre-ensiling treatments[a] on concentrations of liquid- and solid-associated microorganisms at the late time point. Values are provided as Log_{10} transformed rRNA gene copies per mL vessel fluid and g dry matter of feed residue for liquid- and solid-associated microorganisms, respectively.

		Treatment									*P*-value						
Phase	Group	25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM[b]	DM	WI	SA	DM×WI	DM×SA	WI×SA	DM×WI×SA
Liquid	Bacteria	9.33	9.34	9.26	9.35	9.43	9.28	9.27	9.30	0.02	0.968	0.429	0.936	0.749	0.385	0.345	0.719
Liquid	Archaea	7.56	7.31	7.49	7.44	7.68	7.39	7.49	7.31	0.04	0.574	0.651	0.014	0.527	0.532	0.264	0.969
Liquid	An. fungi[c]	–[d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Solid	Bacteria	11.68	11.61	11.54	11.69	11.69	11.60	11.48	11.59	0.03	0.253	0.246	0.262	0.487	0.741	0.094	0.808
Solid	Archaea	10.04	10.01	9.86	10.06	9.98	10.02	9.85	9.97	0.03	0.323	0.119	0.050	0.876	0.977	0.136	0.488
Solid	An. fungi	9.47	8.47	8.95	8.41	9.37	8.60	9.29	8.22	0.17	0.738	0.055	<0.001	0.265	0.418	0.630	0.173

[a]Treatments include different: dry matter (DM) concentrations, i.e. 25 or 35; wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA)

[b]Standard error of the mean

[c]Anerobic fungi

[d]Below the detection limit of the method

Table 3. Effect of time point on concentrations of liquid- and solid-associated microorganisms. Values are provided as Log_{10} transformed rRNA gene copies per mL vessel fluid and g dry matter of feed residue for liquid- and solid-associated microorganisms, respectively.

Phase	Group	Early	Late	SEM[a]	*P*-value
Liquid	Bacteria	9.30	9.32	0.01	0.743
Liquid	Archaea	7.22	7.46	0.12	<0.001
Liquid	Anaerobic fungi	–[b]	–	–	–
Solid	Bacteria	11.44	11.61	0.08	<0.001
Solid	Archaea	9.67	9.97	0.15	<0.001
Solid	Anaerobic fungi	9.01	8.85	0.08	0.036

[a]Standard error of the mean

[b]Below the detection limit of the method

Prokaryotic 16S rRNA gene amplicon sequencing

The prokaryotic 16S rRNA gene sequence data comprised in total 19,728,379 reads that were assigned to 983 OTUs, of which 822 could be assigned up to genus level (Supplementary Table S2). Analysis of the most abundant (>1%) bacterial and archaeal genera revealed that in the liquid phase, 19 and 21 genera had a relative abundance higher than 1% for the ET and LT, respectively (Figures 1A,B). For the solid phase, 26 and 21 genera were among the most abundant genera at the ET and LT, respectively (Figures 1C,D).

Regarding the liquid phase, *Rikenellaceae*_RC9 gut group was the most abundant genus for both time points, whereas *Treponema*_2 was the most abundant taxon for both time points in the solid phase. The results also showed that the family *Prevotellaceae* was overall highly predominant in both phases at ET and LT, and included the following genera: *Prevotella* 1, *Prevotella* 7, *Prevotellaceae*_Ga6A1 group, and YAB2003 group. Likewise, the bacterial genera *Fibrobacter*, *Butyrivibrio*_2, *Roseburia*, *Pseudobutyrivibrio*, and *Lachnospiraceae* NK4A13 group were predominant in both phases and time points. For the solid phase, OTUs belonging to the archaeal genus *Methanobrevibacter* were also among the most abundant taxa for both time points, whereas no archaeal genus was predominant in the liquid phase.

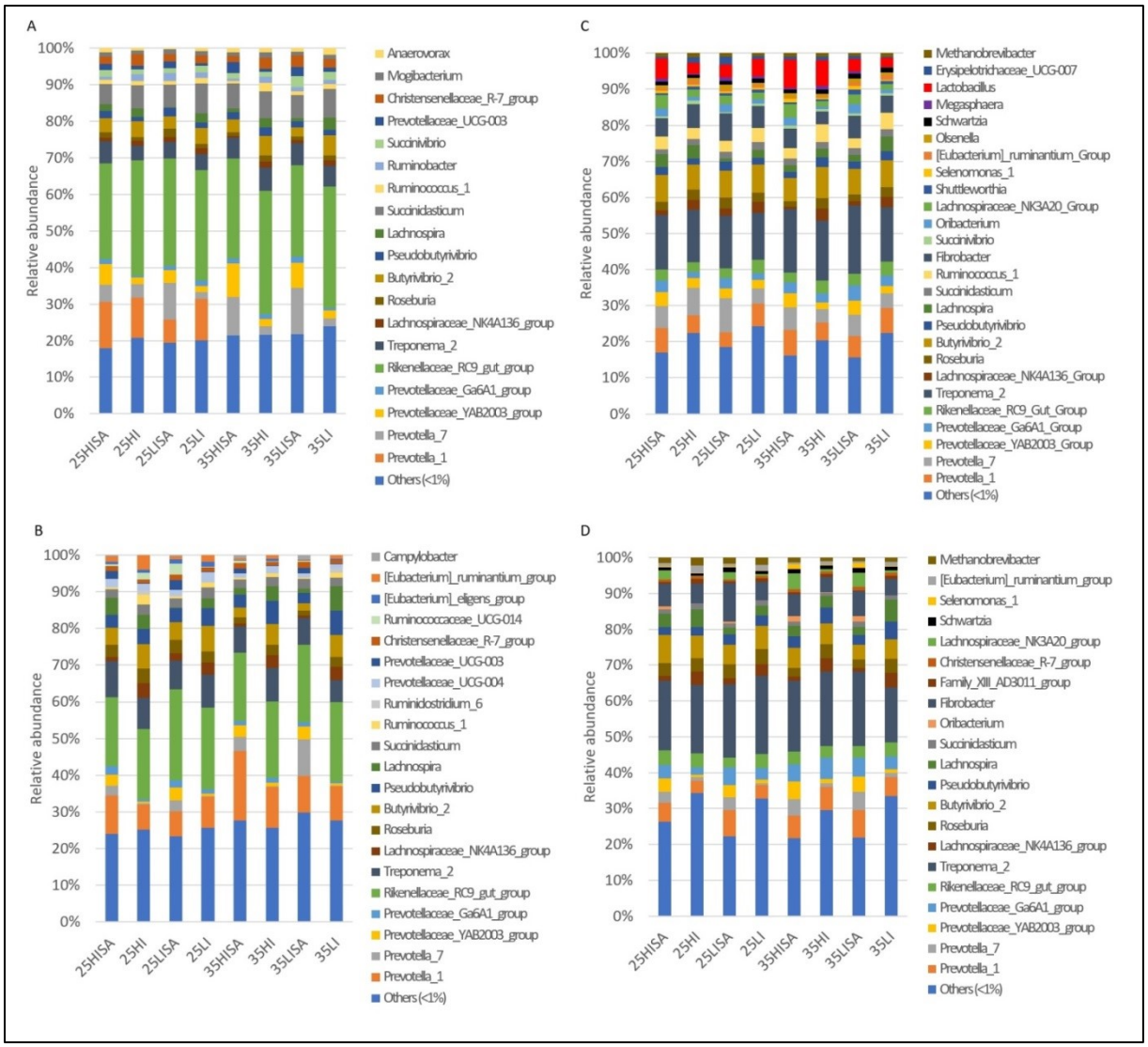

Figure 1. Relative abundances of major prokaryotic genera (>1% relative abundance in at least one alfalfa silage type) and others (<1% relative abundance) in samples for A) liquid phase at the early time point; B) liquid phase at the late time point; C) solid phase at the early time point; D) solid phase at the late time point. Bars represent means (n=4) for each alfalfa silage (AS) type. Abbreviations for each AS type indicate the pre-ensiling treatments including different dry matter concentrations (25 or 35), wilting intensities [low (LI) or high (HI)] and sucrose addition (SA).

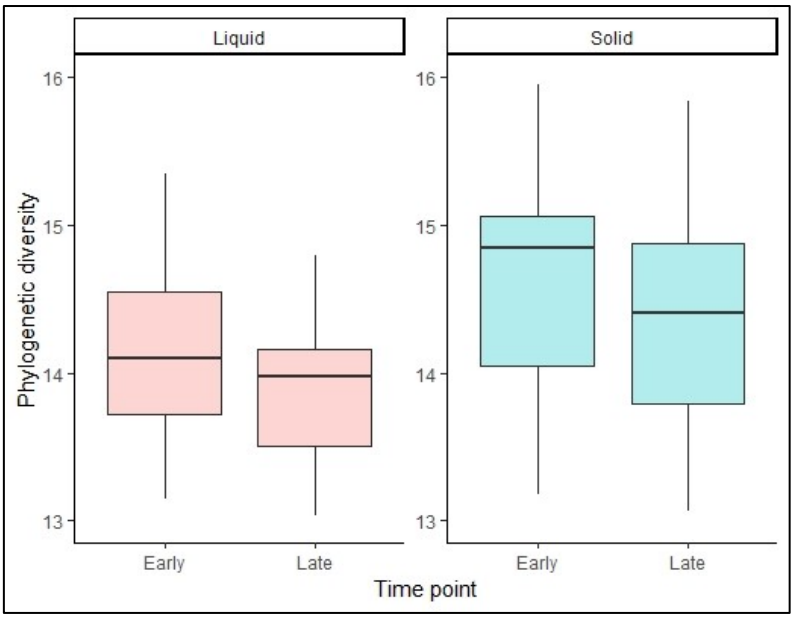

Figure 2. Alpha diversity calculated with the phylogenetic diversity index for the liquid and solid phase and for the early and late time point, respectively. The boxplots show the 25th, 50th and 75th percentiles, with whiskers showing the extremes of the data.

Regarding the influences of pre-ensiling treatments on alpha diversity, no effects on the PD index were observed for the ET in both phases (Table 4 and Supplementary Figure S1). For the LT, however, the pre-ensiling treatment sucrose addition increased the PD index ($P < 0.001$) for liquid-associated communities (Table 4). Also, DM concentration × sucrose addition ($P = 0.002$), as well as wilting intensity × sucrose addition ($P = 0.002$) showed higher PD indices for liquid-associated microbes for sucrose-treated AS, irrespective of DM concentration or wilting intensity. For the solid phase at the LT, sucrose addition showed a tendency to increase the PD index ($P = 0.065$; Table 4). Generally, the PD index of liquid-associated microbes decreased from ET to LT ($P = 0.034$; Figure 2). Similarly, the PD index of solid-associated microorganisms tended to be lower during the LT compared to the ET ($P = 0.060$).

With respect to beta diversity, PCoA analysis at the OTU-level, using weighted UniFrac distances (Figure 3), displayed a clear separation between the liquid and the solid phase along PCoA axis 1 ($P = 0.001$). Regarding time point, ET samples clustered at the top of PCoA axis 2, while LT samples generally clustered at the bottom ($P = 0.001$). Separation of the ET and LT samples was greater for the liquid than for the solid phase. In the solid phase, sucrose-treated and non-treated samples separated for the LT. The same pattern was observed when using unweighted UniFrac distances (Supplementary Figure S2). For the ET, sucrose addition was the only pre-ensiling treatment that had an effect on beta diversity in

the liquid (P = 0.005) and solid (P = 0.011) phase. Sucrose addition also caused a clear effect on beta diversity at the LT for the liquid (P = 0.001) and solid (P = 0.001) phase. For the LT, beta diversity was also affected by interactions of sucrose addition × DM concentration (P = 0.001) and wilting intensity × sucrose addition (P = 0.001), but clear separations were only visible between sucrose-treated and non-sucrose-treated samples along the first PCoA axis (Figure 4). However, for DM concentration × sucrose addition, a weak separation along PCoA axis 1 was found between sucrose-treated low DM AS and sucrose-treated high DM AS (Figure 4A). The same patterns were observed in the corresponding PCoA analysis using unweighted UniFrac distance metrics (Supplementary Figure S3 and Supplementary Table S3).

Constrained RDA analysis showed that only sucrose addition (P = 0.001) and time point (P = 0.001) contributed to explaining the observed variation in the microbial community for both time points in liquid- and solid-associated microorganisms. Time point explained 22.1 and 20.7% of the variation remaining after removal of the between run and between vessel variation for liquid- and solid-associated microorganisms, respectively. Sucrose addition was associated with 10.5 and 11.0% of total variation for liquid- and solid-associated microorganisms, respectively. In addition, the RDA analysis revealed an interaction of time point × sucrose addition for microbes in both phases (both P = 0.001), with sucrose addition having a bigger effect at the LT than at the ET (Figures 5, 6).

In the RDA triplot for the liquid phase, samples of different time points separated along the first canonical axis, i.e., from left to right (Figure 5). Irrespective of sucrose addition the following groups belonging to the *Ruminococcaceae* were generally associated with the ET: UCG-010, UCG-005, and NK4A214. A weaker association with the ET was observed for *Mogibacterium* and *Succiniclasticum*. In general, the UCG004 group belonging to the *Prevotellaceae* was associated with the LT. LT samples clearly separated regarding sucrose addition along the second canonical axis, i.e., from bottom to top. A genus that could only be reliably annotated to the order *Gastranaerophilales* was strongly associated with LT and sucrose addition, whereas the AC2044 group belonging to the *Lachnospiraceae* was strongly associated with LT without sucrose. A weaker association with LT without sucrose was also present for the NK4A136 group belonging to the *Lachnospiraceae*. The NK4A214 group belonging to the *Ruminococcaceae* was positively associated with ET without sucrose.

Table 4. Effect of pre-ensiling treatments[a] on phylogenetic diversity index for liquid-associated and solid-associated microorganisms at early and late time points.

Phase	Time point	Treatment								SEM[b]	*P*-value						
		25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI		DM	WI	SA	DM×WI	DM×SA	WI×SA	DM×WI×SA
Liquid	Early	14.12	13.96	14.05	13.57	13.86	14.03	14.11	14.30	0.08	0.763	0.547	0.792	0.822	0.853	0.933	0.978
Liquid	Late	14.22	12.73	14.00	13.59	13.93	13.39	14.12	12.81	0.20	0.792	0.763	<0.001	0.790	0.002	0.002	0.010
Solid	Early	14.90	14.63	14.68	14.95	14.71	14.83	14.46	15.05	0.07	0.843	0.906	0.843	0.976	0.740	0.807	0.947
Solid	Late	14.86	14.21	14.34	14.32	14.60	14.11	14.67	14.11	0.10	0.972	0.940	0.065	0.989	0.187	0.345	0.808

[a]Treatments include different: dry matter (DM) concentrations, i.e. 25 or 35; wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA)

[b]Standard error of the mean

The RDA triplot for the solid phase showed a similar pattern with samples of different time points separating along the first canonical axis, i.e., from left to right, as well as LT samples separating for sucrose addition along the second canonical axis, i.e., from bottom to top (Figure 6). The genera *Shuttleworthia* and *Lactobacillus* were positively associated with ET. The AC2044 group belonging to the *Lachnospiraceae*, *Synergistes* and *Phocaeicola* were associated with LT without sucrose, and no taxon was associated with LT with sucrose.

Among the most affected genera indicated in the liquid and solid phase RDA plots, respectively, two genera were clearly affected the same way in both phases. These two genera were *Prevotellaceae* UCG-004 (associated with LT) and the AC2044 group belonging to the *Lachnospiraceae* (associated with LT without sucrose).

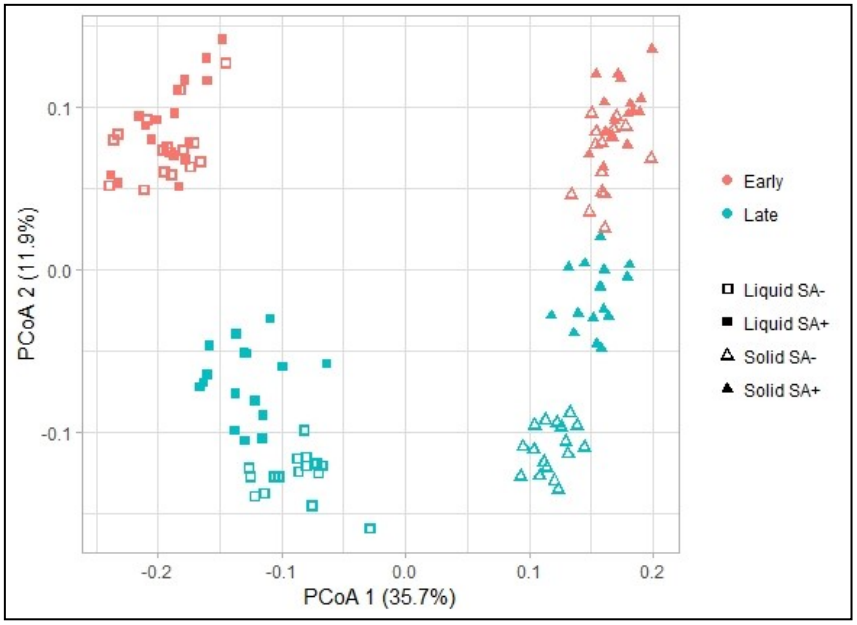

Figure 3. Changes in prokaryotic community composition associated with the time point, phase, and sucrose addition visualized as a principal co-ordinate analysis (PCoA) using weighted UniFrac distance metrics. Symbol shapes indicate the two phases, i.e., liquid and solid, from which the samples originated, colors indicate the different time points, i.e., early and late, and symbol fillings indicate the sucrose addition, i.e. with (SA+) or without (SA-). The percentage of variation explained is indicated on the respective axes.

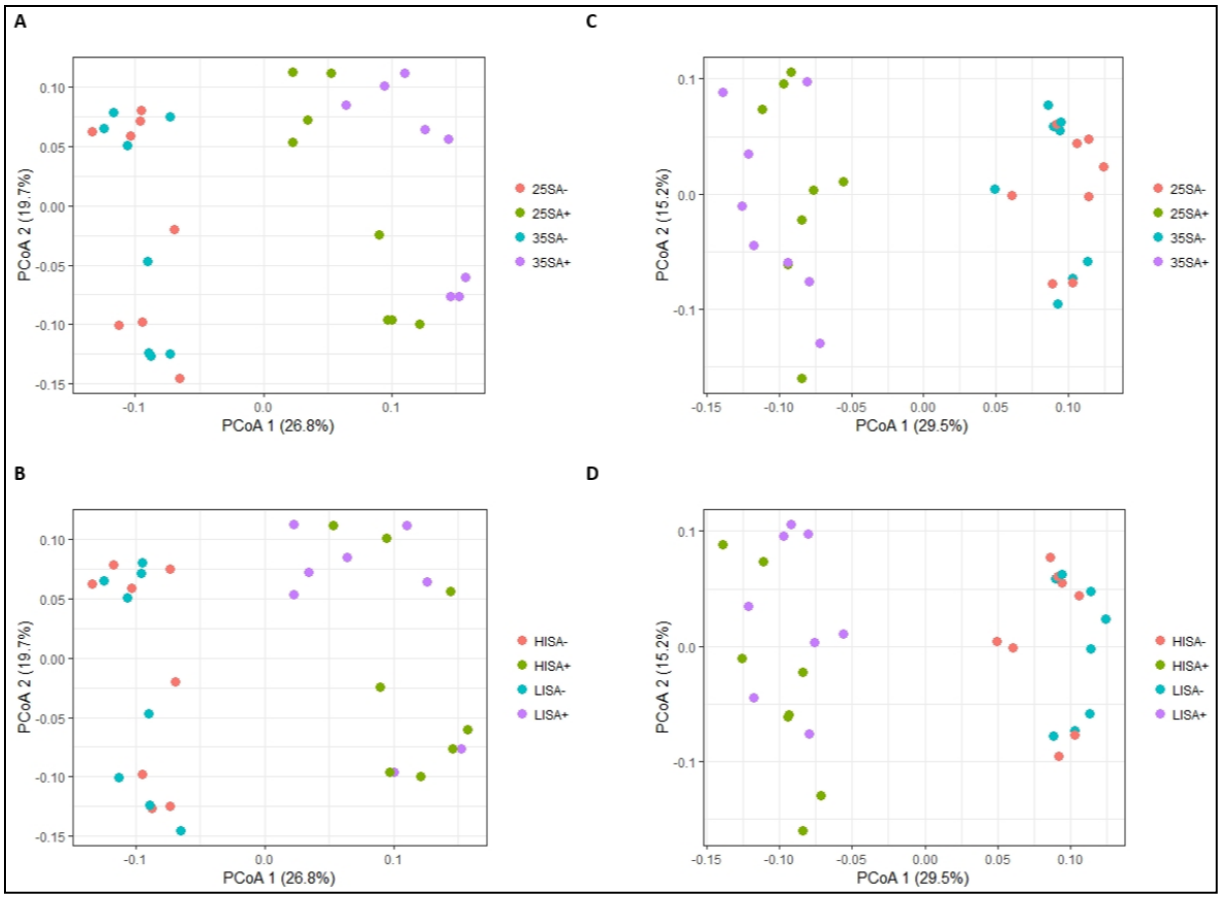

Figure 4. Changes in prokaryotic community composition associated with the interactions of different pre-ensiling treatments at the late time point, visualized as a principal co-ordinate analysis (PCoA) using weighted UniFrac distance metrics for A) treatments dry matter (DM) concentration × sucrose addition in liquid phase samples; B) wilting intensity × sucrose addition in liquid phase samples; C) DM concentration × sucrose addition in solid phase samples; D) and wilting intensity × sucrose addition in solid phase samples. Abbreviations indicate the interactions of treatments including different dry matter concentrations (25 or 35), wilting intensities [low (LI) or high (HI)] and sucrose addition [with (SA+) or without (SA-)]. The percentage of variation explained is indicated on the respective axes.

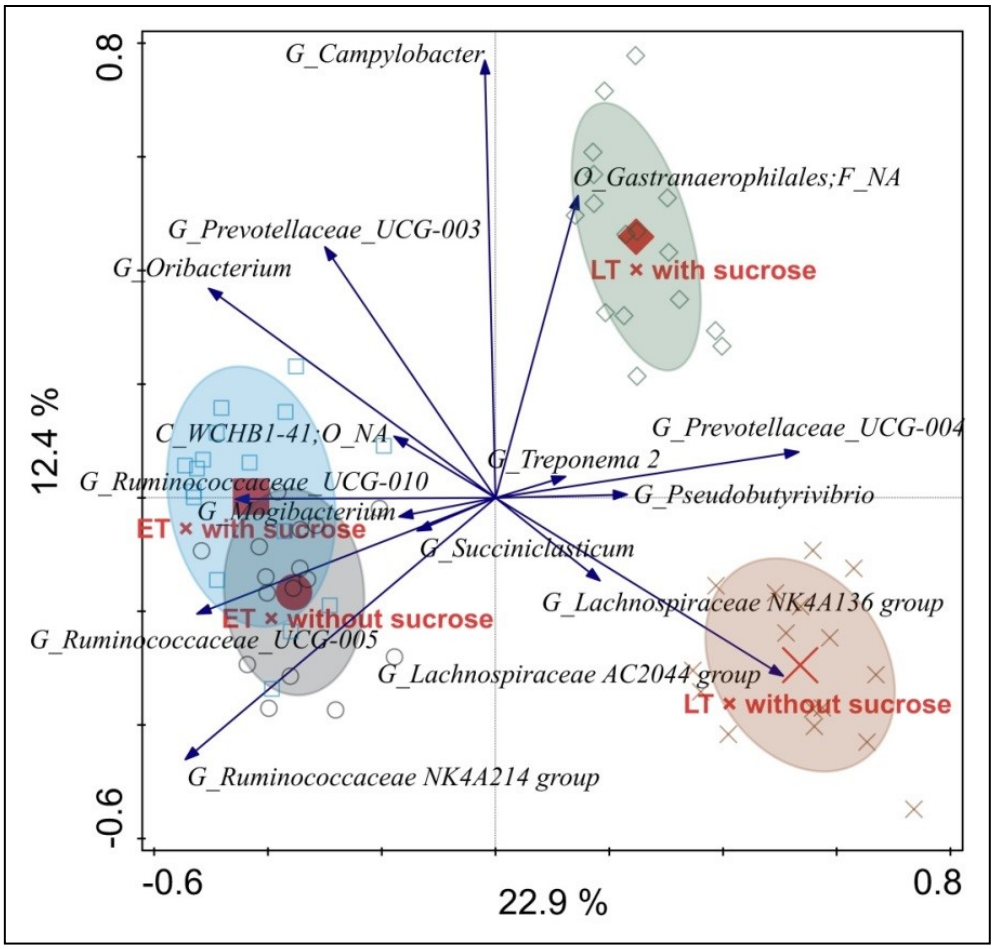

Figure 5. Redundancy analysis triplot illustrating the relationship between the top 15 genus-level phylogenetic groupings of the operational taxonomic units (OTU) for liquid-associated microorganisms explaining the variance of the interaction of time point × sucrose addition with the covariates vessel and experimental run. Arrow labels indicate the taxonomic identification of genus-level phylogenetic groupings, with the level [i.e., phylum (P), class (C), order (O), family (F) or genus (G)]. Abbreviation NA indicates the levels that could not be annotated, for instance "O_*Gastranaerophilales*;F_NA" was reliably assigned to the order *Gastranaerophilales*, but the family could not be annotated. Sample codes for the means indicate the different time points, i.e., early (ET) and late (LT), as well as the sucrose addition, i.e., with sucrose or without sucrose.

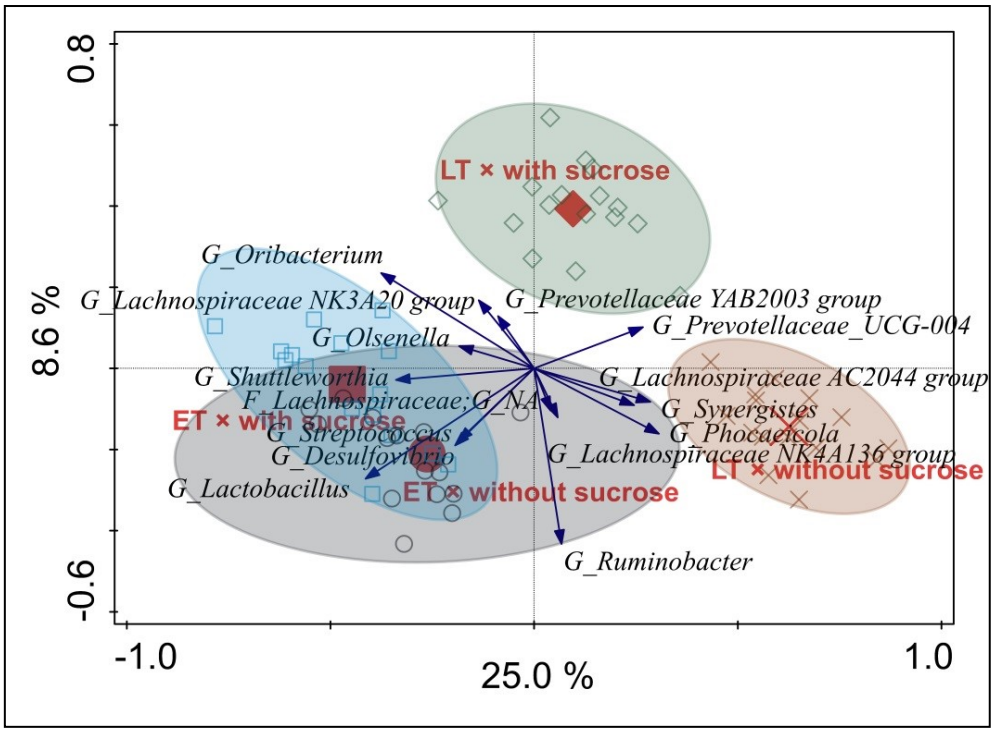

Figure 6. Redundancy analysis triplot illustrating the relationship between the top 15 genus-level phylogenetic groupings of the operational taxonomic units (OUT) for solid-associated microorganisms explaining the variance of the interaction of time point × sucrose addition with the covariates vessel and experimental run. Arrow labels indicate the taxonomic identification of genus-level phylogenetic groupings, with the level [i.e., phylum (P), class (C), order (O), family (F) or genus (G)]. Abbreviation NA indicates the levels that could not be annotated, for instance "F_*Lachnospiraceae*;G_NA" was reliably assigned to the family *Lachnospiraceae*, but the genus could not be annotated. Sample codes for the means indicate the different time points, i.e., early (ET) and late (LT), as well as the sucrose addition, i.e., with sucrose or without sucrose.

Discussion

Absolute abundances of bacteria, archaea, and anaerobic fungi

The qPCR results revealed bacteria to be the most abundant microbial group in both liquid and solid phase, followed by archaea and then anaerobic fungi. This is in line with proportions typically found in the rumen (Puniya et al., 2015; Vaidya et al., 2018). Compared to other Rusitec-based experiments (Martínez et al., 2010; Deckardt et al., 2015; Khiaosa-ard et al., 2015; Wetzels et al., 2018), the abundances of total bacteria and archaea

observed in our study were slightly higher, but within a realistic range (Puniya et al., 2015; van Lingen et al., 2017; Vaidya et al., 2018).

Regarding anaerobic fungi, Deckardt et al. (2015) and Harder et al. (2015) observed 4.65-5.66 Log_{10} gene copies/ml vessel fluid, whereas the concentration observed in this study was below the detection limit for the liquid phase. Furthermore, we quantified this group in the solid phase with an average concentration of 8.9 Log_{10} gene copies/g DM, which is lower than concentrations observed in grass silage-fed dairy cows (Vaidya et al., 2018). Thus, we were most probably able to quantify anaerobic fungi only during their vegetative stage in the solid phase where they physically penetrate plant material, but not during their transient motile stage that is characterized by flagellated zoospores (Gruninger et al., 2014). A possible explanation for this is the known peak in the zoospore density in ruminal fluid being directly after feed intake, i.e., 15–90 min post-feeding (Orpin, 1975, 1976, 1977), which rapidly decreases as the fungal zoospores colonize plant particles (Edwards et al., 2008). Thus, our sampling scheme could explain the low concentration of anaerobic fungi in the liquid phase, since they were quantified in pooled samples consisting of samples that were taken from the liquid phase 2, 4, 12, and 23 h after feed bag exchange.

Anaerobic fungi are important fiber degraders in the rumen (Gordon and Phillips, 1998; Paul et al., 2004). Consequently, the decrease of the anaerobic fungal concentration in the solid phase from ET to LT may partially explain the decline in fiber degradability from ET to LT previously reported (Hartinger et al., 2019b). As protozoa play a key role in the initial colonization and degradation of fibrous structures (Newbold et al., 2015), particularly hemicelluloses (Williams and Coleman, 1985), it is also possible that they too played a role. During the liquid phase sample processing, however, protozoa were separated during the first centrifugation step (Zebeli et al., 2008) and, therefore, did not allow valid assessment of the protozoal concentrations. Furthermore, Rusitec-based studies often report compromised protozoa survival in the vessels (Wallace and Newbold, 1991; Martínez et al., 2010).

Interestingly, the archaeal gene copy numbers were higher at the LT in the liquid and the solid phase, which was not expected since the daily gas production was lower at the LT than the ET (Hartinger et al., 2019b). However, it must be considered that the proportion of methane in the total gas was not determined during the *in vitro* experiment (Hartinger et al., 2019b), and the presence of methanogens may not be strictly correlated with their activity. Therefore, the methane proportion in total gas productions should be measured in future studies assessing the effect of AS pre-ensiling treatments.

During the ET, high DM AS resulted in increased archaeal numbers in the liquid phase and strong tendencies for increased abundances of bacteria and anaerobic fungi in the liquid and

solid phases, respectively. Accordingly, fermentation patterns showed a strong tendency for greater gas productions and higher concentrations of propionate and isobutyrate (Hartinger et al., 2019b), which most likely originated from the tendency for higher microbial concentrations. It is speculated that the microbial concentrations could have been stimulated by the improved quality of high DM AS which resulted in less nutrient degradation during ensiling, as indicated by lower acetic acid and NPN concentrations in these AS (Hartinger et al., 2019a). However, the reduced fiber degradability of the high DM AS in the Rusitec (Hartinger et al., 2019b) contradicts this assumption as more fermentation products occurred with high DM AS, but less fiber was degraded compared to low DM AS. In this context, also the sucrose-promoted growth of anaerobic fungi was not consistent with the reduced degradability of acid detergent fiber in sucrose-treated AS (Hartinger et al., 2019b). However, it must be considered that anaerobic fungi also utilize soluble carbohydrates (Edwards et al., 2008; Gruninger et al., 2014), and likely preferred to metabolize residual sucrose instead of degrading fibrous structures of AS due to catabolite repression (Mountfort and Asher, 1983; Solomon et al., 2016).

Regarding the LT, sucrose addition increased the anaerobic fungal concentration and also high-intensity wilting showed a strong tendency to stimulate them. As high-intensity wilting increased the degradability of neutral detergent fiber (Hartinger et al., 2019b), it is speculated that this effect is at least partly explained by increased anaerobic fungal concentration. If so, then anaerobic fungi may have been more specifically involved in hemicellulose breakdown, as acid detergent fiber degradability was not affected by wilting intensity (Hartinger et al., 2019b). Concerning archaea, incubation of sucrose-treated AS led to their increased concentration in the liquid phase, but a decrease in the solid phase. The increase of liquid-associated archaea might be caused by an indirect effect since sucrose-treated AS incubation led to a higher total SCFA concentration in the vessel fluid (Hartinger et al., 2019b) and, therefore, also more hydrogen that can be used by archaea for methane production. This altogether shows that whilst sucrose addition had the largest influence of the pre-ensiling treatments on microbial concentrations, the phase was key in determining the nature of the effect.

Microbial community composition

For investigating the changes in ruminal microbiota composition, we analyzed the samples from the liquid and solid phase separately. This was done as prior research already showed that clear differences exist in the microbial communities present in liquid and solid rumen content (Henderson et al., 2013; Vaidya et al., 2018). This is also in line with our findings. The number of predominant genera (i.e., those with a relative abundance > 1%) were in a similar range for both phases and time points, and compared well to the number of genera

observed in ruminal *in vivo* samples (Vaidya, 2018). Since the genera *Prevotella*, *Treponema*, *Fibrobacter*, *Ruminococcus*, *Butyrivibrio*, *Pseudobutyrivibrio*, and *Methanobrevibacter* are typically present in the rumen (Bekele et al., 2011; Henderson et al., 2015; Vaidya, 2018; Vaidya et al., 2018), the predominance of these genera in the present study may indicate the existence of a core bacterial community that allows comparisons with *in vivo* assays; although the extrapolation to *in vivo* situation should be made with caution.

Since the genus *Lactobacillus* is of very low abundance in the rumen (Henderson et al., 2015), the predominance of *Lactobacillus* in the solid phase at the ET may have derived from the silages, which typically contain large quantities of this genus (Wen et al., 2017; Zheng et al., 2017). As also deducible from the RDA analysis for the solid-associated microorganisms, *Lactobacillus* was diminished at the LT, which indicated a more adapted and stable microbial community that therefore suppressed the further establishment of exogenously introduced species (Weimer et al., 2015). As the presence of *Shuttleworthia* in the rumen is increased by concentrate feeding (Plaizier et al., 2016), the predominance of this genus at the ET in the solid phase could constitute a remnant of the equilibration period, when hay plus concentrate was incubated in the Rusitec. This observation is again supported by the RDA analysis showing *Shuttleworthia* to be highly associated with the ET. *Selenomonas* harbors various proteolytic and deaminating species (Scheifinger et al., 1976; Wallace, 1985), whose predominance at both time points could be explained by the high provision of N compounds from the AS. It is also a known lactate-utilizing genus in the rumen (Mackie and Gilchrist, 1979), which further supports its high abundance. However, the RDA analysis showed no association of *Selenomonas* with the sucrose treatment that caused higher lactic acid concentrations in the silages. The genus *Megasphaera* also includes various peptidolytic, deaminating and lactate-utilizing members (Scheifinger et al., 1976; Mackie and Gilchrist, 1979; Wallace and McKain, 1991), which is likely to explain its predominance at the ET in the solid phase, but at the same time contradicts its decline toward the LT.

Although total bacterial concentrations were not affected by pre-ensiling treatments at the LT, sequencing data revealed substantial alterations in the bacterial community composition. In accordance with microbial concentration findings, the community composition data revealed that sucrose addition before ensiling had the largest effect of all the pre-ensiling treatments tested. The observed changes were more pronounced at the LT, which is most likely to be due to the longer time that the microorganisms had to adapt to the AS.

We observed a higher PD index for sucrose-treated AS at the LT, and the OTU level PCoA analysis also showed a clear separation between sucrose-treated and non-treated AS in both phases. Likewise, the RDA analysis at the genus level showed that sucrose addition was the only pre-ensiling treatment that significantly explained the observed variation in the microbial

community for both phases and time points, thereby confirming the substantial impact of this pre-ensiling treatment relative to DM and wilting.

The provision of concentrate, i.e., rapidly available dietary energy, increased the microbial diversity of caprine ruminal fluid compared to ruminal fluid from solely forage-fed goats (Belanche et al., 2019b). This finding may explain the higher PD indices in microbial communities deriving from sucrose-treated AS incubations, as these silages had a lower pH as well as higher concentrations of water-soluble carbohydrates and lactic acid (Hartinger et al., 2019a), originating from sucrose metabolism of hetero- and homofermentative lactic acid bacteria during ensiling (McDonald et al., 1991). These soluble metabolites are both rapidly available energy sources for rumen microorganisms. As such, it is perhaps not surprising that the observed effects of sucrose addition were more pronounced in the liquid phase. Conversely, the crude protein and NPN proportions were higher in non-sucrose treated AS (Hartinger et al., 2019a). Since the liquid fraction is dominated by proteolytic bacteria (Plaizier et al., 2018), this may be a further reason why the liquid phase was more affected by the sucrose treatment than the solid phase.

It is important to keep in mind that besides biological causes, also the limited accessibility for sampling the solid phase in the Rusitec system is likely to have had an influence on the observed results. Due to the provision of a nylon bag with fresh feedstuff every 24 h, the microorganisms will also have relocated to the fresh AS, which in consequence may have reduced the observed impact of sucrose addition or any pre-ensiling treatment on the solid-associated population in the feed residues. Therefore, this could have promoted the stronger effect of sucrose addition observed in the liquid phase.

In contrast to RDA analysis, the PCoA analysis revealed further pre-ensiling treatment effects on microbiota beta diversity, i.e., the effects of the interactions of DM concentration and sucrose addition as well as wilting intensity and sucrose addition. These contrasting outcomes between the RDA and PCoA analysis may be partially explained by differences in data analysis approach, as PCoA analysis was performed at the OTU level based on phylogenetically-weighted pairwise distances (UniFrac), whereas RDA analysis was performed at genus level on relative abundance data. For example, OTUs within the same genus responded differently to a pre-ensiling treatment, which hence impedes the observation of an effect at the genus level. However, the pre-ensiling treatments of DM concentration and wilting intensity did not result in clear separation of the samples in the PCoA analysis. For example, this is apparent by the weak separation along the PCoA axis 1 between sucrose-treated low DM AS and sucrose-treated high DM AS (Figure 4A). Consequently, whilst statistically significant, the minor effects of these two pre-ensiling treatment effects were dwarfed by that of sucrose addition.

The second aim of this study was to investigate the effect of incubation time on the microbial community composition. Consistent with the microbial concentration findings, the sequencing data confirmed substantial alterations in the microbial community composition from ET to LT. The overall decrease of prokaryotic PD index from ET to LT may suggest a more adapted microbial community, containing bacteria that can deal better with the high provision of NPN but low availability of dietary energy (Hartinger et al., 2019a). Thus, incubation of solely AS could have caused the less diverse microbial community in both phases, especially at the LT. However, the suggested adaption is not fully reflected in the ruminal fermentation profile as both fiber and organic matter degradability decreased from ET to LT (Hartinger et al., 2019b). Martínez et al. (2010) described a reduced bacterial diversity in the Rusitec with prolonged run time. The decrease of the PD index observed from ET to LT might therefore not necessarily be due to an adaption to the AS, but a general Rusitec-derived effect.

The *Rikenellaceae* RC9 gut group is highly involved in ruminal hemicellulose degradation (Emerson and Weimer, 2017) and its predominance (i.e., highest abundant genus) at both time points in the liquid phase is not in line with the decline of fiber degradability over time. Similarly, growth of *Treponema* species is promoted by hemicelluloses and pectin (Paster and Canale-Parola, 1985; Liu et al., 2014; Emerson and Weimer, 2017) and the predominance of *Treponema* 2 (i.e., highest abundant genus) in the solid phase at both time points does again not match the declining fiber degradability with prolonged Rusitec run time.

The RDA analysis showed that several genera of *Ruminococcaceae*, including the NK4A214 group, had high relative abundances in the liquid phase samples at the ET, where we observed higher daily gas productions and fiber degradability (Hartinger et al., 2019b). Likewise, the abundance of *Ruminococcus* 1 declined in the solid phase with time, as this genus was predominant (relative abundance > 1%) at the ET, but not LT. Genera belonging to the *Ruminococcaceae* harbor major hemicellulolytic, cellulolytic and pectinolytic species (Pettipher and Latham, 1979; Nyonyo et al., 2014) and the *Ruminococcaceae* NK4A214 group was more abundant in the ruminal liquid phase of high performing dairy cows (Tong et al., 2018). Therefore, the higher presence of *Ruminococcaceae* genera, particularly the *Ruminococcaceae* NK4A214 group, might be indicative for enhanced ruminal fermentation and fiber degradation. However, only marginal information about the *Ruminococcaceae* NK4A214 group is available so far (Tong et al., 2018) and our consideration needs therefore further scientific underpinning and for the moment must be regarded with caution. Additionally, the lower presence of *Mogibacterium* and *Succiniclasticum* in the liquid phase at the LT was in line with the reduced fiber degradability since these genera promote ruminal feed degradability (Mi et al., 2018), for instance by the ability of *Mogibacterium* to form phenylacetate, which is needed by several *Ruminococcus albus* strains for cellulose

degradation (Morrison et al., 1990). *Phocaeicola* was associated with the LT without sucrose addition in the solid phase, which could point to an adaptation to the pure AS diet, since this genus was shown to increase when changing sheep from hay-concentrate diets to pasture (Belanche et al., 2019a). Likewise, members of *Lachnospiraceae* are prominent pectin and hemicellulose degraders (Biddle et al., 2013), which could explain the higher presence of several *Lachnospiraceae* genera, such as the AC2044 and NK4A136 groups, in both phases at the LT. However, their high abundance is in striking contrast to the lower fiber degradability at the LT.

The RDA analysis also showed that the YAB2003 group belonging to the *Prevotellaceae* was associated with the ET in the solid phase. Since this group is involved in ruminal hemicellulose fermentation (Emerson and Weimer, 2017), its lower presence at the LT was in line with the lower fiber degradability at the LT. Consequently, although total bacterial concentrations did not decrease from ET to LT, the sequencing data shows alterations in the prokaryotic community composition that, together with the decline in anaerobic fungal concentration, may have contributed to decreased fiber degradation at the LT. Besides fibrolytic members, species of *Prevotellaceae* are also highly active at ruminal proteo- and peptidolysis (Wallace and McKain, 1991; Wallace et al., 1997; Walker et al., 2005), and the higher presence of the *Prevotellaceae* group UCG-004 at the LT in both phases could be the result of an adaption to the high NPN provision by the AS incubation.

Conclusion

The present study demonstrates that differently produced AS influence the ruminal microbiota composition in an *in vitro* Rusitec system. Among all pre-ensiling treatments investigated, sucrose addition had the greatest effect in altering the microbial community composition in both the liquid and the solid phase. Sucrose addition increased the archaeal and anaerobic fungal concentrations in the liquid and solid phase, respectively. Likewise, PD indices were lower without sucrose addition in both phases at the LT and also PCoA analysis showed a stronger separation in community composition for the sucrose addition compared to other pre-ensiling treatments. Thus, in this study we could confirm that sucrose addition is a major driver in shaping the microbial community composition and increasing their abundances. Additionally, the time point of sampling substantially had an effect on the microbiota composition with a lower PD index at the LT and decreased concentrations of anaerobic fungi, but higher archaeal and bacterial concentrations in the solid phase. Therefore, our hypothesis of an altered microbial community composition with prolonged AS incubation could be approved. In general, all observed effects were more pronounced for the liquid than for the solid phase, most probably due to the soluble metabolites released from

the incubated AS, but likely also because of the limited accessibility of the solid phase in the Rusitec system. The observed differences in the microbial composition helped to understand the alterations in the ruminal fermentation patterns, but did not fully explain them. The inclusion of metagenome, transcriptome or proteome analyses may therefore contribute to a deeper understanding of the underlying modes of action by which the pre-ensiling treatments affect the ruminal microbiota activity and fermentation.

Data availability statement

The datasets generated for this study can be found in the European Nucleotide Archive. The codes and files for analysis of the prokaryotic 16S rRNA gene sequence data are deposited as a project available at https://github.com/ThHartinger/Rusitec_Microbiota. In addition, the raw sequence data is deposited in ENA under accession number PRJEB32442.

Ethics statement

The samples analyzed in this study were taken in the course of a previous study (Hartinger et al., 2019b). In brief, the animals used for obtaining liquid and solid ruminal content were kept according to the German Animal Welfare legislation at the Educational and Research Center Frankenforst of the Faculty of Agriculture, University of Bonn, Germany. The experimental procedures and treatments used in the previous study were in accordance with the German guidelines for animal welfare and were approved (file number 84-02.04.2017.A247) by the Animal Care Committee of the state of North Rhine-Westphalia in Germany.

Author contributions

TH, NG, and K-HS conceived and designed the experiment. TH performed the experiment and wrote the manuscript. TH, JE, RG, and CB analyzed the data. All authors interpreted the results, edited the manuscript, read and approved the final version of the manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb. 2019.02761/full#supplementary-material

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

Supplementary Table S1. Effects of dry matter concentration (DM), wilting intensity (WI) and sucrose addition (SA) on *in vitro* rumen fermentation characteristics and fiber degradability of alfalfa silages at the early time point and at the late time point. This Table represents the main fermentation and fiber degradability data presented in Hartinger et al. (2019b) *In vitro* ruminal fermentation characteristics of alfalfa silages in response to different pre-ensiling treatments. Anim. Feed Sci. Technol. 258:114306. doi: 10.1016/j.anifeedsci.2019.114306.

Time point	Item	Treatment[a]									*P*-values					
		25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI	SEM[b]	DM	WI	SA	DM × WI	DM × SA	WI × SA
Early	Gas, mL/day	1433	956	1470	1044	1533	1045	1460	1025	87.4	0.053	0.681	**<0.001**	**0.013**	0.786	0.194
	Ammonia-N, mmol/L	30.0	31.1	29.4	31.7	29.8	30.1	32.1	31.7	0.36	0.186	0.357	**0.006**	**0.018**	0.196	0.735
	Volatile fatty acid concentration, mmol/L															
	Total[c]	143.6	129.2	141.0	135.9	150.5	129.2	144.3	128.6	2.95	0.689	0.501	**0.002**	0.972	0.467	0.568
	Acetate	80.4	76.8	78.8	80.5	79.8	78.1	80.5	76.8	0.57	0.324	0.296	0.329	0.497	0.309	0.741
	Propionate	26.9	17.5	25.6	19.8	31.6	19.0	27.6	20.9	1.77	**0.049**	0.655	**<0.001**	0.681	0.369	0.167
	n-Butyrate	18.6	18.9	18.1	18.1	22.0	14.8	19.1	15.6	0.78	0.386	0.574	**0.004**	0.994	**<0.001**	0.589
	Isobutyrate	1.98	1.73	1.77	2.13	2.17	2.07	1.93	1.85	0.06	**0.010**	0.658	0.227	**0.004**	0.060	0.075
	Isovalerate	7.73	6.81	7.69	7.51	7.06	6.93	6.77	6.05	0.20	**<0.001**	0.812	**0.034**	**0.004**	0.311	0.987
	Degradability, g/kg DM															
	aNDFom[d]	437	387	345	323	326	356	299	323	5.52	**<0.001**	**<0.001**	0.403	**<0.001**	**<0.001**	0.291
	ADFom[e]	361	396	358	361	309	312	318	340	3.78	**<0.001**	0.984	**0.021**	**0.007**	0.587	0.606

Supplementary Table S1 continued.

Time point	Item	Treatment[a]								SEM[b]	*P*-values					
		25HISA	25HI	25LISA	25LI	35HISA	35HI	35LISA	35LI		DM	WI	SA	DM × WI	DM × SA	WI × SA
Late	Gas, mL/day	1407	913	1343	938	1453	970	1490	945	92.4	**<0.001**	0.436	**<0.001**	0.997	0.053	0.424
	Ammonia-N, mmol/L	32.5	33.9	34.5	32.8	31.5	30.6	31.3	30.8	0.50	**0.012**	0.878	0.560	0.756	0.572	0.388
	Volatile fatty acid concentration, mmol/L															
	Total	148.6	129.6	154.7	128.0	155.7	126.0	150.0	122.7	4.97	0.431	0.509	**<0.001**	0.145	0.266	0.629
	Acetate	82.2	80.6	86.3	80.8	84.3	79.6	82.2	79.4	0.84	0.455	0.756	**0.005**	0.299	0.873	0.897
	Propionate	30.1	13.8	30.9	16.0	33.1	18.0	31.4	17.1	2.91	**0.004**	0.822	**<0.001**	0.065	0.494	0.455
	n-Butyrate	15.8	17.2	15.7	13.5	19.6	11.4	18.8	10.5	1.17	0.333	**0.007**	**<0.001**	0.067	**<0.001**	**0.032**
	Isobutyrate	1.99	1.99	2.05	2.02	1.99	2.04	1.84	1.85	0.03	0.647	0.733	0.946	0.267	0.719	0.957
	Isovalerate	11.3	8.49	11.9	8.58	9.59	8.23	8.56	7.29	0.56	**<0.001**	0.192	**<0.001**	**0.038**	**<0.001**	0.916
	Degradability, g/kg DM															
	aNDFom	325	342	297	267	270	278	237	264	4.31	**<0.001**	**0.002**	0.564	0.155	0.204	0.492
	ADFom	234	285	269	275	204	235	223	247	3.49	**0.003**	0.157	**0.016**	0.884	0.973	0.191

[a]Treatments include different: DM concentrations, i.e. 250 g/kg DM (25) or 350 g/kg DM (35); wilting intensities, i.e. low (LI) or high (HI); and sucrose addition (SA).

[b]Standard error of the mean.

[c]Including acetate, propionate, n-butyrate, n-valerate, n-caproate, isobutyrate, and isovalerate.

[d]NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

[e]ADF expressed exclusive of residual ash.

Supplementary Table S2.1. Relative abundances of observed genera (%) in samples taken from the liquid phase during early time point with treatments including 25% dry matter concentration and different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.18	0.17	0.30	0.00	0.19	0.14	0.00	0.17	0.66	0.14	0.00	0.00	0.11	0.23	0.00	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	0.41	0.16	0.32	0.13	0.00	0.20	0.45	2.77	0.21	0.00	0.50	0.41	0.21	0.41	0.00	1.04
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	1.11	0.46	0.17	0.26	0.33	0.23	0.28	0.17	1.79	1.15	0.17	0.61	0.82	0.27	0.51	0.20
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	9.90	14.99	15.04	5.31	6.61	8.38	14.34	7.63	5.94	4.90	8.22	4.19	9.89	17.82	4.15	7.01
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	0.00	4.54	2.38	9.53	3.49	4.91	0.00	4.24	0.18	6.95	5.41	23.54	0.25	3.16	0.47	2.77
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	7.78	5.97	3.54	2.48	1.48	2.05	0.71	1.85	4.76	3.16	2.46	2.03	2.03	2.00	0.53	1.18
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	1.01	2.39	3.32	1.67	16.34	4.49	1.85	0.98	2.26	12.60	3.38	1.55	1.76	3.97	16.21	3.64
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	2.97	12.37	10.01	2.97	5.04	15.95	5.64	4.40	2.57	2.93	4.34	3.04	4.71	7.32	5.37	6.26
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	0.87	1.19	0.88	1.00	0.42	2.58	1.25	2.38	0.79	0.27	1.15	0.71	0.21	1.80	0.35	1.28
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.00	0.20	0.27	0.00	0.15	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	0.63	0.00	0.00	0.18	0.22	0.00	0.21	0.77	0.44	0.26	0.00	0.18	0.52	0.00	0.16	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.00	0.00	0.00	0.90	0.49	0.00	0.40	0.35	0.00	0.75	0.48	1.10	0.00	0.00	0.26	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	20.90	15.86	16.87	39.19	30.33	14.52	26.72	34.35	13.81	31.57	27.94	32.95	22.18	13.14	33.78	34.68
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.32	1.14	1.16	0.39	0.00	0.63	0.86	1.00	0.00	0.17	0.13	0.60	0.47	0.93	0.00	0.36
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	2.47	1.24	0.77	0.63	0.30	0.38	0.32	0.63	1.68	0.82	1.12	0.46	3.12	0.46	1.06	0.36
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	0.00	0.27	0.81	0.00	0.00	0.73	0.20	0.18	0.00	0.00	0.21	0.00	0.28	1.26	0.00	0.21

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.17	0.00	0.17	0.00	0.15	0.16	0.00	0.18	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.24
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	1.81	1.31	1.75	0.95	0.36	0.53	1.27	0.97	2.39	1.67	1.46	1.03	0.63	0.84	0.42	0.72
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.26	0.38	0.64	0.23	0.52	0.79	0.95	1.24	0.00	0.36	0.63	0.19	0.29	0.74	0.93	0.41
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.00	0.00	0.13	0.13	0.00	0.00	0.00	0.96	0.14	0.00	0.00	0.19	0.00	0.00	0.00	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	6.68	4.01	5.29	5.24	2.73	3.65	3.85	3.30	2.80	3.45	5.21	4.74	4.76	3.96	3.27	2.97
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.16	0.00	0.00	0.50	0.00	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.22
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.15	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.13	0.14	0.00	0.00	0.12	0.15	0.13	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.00	0.09	0.14	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.52	0.00	0.00	0.38	0.00	0.00	0.25	0.00	0.46	0.00	0.18	0.17	0.18	0.00	0.00	0.60
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	0.00	0.11	0.00	0.00	0.00	0.00	0.10	0.14	0.00	0.00	0.10	0.00	0.00	0.11	0.00	0.00
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.00	0.00	0.27	0.22	0.00	0.00	0.00	0.15	0.00	0.11	0.14	0.30	0.00	0.00	0.00	0.09
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.44	0.39	5.26	0.28	2.08	0.56	1.23	0.40	1.22	2.50	0.98	0.00	0.68	0.38	5.20	0.21
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	1.05	0.97	0.72	0.48	0.36	0.86	0.45	0.44	2.39	0.39	0.46	0.23	0.85	0.57	0.43	0.75

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	1.29	0.97	0.37	0.65	1.09	1.53	1.15	0.79	1.60	1.04	1.26	0.79	1.67	1.11	1.26	1.36
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.18	0.35	0.00	0.00	0.00	0.28	0.00	0.00	0.32	0.00	0.00	0.00	0.15	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	1.49	0.94	0.21	0.82	0.87	1.56	2.54	0.51	3.57	0.50	1.03	0.57	2.85	1.55	0.66	1.69
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	2.34	1.47	0.69	0.84	0.98	1.36	0.78	0.47	4.35	1.03	2.06	0.84	1.92	0.88	0.29	1.18
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	2.58	2.67	0.96	1.20	0.83	1.50	0.86	0.60	4.55	1.14	1.68	1.05	1.94	1.06	0.49	1.72
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.52	0.47	0.46	0.79	0.98	0.44	1.17	0.13	0.94	0.96	1.17	0.52	0.50	0.33	0.90	1.01
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.55	0.44	0.00	0.23	0.17	0.37	0.25	0.15	1.31	0.18	0.48	0.00	0.60	0.00	0.16	0.32
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	0.18	0.00	1.41	0.11	0.50	0.14	1.04	0.57	0.51	0.22	0.94	0.50	0.55	0.27	0.16	0.86
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.33	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.00	0.15	0.00	0.31	0.16	0.00	0.18
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.63	0.87	0.62	0.57	0.72	1.00	0.54	0.36	1.96	0.68	0.62	0.33	0.79	0.73	0.58	0.51
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	4.52	3.63	2.46	2.94	3.41	4.50	3.58	2.92	2.49	3.36	3.72	2.82	5.25	3.55	3.38	2.89
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	0.36	1.18	4.74	0.25	0.55	4.29	0.63	2.77	0.00	0.00	0.18	0.15	1.80	5.65	0.39	0.27
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	0.22	0.00	0.00	0.31	0.00	0.17	0.00	0.90	0.26	0.00	0.00	0.26	0.00	0.17	0.00	2.31
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	0.32	0.47	0.11	0.00	0.12	0.45	0.00	0.09	0.60	0.20	0.10	0.00	0.24	0.16	0.14	0.23
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	0.28	0.70	0.00	0.13	0.17	0.44	0.00	0.09	0.62	0.00	0.00	0.11	0.43	0.44	0.22	0.22
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.12	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.14	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	0.48	0.00	0.13	0.15	0.26	0.00	0.30	0.13	0.18	0.19	0.39	0.11	0.67	0.13	0.22	0.00
Bacteria	Tenericutes	Unknown_Class	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	1.33	0.55	0.46	1.67	0.74	0.73	2.24	0.47	1.05	0.48	0.72	1.22	1.22	0.57	0.49	2.96
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.62	0.41	0.46	0.80	0.52	0.50	1.27	0.88	0.84	0.45	0.86	0.50	0.71	0.45	0.48	1.17
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	0.49	0.47	0.35	0.16	0.00	0.15	0.00	0.17	1.10	0.00	0.00	0.00	0.23	0.13	0.00	0.14
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.35	0.24	0.22	0.20	0.26	0.20	0.00	0.00	0.83	0.40	0.19	0.00	0.30	0.00	0.21	0.14
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.37	0.12	0.27	0.00	0.20	0.15	0.20	0.17	0.00	0.34	0.16	0.00	1.01	0.15	0.47	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	0.95	0.50	0.45	0.35	0.37	0.71	0.89	0.48	1.26	0.14	0.78	0.37	1.05	0.46	0.40	0.70
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.00	0.13	0.19	1.16	0.62	0.13	0.72	0.90	0.00	0.48	1.16	0.78	0.00	0.00	0.61	0.97
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.00	0.00	0.00	0.23	0.00	0.12	0.08	0.21	0.00	0.00	0.13	0.00	0.18	0.00	0.00	0.23
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.09	0.00	0.18	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.10
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.12	0.22	0.00	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.31	0.35	0.24	0.00	0.18	0.51	0.23	0.34	0.75	0.14	0.16	0.00	0.41	0.32	0.30	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.52	0.13	0.13	0.28	0.43	0.00	0.28	0.41	0.74	0.41	0.23	0.29	0.45	0.00	0.35	0.48
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.10	0.00	0.12	0.11	0.00	0.00	0.00	0.00	0.20	0.13	0.17	0.12	0.00	0.28	0.11	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.00	0.11	0.14	0.00	0.19	0.00	0.00	0.17	0.40	0.00	0.00	0.00	0.00	0.18
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	6.74	3.48	5.02	4.36	4.19	3.82	6.36	6.65	8.06	4.61	5.21	4.40	8.44	9.81	5.76	4.57
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.32	0.29	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.18	0.00	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.58	0.39	0.55	0.56	0.42	0.65	0.53	0.19	0.30	0.42	0.76	0.33	0.53	0.47	0.43	0.48
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	0.82	0.65	0.96	0.51	0.51	0.81	0.69	0.95	0.40	0.48	0.89	0.49	0.97	1.37	0.61	0.52
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	<empty>	Unknown_Genus	2.34	2.72	2.56	1.68	1.54	2.89	3.22	1.13	1.25	2.28	2.39	0.98	2.14	2.32	1.66	0.94
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	1.29	0.54	0.67	0.45	0.38	0.44	0.25	0.63	1.12	0.83	0.62	0.44	1.05	0.76	0.74	0.34
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.49	0.19	0.11	0.00	0.00	0.18	0.00	0.00	0.24	0.00	0.22	0.00	0.23	0.17	0.00	0.15
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.16	0.00	0.00	0.37	0.00	0.00	0.11	0.00	0.30	0.00	0.23	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.98	0.81	0.63	0.39	2.91	2.00	1.12	0.28	5.58	0.92	0.37	1.12	1.51	0.93	1.90	0.36
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.32	0.00	0.28	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.45	0.00	0.00	0.00	0.28	0.36	0.00	0.09	0.00	0.00	0.00	0.00	0.31	0.00	0.12
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.10	0.00	0.25	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.20	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.12	0.10	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	1.87	1.25	1.45	2.61	2.30	1.49	3.71	3.23	1.01	1.93	2.66	1.57	1.67	1.02	1.66	2.55
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.23	0.13	0.18	0.00	0.00	0.17	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.14	0.00	0.31
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.93	1.54	0.89	0.94	0.83	1.40	0.79	0.62	2.48	0.66	1.03	0.64	1.73	1.48	0.74	0.56
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.21	0.23	0.25	0.22	0.46	0.73	0.57	0.35	0.59	0.26	0.34	0.16	0.69	0.58	0.44	0.61
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	0.91	1.47	1.36	0.22	0.41	1.06	0.16	0.34	0.54	0.41	0.20	0.18	0.77	1.02	0.45	0.28
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.10	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.00	0.00	0.11	0.24	0.00	0.10	0.14	0.12	0.18	0.10	0.00	0.00	0.00	0.12	0.11

Supplementary Table S2.2. Relative abundances of observed genera (%) in samples taken from the liquid phase during early time point with treatments include 35% dry matter concentration and different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.38	0.20	0.25	0.23	0.19	0.19	0.19	0.00	0.00	0.21	0.00	0.12	0.16	0.32	0.26	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	0.48	0.21	0.58	0.61	0.00	0.67	0.00	0.11	2.11	0.00	0.11	2.18	0.75	0.00	1.61	0.40
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	4.09	1.09	0.43	0.53	0.56	0.62	0.68	0.11	0.49	0.68	0.12	0.31	0.85	0.50	0.38	0.13
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	16.99	16.00	18.08	13.24	9.31	14.39	5.43	7.19	7.92	7.13	7.65	4.14	9.95	9.91	9.14	7.43
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	0.00	0.00	7.15	8.95	0.00	0.85	2.75	3.51	4.40	18.90	16.03	13.26	1.59	1.25	1.28	2.05
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	9.96	12.17	5.16	8.89	2.67	1.46	0.95	0.82	3.04	3.44	2.27	2.12	1.33	1.44	2.15	0.87
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	1.86	1.68	4.59	0.63	0.95	2.81	23.79	4.35	0.00	9.51	2.81	1.22	2.77	22.12	1.99	1.56
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	4.22	2.46	12.02	29.52	3.34	13.18	4.60	4.18	22.14	2.27	2.71	3.93	15.41	5.17	25.20	3.26
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	1.15	0.76	1.27	0.00	0.29	2.50	0.33	0.96	0.13	0.27	0.82	1.23	1.59	0.38	0.74	2.55
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.00	0.00	0.13	0.00	0.00	0.45	0.16	0.00	0.00	0.00	0.00	0.00	0.59	0.12	0.13	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	0.24	0.27	0.00	0.19	0.26	0.00	0.22	0.27	0.51	0.39	0.14	0.34	0.00	0.29	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.64	0.00	0.39	0.92	1.12	0.00	0.22	0.00	0.44
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	15.02	16.02	11.68	7.62	16.83	15.70	26.84	41.62	13.76	19.01	28.32	43.33	14.04	22.80	16.01	39.39
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.00	0.32	1.20	0.68	0.00	2.56	0.49	0.66	1.02	0.35	0.12	0.76	1.25	0.51	0.57	0.30
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	1.81	3.17	0.70	0.66	2.88	0.32	0.43	0.37	0.92	0.61	0.67	0.26	0.41	0.52	0.44	0.59
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	0.00	0.00	0.58	0.21	0.31	0.61	0.00	0.17	0.43	0.00	0.17	0.00	0.76	0.19	1.19	0.34
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.15	0.00	0.19	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.17	0.49	0.23
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	2.56	1.95	2.03	2.00	0.68	0.65	0.43	0.40	2.79	1.62	1.40	1.81	0.88	0.46	1.28	0.62
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.22	0.32	0.89	0.00	0.44	0.54	0.46	0.27	0.00	0.36	0.49	0.23	0.63	0.77	0.70	0.97
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.14	0.00	0.11	0.00	0.77	0.16	0.00	0.14	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.23
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	4.35	5.56	4.33	3.29	8.95	3.33	2.34	4.48	5.01	3.08	5.04	3.67	2.99	3.29	3.62	6.11
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.12	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.16	0.49	0.00	0.00	0.00	0.44
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.10	0.19	0.00	0.16	0.17	0.00	0.00	0.20	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.25	0.17	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.10	0.00	0.25	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.38	0.11	0.00	0.00	0.23	0.00	0.00	0.27	0.00	0.00	0.39	0.49	0.00	0.00	0.00	0.33
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	0.15	0.14	0.24	0.11	0.00	0.12	0.00	0.00	0.14	0.00	0.14	0.00	0.00	0.00	0.13	0.11
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.00	0.00	0.09	0.19	0.00	0.00	0.00	0.00	0.21	0.46	0.26	0.71	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.53	0.89	0.41	3.59	0.46	0.35	2.99	0.25	3.38	4.45	0.61	0.00	0.38	2.16	2.73	0.88
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	1.05	1.21	0.69	0.44	1.31	0.59	0.46	0.55	1.07	0.83	0.65	0.39	0.96	0.47	0.35	0.34
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	0.98	0.36	0.45	0.11	1.79	1.40	1.29	0.84	0.76	0.53	0.61	0.21	1.43	1.14	0.71	0.94
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.16	0.22	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.43	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	0.99	0.72	0.66	0.17	2.27	1.31	0.33	0.56	0.55	0.39	0.52	0.15	1.49	0.67	0.58	0.81
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	1.39	1.19	0.62	0.46	2.83	0.60	0.61	0.77	0.96	0.51	0.75	0.16	0.77	0.44	0.78	1.47
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	2.67	2.50	1.48	0.77	4.17	1.13	0.65	1.07	1.37	0.71	0.83	0.63	1.36	0.63	1.02	1.34
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.36	0.47	0.50	0.37	0.77	0.30	0.66	0.73	0.60	0.78	0.86	0.50	0.44	0.84	0.54	1.49
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.40	0.52	0.12	0.00	0.81	0.15	0.28	0.22	0.36	0.10	0.25	0.00	0.42	0.26	0.19	0.35
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	0.00	0.13	0.00	0.42	0.52	0.12	0.58	0.14	2.11	0.45	0.00	0.00	0.31	1.16	1.21	1.10
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.34	0.29	0.00	0.00	0.32	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.94	1.01	0.49	0.58	1.00	0.73	0.85	0.54	0.00	0.89	0.62	0.43	1.20	1.13	0.53	0.46
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	4.47	3.03	2.77	1.52	5.72	5.19	2.62	2.64	0.88	1.82	1.66	1.09	5.56	3.53	2.92	3.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	0.00	0.11	1.60	1.55	1.04	5.56	0.23	0.60	2.29	0.00	0.00	0.00	4.29	0.23	4.81	0.21
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.17
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	0.28	0.37	0.20	0.12	0.36	0.16	0.13	0.11	0.11	0.21	0.10	0.00	0.27	0.12	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	0.38	0.49	0.28	0.00	1.12	0.31	0.00	0.00	0.20	0.00	0.00	0.00	0.41	0.00	0.14	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.15	0.19	0.00	0.00	0.13	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	0.25	0.39	0.10	0.00	0.41	0.00	0.20	0.14	0.16	0.44	0.28	0.00	0.13	0.21	0.00	0.36
Bacteria	Tenericutes	Unknown_Class	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.00	0.79	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	0.80	0.76	0.30	0.37	2.85	0.66	0.71	2.51	0.73	0.61	0.73	0.39	0.76	0.76	0.52	1.30
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.56	0.64	0.37	0.31	0.85	0.51	0.59	0.93	0.67	0.42	0.80	0.70	0.50	0.54	0.38	0.93
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	0.76	1.79	0.64	0.51	0.29	0.11	0.00	0.00	0.98	0.23	0.23	0.20	0.17	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.25	0.50	0.21	0.19	0.33	0.00	0.13	0.00	0.32	0.39	0.29	0.00	0.16	0.11	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.11	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.14	0.36	0.20	0.17	0.66	0.32	0.00	0.00	0.25	0.00	0.37	0.00	0.15	0.17	0.30	0.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	1.17	1.29	0.46	0.82	0.90	0.48	0.56	0.20	1.40	0.60	0.52	0.28	0.31	0.65	0.74	0.46
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.00	0.11	0.00	0.00	0.00	0.11	0.25	0.81	0.20	0.28	1.46	1.38	0.24	0.48	0.17	0.75
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.24	0.21	0.00	0.11	0.24	0.10	0.13	0.14	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.14	0.00	0.11	0.00	0.13	0.00	0.12	0.00	0.00	0.10	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.00	0.00	0.31	0.00	0.00	0.18	0.00	0.00	0.13	0.00	0.00	0.00	0.10	0.00	0.13	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.18	0.33	0.32	0.00	0.34	0.36	0.17	0.00	0.12	0.14	0.18	0.00	0.37	0.15	0.15	0.11
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.25	0.41	0.00	0.16	0.66	0.00	0.47	0.37	0.28	0.44	0.21	0.24	0.00	0.58	0.12	0.28
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.28	0.00	0.19	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.10	0.00	0.29	0.11	0.00	0.13	0.10	0.00	0.00	0.16	0.16	0.00	0.25	0.00	0.12	0.21
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.24	0.35	0.00	0.00	0.11	0.00	0.28
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	7.18	4.52	5.52	2.58	4.95	6.64	5.74	4.84	4.33	5.60	5.39	3.96	8.29	2.95	4.71	5.86
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	0.00	0.00	0.00	0.00	0.18	0.00	0.16	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.00	0.00	0.00	0.00	0.09	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.11	0.11
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.00	0.42	0.23	0.00	0.17	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.12	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.25	0.50	0.55	0.36	0.37	0.47	0.31	0.53	0.54	0.41	0.54	0.36	0.29	0.61	0.41	0.68
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	0.45	0.41	0.84	0.28	0.50	0.96	0.76	0.51	1.36	0.41	0.53	0.50	1.02	0.53	0.54	1.01
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	<empty>	Unknown_Genus	1.85	2.63	2.33	2.14	1.88	1.98	1.51	1.01	1.89	1.96	2.91	1.19	1.94	2.56	1.94	1.76
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.11	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	1.22	0.00	0.00	0.00	0.00	0.00	1.05	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.11	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.18	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.17	0.12	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	1.05	1.11	0.73	0.46	1.12	0.52	0.61	0.31	0.99	1.02	0.44	0.29	0.86	0.26	0.65	0.51
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.83	0.35	0.34	0.11	0.27	0.21	0.00	0.00	0.00	0.00	0.00	0.17	0.12	0.13	0.00	0.15
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.18	0.23	0.14	0.14	0.10	0.10	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.10	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.11	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.72	0.38	0.39	0.37	3.01	0.59	1.30	0.41	0.48	2.60	0.24	0.13	0.71	1.28	0.57	0.63
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.12
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.42	0.33	0.00	0.18	0.10	0.12	0.00	0.00	0.00	0.20	0.48	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	0.83	2.19	1.09	0.75	2.01	1.43	2.12	3.36	1.24	2.13	3.99	2.04	1.13	2.44	0.83	2.12
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.00	0.00	0.00	0.00	0.61	0.31	0.00	0.14	0.16	0.00	0.00	0.00	0.09	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.79	1.01	0.91	0.31	1.62	1.20	0.71	1.05	0.79	0.59	0.84	0.54	1.31	0.94	0.57	0.66
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.00	0.00	0.00	0.00	0.28	0.21	0.36	0.32	0.19	0.00	0.00	0.00	0.23	0.26	0.15	0.24
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	1.03	0.94	1.29	1.06	0.82	2.42	0.28	0.00	1.69	0.24	0.00	0.00	2.89	0.34	1.34	0.26
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.13	0.17	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.11	0.10	0.18	0.00	0.17	0.00	0.24	0.10	0.00

Supplementary Table S2.3. Relative abundances of observed genera (%) in samples taken from the liquid phase during late time point with treatments including 25% dry matter concentration with different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.27	0.38	0.65	0.43	0.21	0.00	0.33	0.24	0.52	0.54	0.22	0.46	0.20	0.22	0.18	0.19
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.80	0.00	0.00	0.19
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	1.20	0.52	0.24	2.56	0.24	0.20	0.87	0.72	1.00	0.22	0.78	2.30	1.28	0.14	0.20	1.03
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	0.37	1.75	0.28	0.43	0.35	1.08	0.16	0.48	0.38	0.82	1.10	0.48	0.15	0.48	0.66	0.32
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	11.05	11.27	13.22	6.54	11.87	6.04	5.03	4.94	10.04	6.64	6.26	4.84	5.64	15.14	5.83	7.76
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	0.17	0.96	2.66	6.52	0.32	0.00	0.14	0.39	0.00	6.43	2.71	2.41	0.00	0.13	0.00	0.21
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	2.99	4.21	2.75	2.44	0.99	0.32	0.37	0.51	8.02	1.80	2.15	2.22	0.78	0.85	0.35	0.50
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	3.34	2.41	1.42	2.06	2.34	0.43	2.05	2.12	3.55	0.99	3.17	2.76	0.51	3.13	0.75	1.01
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	3.73	4.45	9.45	3.24	10.43	4.96	1.85	10.51	2.12	6.18	2.25	3.38	5.09	8.89	4.83	7.43
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	0.49	0.12	0.00	1.37	0.00	0.36	0.55	1.85	0.00	0.65	0.22	0.20	0.11	0.00	0.29	0.41
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.21	0.45	0.33	0.00	0.38	0.82	0.59	0.41	0.31	0.34	0.45	0.21	0.27	0.60	0.62	0.17
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	0.68	0.88	0.89	0.36	0.39	0.60	0.53	0.77	0.65	0.45	0.47	0.50	0.75	0.45	0.30	0.38
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.00	0.00	0.00	0.75	0.00	0.26	0.21	0.00	0.33	0.46	0.53	0.67	0.00	0.00	0.47	0.42
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	20.31	14.97	13.17	27.20	5.56	23.12	32.24	15.55	18.98	18.71	29.11	32.54	18.00	9.40	31.83	29.91
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.30	0.20	0.12	0.45	0.00	0.41	0.18	0.38	0.23	0.16	0.16	0.31	0.17	0.00	0.20	0.53
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	6.67	0.71	0.61	0.78	0.32	1.09	0.54	1.04	2.92	2.20	1.57	0.89	1.35	0.67	0.74	2.25
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	1.57	1.94	3.74	1.85	2.99	1.06	1.43	1.63	1.44	1.43	1.46	2.15	3.18	2.47	1.38	3.36
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.29	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	2.21	2.29	2.69	1.75	0.00	0.40	0.16	0.27	3.06	2.44	2.89	1.99	0.34	0.00	0.22	0.48
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.17	0.30	0.23	0.16	0.32	0.24	0.23	0.29	0.23	0.28	0.18	0.20	0.43	0.48	0.27	0.60
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.18	0.10	0.24	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.00	0.00	0.00	0.39	0.00	0.27	0.34	0.16	0.00	0.51	0.67	0.39	0.00	0.00	0.49	0.29
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	8.43	9.30	11.08	9.88	8.28	8.36	8.19	8.75	9.61	8.70	7.22	5.47	9.15	7.63	8.12	11.14
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.25	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.57	0.67	0.14	0.00	0.29	0.00	0.00	0.28	0.20	0.19	0.20	0.24	0.27	0.16	0.00	0.14
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.21	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	0.44	0.00	0.00	0.22	0.00	0.00	0.00	0.42	0.39	0.18	0.17	0.45	0.00	0.00	0.16	0.10
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.17	0.17	0.46	0.92	0.00	0.00	0.00	0.00	0.13	0.79	0.31	0.47	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.19	0.25	0.36	0.21	1.28	1.27	0.66	0.43	0.21	0.20	0.25	0.00	0.38	0.64	1.35	0.43
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	0.33	0.38	0.63	0.28	0.00	0.00	0.00	0.00	0.44	0.25	0.24	0.00	0.00	0.15	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	1.42	0.80	0.95	1.81	2.60	6.58	3.94	2.82	1.09	2.68	2.32	2.52	3.22	1.64	4.67	3.45
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.00	0.00	0.00	0.00	0.28	0.28	0.16	0.00	0.00	0.00	0.17	0.00	0.11	0.24	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.00	0.00	0.00	0.00	0.28	0.00	0.18	0.00	0.00	0.00	0.13	0.00	0.00	0.17	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	1.16	0.81	0.64	1.31	2.56	2.89	2.56	2.41	1.04	1.00	1.79	0.83	2.17	1.63	1.43	3.01
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.16	0.13	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	3.29	5.11	2.37	2.48	4.37	4.91	3.62	2.89	4.12	4.04	4.24	1.97	4.31	2.69	2.92	2.12
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	4.58	4.23	3.04	1.81	5.05	5.71	2.91	3.00	5.50	4.36	3.55	2.19	4.93	5.34	5.78	3.32
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.29	0.34	0.21	0.26	0.14	0.17	0.13	0.00	0.37	0.36	0.33	0.25	0.17	0.17	0.00	0.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.13	0.15	0.15	0.00	0.00	0.00	0.00	0.00	0.12	0.13	0.13	0.00	0.15	0.15	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.00	0.20	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.36	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.16	0.26	0.15	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	0.00	3.84	1.44	0.30	6.15	3.18	1.74	4.66	0.00	0.98	1.79	0.42	1.28	3.31	1.96	0.94

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.12	0.13	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.30	0.36	0.24	0.31	0.00	0.23	0.25	0.20	0.38	0.33	0.38	0.15	0.34	0.32	0.22	0.17
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	5.67	5.19	4.20	3.77	6.70	6.49	7.47	6.12	6.95	4.02	4.82	3.40	8.80	6.20	8.37	4.74
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.16	0.12	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.11
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	0.16	7.23	10.88	0.30	9.05	0.48	0.66	4.91	0.27	0.21	0.11	0.12	1.52	8.55	0.36	0.26
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	1.23	0.38	0.00	1.24	0.00	1.39	0.63	1.63	2.64	0.16	0.45	1.23	2.15	0.00	1.33	1.60
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	0.11	0.51	0.33	0.26	0.25	0.16	0.16	0.11	0.12	0.41	0.39	0.18	0.22	0.27	0.18	0.12
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.24	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.15	0.00	0.00	0.00	0.82	0.69	0.27	0.35	0.16	0.00	0.11	0.00	0.68	0.65	0.18	0.73
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	1.16	0.44	0.27	0.00	0.00	0.00	0.14	7.23	0.83	0.09	0.00	10.98	0.25	0.33	0.00	0.00
Bacteria	Tenericutes	Unknown_Class	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	3.34	0.00	1.16	0.00	0.32	0.00	2.19	0.38	1.91	0.00	0.16	1.28	0.90
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	0.00	3.39	1.69	0.00	0.14	0.84	0.33	0.00	0.55	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.12	0.00	0.00	0.00	0.25	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	0.64	0.37	0.25	0.55	1.07	1.49	6.44	1.31	0.77	0.41	0.13	0.24	2.72	1.21	0.39	1.02
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.16	0.00	0.00	0.66	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	0.16	0.23	0.00	0.16	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.14	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.56	0.36	0.00	0.00	0.64	0.27	0.52	0.00	0.48	0.61	0.14	0.00	0.44	0.71	0.23	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	0.00	0.00	0.22	0.28	0.12	0.00	0.35	0.08	0.00	0.00	0.51	0.34	0.00	0.13	0.00	0.11
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.18	0.22	0.25	0.40	0.11	0.00	0.25	0.36	0.00	0.28	0.55	0.28	0.26	0.55	0.45	0.24
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.00	0.15	0.13	0.32	0.13	0.00	0.00	0.11	0.12	0.16	0.00	0.20	0.27	0.18	0.00	0.13
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.12	0.12	0.00	0.00	0.27	0.21	0.17	0.00	0.12	0.00	0.09	0.00	0.26	0.16	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.00	0.32	0.10	0.18	0.45	0.00	0.14	0.00	0.00	0.23	0.18	0.10	0.10	0.19	0.23	0.16
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.00	0.19	0.00	0.37	0.45	0.00	0.00	0.31	0.23	0.18	0.14	0.00	0.11	0.38
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	3.01	2.27	2.73	1.61	4.30	1.89	3.06	2.28	2.23	3.10	2.99	1.64	2.73	3.77	3.01	2.18
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.16	0.35	0.15	0.11	0.00	0.00	0.00	0.00	0.16	0.00	0.16	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.12	0.13	0.16	0.00	0.14	0.00	0.00	0.13	0.00	0.11	0.14	0.00	0.13	0.13	0.00	0.12
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.00	0.39	0.14	0.10	0.63	0.10	0.00	0.00	0.15	0.00	0.13	0.00	0.13	0.25	0.28	0.12
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.00	0.25	0.18	0.41	0.00	1.51	0.36	0.00	0.33	2.66	1.49	0.32	1.03	0.00	2.55	0.26
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.12	0.00	0.00
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	1.86	0.99	1.04	0.46	2.98	1.12	1.14	2.51	0.53	0.69	0.99	0.44	1.33	2.68	0.94	1.36

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	Unknown_Family	Unknown_Genus	1.94	1.57	1.40	1.17	0.69	0.23	0.39	0.61	1.27	2.07	1.20	1.03	0.96	0.85	0.57	0.44
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	0.39	0.42	0.27	0.44	0.50	0.29	0.30	0.33	0.41	0.64	0.59	0.30	0.50	0.34	0.61	0.55
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.74	0.31	0.26	0.41	0.00	0.00	0.00	0.00	0.84	0.44	0.54	0.31	0.00	0.00	0.00	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.31	0.25	0.21	0.00	0.00	0.00	0.00	0.18	0.35	0.00	0.11	0.00	0.19	0.12	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.27	0.19	0.00	0.00	0.38	0.00	0.00	0.32	0.35	0.16	0.00	0.00	0.29	0.16	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.31	0.26	0.11	0.42	0.89	0.73	0.48	0.41	0.30	0.38	0.51	0.24	1.04	1.13	1.09	0.22
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.18	0.14	0.00	0.00	0.00	0.00	0.89	0.17	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.27	0.00	0.30	0.30	0.00	0.00	0.00	0.00	0.22	0.48	0.00	0.00	0.54	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.13	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.27	0.00	0.19	0.00	0.17	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	1.05	0.62	0.74	2.92	0.78	1.24	1.10	0.67	0.62	2.03	1.64	1.75	1.51	1.36	0.88	1.08
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.40	0.43	0.50	0.30	0.28	0.21	0.17	0.15	0.34	0.44	0.32	0.26	0.51	0.45	0.23	0.18
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.28	0.00	0.12	0.58	0.24	0.34	0.24	0.19	0.36	0.13	0.34	0.27	0.62	0.35	0.22	0.31
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	0.46	0.26	0.39	0.13	0.00	0.00	0.00	0.00	0.39	0.31	0.30	0.10	0.10	0.11	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.13	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.12	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.17	0.00	0.00

Supplementary Table S2.4. Relative abundances of observed genera (%) in samples taken from the liquid phase during late time point with treatments including 35% dry matter concentration and different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.34	0.37	0.50	0.26	0.79	0.23	0.32	0.27	0.53	0.35	0.24	0.71	0.20	0.21	0.19	0.35
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	0.97	0.32	0.63	1.77	0.59	0.59	0.20	1.44	1.12	0.30	0.87	1.33	0.18	0.40	0.23	0.26
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	0.25	0.90	0.34	0.30	0.36	0.51	0.46	0.10	0.66	0.59	0.24	0.29	0.43	0.79	0.14	0.39
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	22.12	30.92	16.24	6.54	10.99	14.44	7.57	11.39	8.97	6.88	6.45	17.83	13.54	12.45	5.68	5.33
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	0.25	1.67	2.23	10.91	0.09	0.24	0.36	0.10	2.93	17.76	18.51	0.45	0.17	0.47	0.00	0.15
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	3.87	2.46	4.51	1.99	1.13	1.03	1.07	0.83	3.56	2.00	1.63	6.79	0.91	0.94	0.40	0.52
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	3.40	1.94	2.15	1.44	0.87	2.57	1.17	0.13	1.38	0.51	2.95	3.23	5.76	0.40	0.34	1.38
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	3.06	14.72	19.62	4.05	7.65	9.91	3.64	7.06	31.56	5.86	1.61	3.41	6.48	23.12	4.79	4.17
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	0.00	1.05	0.64	0.90	0.00	1.48	0.34	0.41	0.52	0.41	0.47	0.11	0.52	0.17	0.24	0.32
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.00	0.16	0.00	0.15	0.30	0.57	1.41	0.13	0.00	0.24	0.52	0.00	0.63	0.21	0.23	1.01
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	0.22	0.16	0.23	0.22	0.00	0.15	0.33	0.14	0.71	0.25	0.25	0.12	0.15	0.69	0.00	0.21
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.30	0.00	0.00	0.87	0.00	0.00	0.37	0.50	0.00	0.17	0.78	0.23	0.00	0.00	0.26	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	19.66	9.14	10.83	34.56	15.04	7.62	30.64	29.91	7.88	26.72	31.13	18.38	6.99	5.33	37.19	36.87
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.00	0.00	0.00	0.21	0.27	0.20	0.25	0.33	0.14	0.00	0.00	0.00	0.17	0.26	0.15	0.20
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	3.22	0.32	0.51	0.69	1.50	0.53	0.91	2.59	0.47	0.84	0.43	3.33	0.60	0.38	0.33	0.48
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	1.31	0.91	0.56	1.77	2.72	1.75	1.46	1.95	0.30	1.44	1.66	0.99	1.50	0.91	1.14	1.39

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.00	0.00	0.00	0.10	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.43	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	1.52	0.95	1.57	1.25	0.20	0.00	0.19	0.31	0.88	1.56	1.03	2.35	0.00	0.00	0.00	0.16
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.28	0.18	0.19	0.27	0.60	0.63	0.75	0.64	0.00	0.26	0.34	0.24	0.77	0.40	0.82	1.10
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.00	0.00	0.12	0.42	0.00	0.16	0.00	0.21	0.16	0.68	0.39	0.00	0.00	0.16	0.00	0.15
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.00	0.00	0.00	0.26	0.19	0.00	0.40	0.41	0.24	0.27	0.23	0.11	0.00	0.00	0.35	0.29
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	8.71	5.23	8.27	6.48	10.81	7.78	8.78	9.05	8.19	6.79	5.74	8.69	5.61	7.04	4.13	7.51
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.39	0.71	0.33	0.15	0.25	0.30	0.00	0.00	0.53	0.20	0.14	0.84	0.43	0.14	0.00	0.11
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.13	0.13	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	0.20	0.00	0.21	0.24	0.00	0.00	0.00	0.13	0.20	0.17	0.00	0.46	0.00	0.10	0.00	0.00
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.24	0.49	0.79	1.76	0.00	0.00	0.00	0.00	0.41	1.79	1.62	0.59	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.00	0.00	0.00	0.00	0.35	1.34	0.85	0.59	0.27	0.20	0.21	0.23	2.18	0.55	0.44	0.50
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	0.52	0.96	0.61	0.32	0.00	0.00	0.00	0.00	0.41	0.53	0.70	0.62	0.30	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	0.97	0.70	0.87	0.73	1.96	1.53	4.62	6.08	1.07	0.40	0.47	0.85	2.45	2.63	5.32	4.14
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	0.84	0.17	1.18	0.49	1.91	1.74	2.49	1.88	1.25	0.35	0.55	0.62	1.37	2.45	3.68	1.71
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.00	0.00	0.00	0.00	0.35	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	2.27	1.65	1.71	1.00	3.30	1.61	4.17	2.10	1.31	1.13	1.31	1.57	2.39	1.97	3.14	2.99
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	5.32	3.00	2.94	2.74	9.10	5.13	7.22	3.53	2.53	1.80	2.57	4.57	8.83	5.79	6.01	6.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.52	0.24	0.41	0.30	0.53	0.14	0.44	0.38	0.31	0.35	0.57	0.41	0.25	0.29	0.33	0.40
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.00	0.00	0.12	0.00	0.26	0.19	0.00	0.00	0.19	0.00	0.00	0.24	0.14	0.14	0.17	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.00	0.12	0.00	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.25	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	0.00	1.35	0.57	0.00	0.49	1.52	2.00	0.23	0.62	0.16	0.37	0.00	0.79	0.66	0.92	1.38
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.17	0.25	0.36	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.40	0.23	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.47	0.60	0.48	0.20	0.76	0.29	0.53	0.29	0.50	0.22	0.61	0.35	1.48	1.24	0.54	0.56
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	3.54	2.75	2.55	1.49	8.82	4.01	4.96	4.75	2.44	1.13	1.28	2.62	3.64	9.45	6.04	4.60
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.16	0.14
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.19	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	0.00	3.22	4.36	0.00	0.76	14.39	0.28	0.56	4.48	0.00	0.13	0.00	14.31	11.72	0.21	0.27
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	0.29	0.19	0.00	0.67	0.58	0.32	0.55	1.08	0.00	0.00	0.00	0.24	0.00	0.00	0.84	0.98
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	0.13	0.64	0.32	0.14	0.16	0.18	0.00	0.12	0.31	0.28	0.20	0.24	0.27	0.25	0.24	0.18
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	0.00	0.12	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.30	0.16
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.11	0.00	0.00	0.00	0.49	0.38	0.18	0.45	0.00	0.00	0.00	0.11	0.60	0.21	0.18	0.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	0.74	0.46	0.68	0.78	0.18	0.24	0.00	0.00	0.00	0.39	0.00	0.98	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Unknown_Class	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.43	2.97	0.00	0.00	1.29	2.39	0.52	1.88	0.65	0.45	0.00	0.00	0.26	2.14
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	0.24	0.16	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00	3.63	1.05
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.00	0.27	0.00	0.00	0.10	0.19	0.17	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	1.12	0.46	0.14	0.21	1.33	0.89	0.89	0.79	0.30	0.54	0.19	0.74	1.53	0.80	1.83	1.10
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.00	0.00	0.00	0.00	0.23	0.18	0.23	0.19	0.00	0.00	0.00	0.00	0.17	0.19	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	0.44	0.50	0.24	0.41	0.00	0.11	0.00	0.00	0.36	0.31	0.32	0.51	0.13	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.19	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.28	0.11	0.00	0.00	0.41	0.39	0.17	0.00	0.00	0.00	0.18	0.12	0.00	0.00	0.18	0.35
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	0.00	0.13	0.20	0.50	0.00	0.10	0.00	0.14	0.52	0.16	0.47	0.12	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.49	0.14	0.55	0.57	0.19	0.41	0.22	0.18	0.27	0.52	0.59	0.25	0.38	0.35	0.98	0.30
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.15	0.00	0.00	0.12	0.12	0.12	0.00	0.17	0.15	0.00	0.00	0.12	0.16	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.10	0.11	0.00	0.00	0.16	0.15	0.00	0.00	0.00	0.00	0.25	0.00	0.21	0.23	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.00	0.12	0.11	0.00	0.35	0.28	0.16	0.15	0.20	0.15	0.00	0.13	0.62	0.00	0.13	0.14

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.26	0.15	0.00	0.00	0.32	0.15	0.32	0.19	0.27	0.00	0.00	0.00	0.29	0.28
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	2.18	2.61	2.87	1.87	2.64	4.45	1.67	1.45	2.27	2.97	2.21	3.21	4.84	1.21	1.58	1.68
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.12	0.28	0.32	0.00	0.00	0.00	0.00	0.00	0.19	2.90	0.36	1.54	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.00	0.00	0.00	0.00	0.12	0.15	0.00	0.00	0.13	0.00	0.00	0.10	0.37	0.00	0.00	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.00	0.14	0.19	0.00	0.52	0.30	0.36	0.00	0.17	0.00	0.11	0.27	0.12	0.00	0.19	0.30
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.42	0.00	0.00	0.26	1.06	0.19	1.62	0.51	0.17	0.54	0.57	0.48	0.00	0.18	0.82	1.59
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.00	0.00	0.00	0.00	0.14	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	0.36	0.43	0.30	0.22	0.72	2.34	0.43	0.35	0.44	0.45	0.34	0.49	1.48	0.98	0.34	0.42
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	Unknown_Family	Unknown_Genus	2.23	1.75	1.03	1.24	1.39	0.62	0.28	0.33	1.49	1.51	0.90	1.50	0.50	0.37	0.63	0.31
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.16	0.15	0.16	0.22	0.00	0.00	0.00	0.00	0.11	0.10	0.00	0.41	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	0.54	0.42	0.33	0.39	0.58	0.80	0.55	0.56	0.28	0.53	0.29	0.77	1.45	0.28	0.32	0.42
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.75	0.66	0.59	0.31	0.00	0.00	0.00	0.17	0.24	0.24	0.14	1.04	0.00	0.00	0.00	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.29	0.21	0.00	0.00	0.19	0.15	0.00	0.00	0.00	0.00	0.00	0.22	0.27	0.15	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.31	0.18	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.15	0.15	0.35	0.00	0.72	0.96	0.48	0.58	0.19	0.17	0.00	0.16	1.41	0.65	0.98	0.52
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.10	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.12	0.00	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.24	0.85	0.00	0.00	0.00	0.11	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.36	0.00	0.71	0.24	0.32	0.00	0.00	0.00	0.00	0.22	0.00	0.28	0.12	0.00	0.00	0.12
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.00	0.36	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	0.96	0.62	1.44	2.62	0.93	1.04	1.32	0.60	1.22	1.69	3.15	0.71	0.46	0.72	1.46	1.49
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.43	0.32	0.62	0.43	0.66	0.38	0.40	0.29	0.38	0.41	0.57	0.38	0.52	0.38	0.36	0.45
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.29	0.17	0.40	0.40	0.50	0.35	0.46	0.47	0.24	0.20	0.28	0.23	0.13	0.20	0.43	0.36
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	0.57	0.66	0.63	0.38	0.12	0.00	0.11	0.00	0.81	0.45	0.46	0.52	0.00	0.26	0.11	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.11	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary Table S2.5. Relative abundances of observed genera (%) in samples taken from the solid phase during early time point with treatments including 25% dry matter concentration and different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.11	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.26	0.00	0.16	0.14	0.00	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.24	0.19	0.00	0.18	0.00	0.00	0.24	0.00	0.00	0.22	0.33	0.17	0.00	0.39	0.22	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	0.91	1.59	0.71	0.83	0.92	0.65	2.01	1.28	0.62	1.46	1.35	0.28	0.54	1.75	0.70	0.60
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	0.69	0.62	0.87	0.00	0.23	0.00	0.35	1.53	0.31	0.27	0.71	0.74	0.80	0.52	0.84	0.45
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	0.29	0.13	0.00	0.31	0.11	0.18	0.00	0.00	0.32	0.34	0.17	0.11	0.22	0.16	0.15	0.40
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	9.34	7.56	4.78	2.50	5.06	5.96	5.36	5.36	4.37	4.14	5.71	5.96	7.10	5.84	4.54	6.24
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	0.00	9.36	4.44	24.76	10.74	10.48	11.44	0.30	5.81	0.90	0.46	3.85	1.44	10.01	8.60	1.61
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	6.61	3.34	3.74	2.08	2.04	3.11	2.49	1.26	3.04	2.98	0.70	1.85	3.49	2.85	1.94	1.16
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	0.31	0.49	0.86	0.70	0.57	0.69	0.38	1.51	0.76	0.25	0.94	0.87	0.39	0.55	0.39	1.78
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	0.35	0.49	0.00	0.21	0.22	0.31	0.70	0.14	0.69	1.39	0.30	0.40	0.68	1.08	0.00	0.46
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	2.15	1.39	1.55	1.52	1.16	1.09	1.15	3.62	1.55	4.18	2.57	2.45	3.18	2.13	1.43	1.42
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	0.14	0.00	0.00	0.00	0.00	0.00	0.13	0.30	0.00	0.46	0.00	0.00	0.15	0.12	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.20	0.12	0.30	0.18	0.00	0.11	0.20	0.11	0.11	0.22	0.48	0.11	0.12	0.29	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.00	0.20	0.34	0.00	0.00	0.18	0.38	0.00	0.31	0.00	0.28	0.00	0.00	0.38	0.00	0.16

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	0.90	4.81	4.11	0.90	0.39	1.44	11.25	2.58	2.79	0.49	2.31	0.96	0.64	7.55	0.94	2.83
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	2.49	0.11	0.00	0.00	0.00	0.00	0.00	0.34	0.00	2.56	0.24	0.00	7.43	0.00	0.00	0.14
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	3.84	3.15	3.51	2.04	2.04	2.33	1.97	4.37	3.98	1.57	7.13	3.37	4.83	2.97	2.31	3.65
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.19	0.13	0.23	0.18	0.00	0.38	0.13	0.92	0.32	0.35	0.29	0.33	0.31	0.15	0.15	0.33
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	4.38	2.68	2.96	2.59	2.66	1.56	1.16	2.46	2.65	2.20	4.97	1.33	2.41	1.36	1.67	2.78
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	0.00	0.00	0.31	0.00	0.00	0.00	0.26	0.34	0.00	0.00	0.76	0.18	0.20	0.58	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.39	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.21	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.17	0.28	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.24	0.00	0.27	0.13	0.17	0.17	0.00	0.30	0.27	0.09	1.71	0.11	0.11	0.00	0.26	0.14
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.00	0.10	0.00	0.15	0.20	0.15	0.00	0.11	0.46	0.00	0.67	0.21	0.00	0.00	0.23	0.24
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	14.85	12.69	12.33	19.20	20.11	14.92	11.38	8.43	15.88	8.52	20.22	12.36	13.56	8.49	29.91	17.36
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.18	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.14	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.14	0.00	0.11	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.23	0.00	0.00	0.14	0.22	0.00	0.00	0.16	0.11	0.35	0.00	0.37	0.17	0.00	0.10	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	4.91	4.13	6.82	5.46	4.35	8.53	2.95	6.67	8.80	3.31	8.31	5.76	3.14	5.12	4.48	10.08
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.24	0.31	1.57	0.00	0.30	0.77	0.54	2.54	0.44	0.46	0.51	0.56	0.40	0.49	0.00	1.41
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	1.89	3.26	2.19	0.61	1.28	0.84	1.43	1.01	2.64	3.85	0.00	0.97	1.42	0.81	2.00	1.33
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	1.37	1.06	0.96	1.63	1.95	3.81	1.95	3.33	1.69	2.70	3.12	4.35	2.55	1.84	0.86	3.60
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.00	0.21	0.44	0.00	0.00	0.00	0.35	0.53	0.00	0.49	0.00	0.00	0.25	0.22	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	2.12	1.36	1.35	1.46	1.46	2.84	2.92	4.90	1.63	3.30	3.44	3.49	4.05	3.46	0.92	2.23
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.00	0.14	0.15	0.00	0.00	0.18	0.35	0.22	0.18	0.16	0.00	0.21	0.00	0.17	0.00	0.20
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.22	0.43	0.34	0.19	0.35	0.42	0.86	0.39	0.21	0.53	0.00	0.41	0.30	0.46	0.31	0.17
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	2.61	2.15	1.91	2.18	3.02	3.29	2.00	4.31	2.14	5.10	2.83	2.87	2.83	1.70	1.06	2.77
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	2.19	1.92	1.66	2.08	2.77	1.78	1.18	2.45	1.74	4.03	3.20	3.10	1.83	1.53	1.88	1.46
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.27	0.17	0.25	0.22	0.22	0.32	0.17	0.33	0.18	0.52	0.26	0.31	0.31	0.22	0.23	0.33
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.14	0.00	0.37	0.00	0.35	0.00	0.24	0.16	0.25
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.23
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	0.00	0.38	1.83	0.56	0.55	1.11	0.78	1.77	0.48	0.15	0.71	1.77	0.19	1.09	0.00	0.70
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.53	0.64	0.54	0.42	0.70	0.54	0.48	0.43	0.75	1.05	0.35	0.56	0.59	0.54	0.63	0.50
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	7.96	6.20	6.91	7.93	8.80	6.75	7.11	6.59	10.48	4.59	3.29	7.07	9.49	5.80	5.82	8.58
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.58	0.93	0.42	0.00	0.00	0.36	0.00	0.11	0.00	0.33	0.00	0.00	0.25

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.13	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	0.58	3.92	8.05	0.74	1.36	2.88	4.46	1.73	0.62	0.75	1.09	1.90	1.23	3.63	0.48	1.65
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	0.11	0.21	0.00	0.32	0.68	0.00	0.14	0.00	0.00	0.50	0.30	3.01	0.00	0.00	0.38	0.19
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	2.86	6.37	2.94	1.61	2.51	1.58	4.11	1.15	3.07	2.15	0.43	2.40	1.66	1.73	2.38	1.67
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	0.14	1.21	0.27	0.17	0.19	2.10	0.77	0.00	2.40	0.38	0.00	0.35	0.43	0.36	0.13	2.49
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.00	0.00	0.00	0.35	0.28	0.36	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.22	0.00	0.15	0.15	0.35	0.11	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.11	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.14
Bacteria	Tenericutes	Unknown_Class	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.19	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	2.25	2.78	4.00	4.81	5.04	3.03	2.73	4.21	2.88	2.84	5.78	6.79	3.17	2.35	2.67	3.39
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.53	0.61	0.52	0.37	0.39	0.36	0.48	0.65	0.41	0.77	0.40	0.75	0.61	0.49	0.33	0.37
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	0.66	1.04	0.70	0.30	0.60	0.20	0.90	0.00	0.29	1.17	0.00	0.34	0.45	0.68	0.82	0.14
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.16	0.16	0.17	0.00	0.17	0.16	0.00	0.00	0.19	0.31	0.00	0.15	0.17	0.22	0.00	0.16
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.14	0.19	0.00	0.00	0.00	0.00	0.46	0.11	0.00	0.46	0.00	0.00	0.14	0.22	0.00	0.11
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.00	0.17	0.18	0.00	0.00	0.14	0.21	0.15	0.16	0.00	0.25	0.00	0.24	0.15	0.00	0.14
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.12	0.16	0.10	0.00	0.14	0.12	0.22	0.50	0.14	0.50	0.00	0.23	0.10	0.19	0.12	0.23
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.48	0.31	0.38	0.28	0.21	0.12	0.35	0.14	0.43	0.57	0.36	0.15	0.52	0.80	0.23	0.33
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.00	0.00	0.00	0.00	0.97	0.00	1.18	0.70	0.00	0.00	0.00	0.00	0.00	0.00	1.18
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	2.08	1.13	1.79	1.29	0.83	1.29	1.22	2.08	2.22	2.15	1.83	1.55	2.23	3.44	1.31	1.79
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	1.11	0.82	0.68	0.56	0.60	1.02	0.41	0.77	0.93	2.24	0.16	0.25	0.69	0.30	0.45	0.71
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.13	0.12	0.13	0.19	0.19	0.10	0.00	0.00	0.18	0.12	0.00	0.16	0.00	0.19	0.18	0.20
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.19	0.14	0.15	0.12	0.14	0.00	0.10	0.32	0.11	0.13	0.00	0.35	0.28	0.00	0.14	0.11
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	0.57	0.21	0.41	0.24	0.16	0.16	0.22	0.48	0.28	0.29	0.29	0.22	0.56	0.54	0.20	0.22
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	Unknown_Family	Unknown_Genus	0.38	0.31	0.61	0.11	0.14	0.10	0.19	0.49	0.43	0.13	0.14	0.00	0.47	0.45	0.10	0.28
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0.78	0.34	0.37	0.31	0.25	0.13	0.21	0.30	0.36	0.36	1.38	0.25	0.38	0.34	0.31	0.17
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.14	0.00	0.00	0.00	0.50	0.00	0.00	0.34	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.00	0.14	0.17	0.00	0.00	0.00	0.16	0.00	0.00	0.16	0.00	0.11	0.13	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	1.32	0.83	1.13	0.96	0.86	0.70	0.64	0.36	1.11	1.00	1.21	0.71	0.97	1.88	1.02	1.22
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.13	0.11	0.12	0.15	0.20	0.00	0.10	0.15	0.10	0.36	0.10	0.14	0.12	0.15	0.10	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.37	0.00	0.00	0.00	0.00	1.39	0.00	0.91	0.97	0.86	0.25	0.00	0.26	0.00	0.00	0.83
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.33	0.00	0.00	0.00	0.10	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.26
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.20	0.21	0.10	0.16	0.19	0.35	0.65	0.72	0.10	0.56	0.74	0.29	0.56	0.54	0.00	0.31
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.19	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	2.17	0.00	0.00	1.35	1.55	0.80	0.00	0.39	0.71	1.19	0.21	0.00	1.35	0.00	0.00	0.96
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	0.83	0.86	1.11	0.35	0.36	0.63	0.69	1.11	0.88	0.16	1.01	0.67	1.10	0.66	0.30	0.25
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.49	0.31	0.31	0.00	0.14	0.11	0.16	0.00	0.10	0.63	0.00	0.15	0.44	0.10	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.19	0.18	0.13	0.14	0.19	0.12	0.28	0.11	0.15	0.00	0.26	0.33	0.22	0.21	0.19	0.22
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	4.42	2.90	6.31	2.89	8.29	2.21	4.07	3.30	1.71	6.29	0.00	7.55	2.40	7.73	14.10	1.79

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.16	0.13	0.14	0.00	0.11	0.11	0.16	0.17	0.13	0.35	0.19	0.11	0.20	0.14	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.27	0.54	0.36	0.26	0.27	0.00	0.44	0.32	0.28	0.96	0.41	0.27	0.34	0.38	0.24	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.26	0.45	0.39	0.16	0.16	0.15	0.89	0.51	0.10	0.58	1.06	0.78	0.57	0.38	0.12	0.12
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	1.87	0.00	0.00	0.00	0.00	3.08	0.00	3.15	0.97	4.71	0.00	0.00	1.07	0.00	0.00	2.12
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.16	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.10
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.16	0.00	0.00	0.00	0.00	0.14	0.00	0.61	0.12	0.00	0.00	0.00	0.12	0.00	0.00	0.10
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary Table S2.6. Relative abundances of observed genera (%) in samples taken from the solid phase during early time point with treatments including 35% dry matter concentration and different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.15	0.16	0.00	0.00	0.11	0.15	0.16	0.00	0.00	0.00	0.00	0.00	0.10	0.12	0.00	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.25	0.31	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.16	0.00	0.15
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	1.06	1.29	0.68	1.12	1.44	0.10	1.36	0.80	0.81	0.62	0.52	1.46	1.17	0.83	0.63	0.79
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	1.25	0.25	0.66	0.78	1.68	0.46	0.31	0.85	0.29	1.36	0.21	0.11	1.33	0.61	0.32	0.31
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	0.16	0.87	0.13	0.00	0.51	0.43	0.22	0.12	0.00	0.14	0.20	0.24	0.47	0.27	0.24	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	4.58	13.60	5.32	5.08	4.23	4.17	6.11	2.82	4.32	5.52	5.20	8.78	3.37	5.27	13.87	4.70
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	5.96	0.00	10.81	6.28	2.06	8.84	0.00	8.47	7.23	10.37	6.28	0.00	4.97	4.62	5.75	4.28
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	3.32	6.76	3.26	3.06	2.26	2.31	1.85	3.86	2.84	3.32	3.73	5.75	1.63	2.39	3.57	1.12
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	0.54	0.44	1.46	0.43	1.05	0.67	0.37	1.65	0.16	1.13	0.54	0.56	2.02	1.21	0.46	0.69

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	1.61	0.30	0.00	0.00	0.71	0.76	0.75	0.00	0.00	0.00	0.38	0.32	0.71	0.19	0.42	0.26
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	2.02	2.22	2.90	1.60	1.51	1.71	2.65	0.88	1.22	1.92	1.35	1.76	1.40	2.02	0.99	1.41
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	0.19	0.15	0.00	0.14	0.25	0.00	0.17	0.32	0.00	0.00	0.00	0.31	0.15	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.15	0.10	0.12	0.15	0.10	0.00	0.15	0.26	0.14	0.23	0.00	0.14	0.20	0.16	0.00	0.20
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.23	0.00	0.00	0.00	0.51	0.30	0.00	0.00	0.00	0.00	0.13	0.00	0.36	0.17	0.17	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	3.05	0.38	2.32	2.20	5.77	3.76	0.38	1.83	0.80	3.18	1.68	0.47	5.38	4.46	1.42	0.85
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	0.00	0.47	0.12	0.00	0.12	0.00	3.65	0.14	0.00	0.00	0.00	2.60	0.23	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	3.66	2.31	2.85	2.98	2.79	3.82	5.19	2.11	2.94	3.06	3.13	4.06	2.58	2.68	3.13	2.62
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.36	0.37	0.41	0.27	0.31	0.30	0.23	0.39	0.38	0.41	0.19	0.29	0.42	0.34	0.28	0.20
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	3.45	3.33	3.85	3.86	1.93	2.75	3.47	1.70	4.30	3.72	3.69	4.78	1.99	2.02	2.57	1.71
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	0.33	0.00	0.12	0.11	0.28	0.00	0.20	0.00	0.00	0.20	0.00	0.00	0.21	0.35	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.29	0.10	0.37	0.43	0.32	0.00	0.15	0.33	0.27	0.28	0.21	0.13	0.31	0.28	0.14	0.13
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.00	0.00	0.19	0.43	0.00	0.17	0.13	0.29	0.59	0.13	0.37	0.00	0.00	0.23	0.18	0.65
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	14.24	12.61	13.26	14.95	12.84	17.06	17.51	23.72	22.35	14.20	20.48	18.75	11.04	12.60	16.16	18.61
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.15	0.22	0.16	0.00	0.00	0.00	0.09	0.00	0.00	0.14	0.00	0.27	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.18	0.00	0.16	0.00	0.00	0.00	0.18	0.00	0.10
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.00	0.17	0.00	0.25	0.00	0.00	0.16	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	5.37	4.98	6.70	13.35	3.14	2.66	4.74	8.06	5.67	5.51	5.81	7.85	2.93	5.15	2.16	3.80
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.16	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.19	0.00	3.41	0.55	0.73	0.91	0.23	0.45	0.44	1.12	0.83	0.15	0.59	1.35	0.76	0.56
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	2.27	2.65	1.99	1.64	0.87	1.13	0.57	1.01	2.94	2.05	2.58	2.57	1.26	1.11	1.46	0.36
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	0.81	0.60	0.73	1.65	2.60	4.67	2.78	1.49	0.89	1.33	1.31	0.86	3.18	1.90	3.86	3.24
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.32	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.00	0.00	0.41	0.20	0.13	0.00	0.00	0.12	0.00	0.21	0.00	0.19	0.20	0.24	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	1.68	1.38	1.21	1.46	2.92	1.93	2.20	2.52	0.86	1.66	1.30	0.74	3.57	2.92	1.57	2.14
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.18	0.00	0.00	0.00	0.00	0.16	0.00	0.38	0.46	0.13	0.00	0.14	0.19	0.00	0.21	0.16
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.23	0.30	0.32	0.25	0.28	0.26	0.21	0.24	0.36	0.31	0.25	0.20	0.31	0.34	0.28	0.39
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	1.51	1.61	1.45	3.06	1.94	2.96	3.05	2.02	2.60	2.09	1.55	1.61	2.96	2.49	2.54	3.91
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	1.75	1.83	1.40	2.06	2.19	2.62	3.43	1.10	1.88	2.16	1.73	2.21	2.93	1.93	2.12	2.82
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.28	0.16	0.53	0.33	0.24	0.36	0.35	0.12	0.39	0.33	0.31	0.36	0.33	0.33	0.24	0.34
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.24	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.21	0.24	0.00	0.21	0.21	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.27	0.14	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	0.17	0.00	1.19	1.08	0.76	0.95	0.20	1.04	0.22	3.40	0.80	0.00	1.02	2.52	1.60	0.63

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.00	0.00	0.00	0.23	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.12
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.15	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.12	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.77	0.80	0.57	0.62	0.47	0.67	0.34	0.59	0.80	0.00	0.69	0.71	0.73	0.53	0.92	0.60
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	7.99	7.29	5.05	7.00	10.18	7.97	7.88	7.46	10.17	3.77	6.68	7.76	9.73	7.43	9.22	8.30
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.32	0.64
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.16	0.18	0.00	0.00	0.17	0.00	0.15	0.00	0.00	0.00	0.00	0.15	0.17	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	2.22	0.46	3.92	1.10	6.25	1.10	1.00	5.61	0.66	5.15	1.11	0.39	5.14	7.14	2.71	1.09
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	5.02	3.69	3.47	2.05	1.71	1.48	1.12	1.28	3.24	1.20	3.47	3.02	2.18	1.71	1.45	1.63
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	1.08	0.33	0.55	0.12	0.30	0.17	1.35	0.31	0.27	0.56	2.63	2.19	0.53	0.19	2.76	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.00	0.00	0.00	0.26	0.58	0.00	0.27	0.67	0.00	0.00	0.00	0.00	0.65	0.19	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.21	0.22	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Unknown_Class	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	2.95	2.07	3.44	1.68	3.70	3.40	4.59	2.26	2.04	4.13	2.33	2.68	4.19	5.72	2.84	7.90
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.62	0.51	0.45	0.34	0.49	0.42	0.60	0.34	0.33	0.51	0.39	0.55	0.47	0.45	0.35	0.51
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	1.27	1.40	0.91	0.30	0.51	0.17	0.29	1.04	1.10	0.69	0.65	1.33	0.83	0.28	0.20	0.31

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.16	0.00	0.20	0.16	0.18	0.19	0.00	0.16	0.18	0.21	0.16	0.14	0.22	0.14	0.16	0.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.24	0.12	0.00	0.00	0.17	0.00	0.11	0.22	0.00	0.00	0.11	0.34	0.29	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.15	0.00	0.17	0.00	0.15	0.17	0.00	0.00	0.00	0.19	0.00	0.00	0.18	0.21	0.17	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.30	0.11	0.00	0.48	0.12	0.00	0.12	0.00	0.13	0.00	0.00	0.11	0.12	0.00	0.00	0.14
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.74	0.30	0.27	0.60	0.50	0.49	0.36	0.39	0.29	0.11	0.26	0.26	0.47	0.32	0.16	0.20
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.00	0.00	0.17	0.00	1.01	0.35	0.00	0.97	0.00	1.72	0.00	0.00	0.00	0.77	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	2.46	2.25	1.74	1.63	2.07	2.41	1.77	2.22	1.51	1.55	2.57	1.46	2.48	2.64	0.97	1.17
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	0.75	1.94	1.09	1.17	0.36	1.30	0.50	0.48	1.19	0.47	1.45	1.07	0.68	0.71	0.97	0.38
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.12	0.18	0.11	0.12	0.12	0.19	0.00	0.00	0.13	0.12	0.17	0.14	0.17	0.28	0.12	0.17
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.11	0.17	0.12	0.00	0.12	0.15	0.15	0.17	0.13	0.13	0.10	0.17	0.12	0.11	0.10	0.15
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	0.57	0.21	0.27	0.27	0.45	0.29	0.30	0.36	0.18	0.58	0.15	0.15	0.35	0.35	0.19	0.13

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	Unknown_Family	Unknown_Genus	0.38	0.00	0.25	0.38	0.26	0.12	0.15	0.12	0.11	0.13	0.00	0.32	0.13	0.25	0.10	0.10
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0.78	0.31	0.29	0.28	0.38	0.33	0.35	0.32	0.23	0.87	0.21	0.25	0.35	0.47	0.17	0.25
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.14	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.94	0.00	0.00	0.70
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.14	0.19	0.21	0.00	0.00	0.00	0.11	0.00	0.00	0.13	0.00	0.18	0.00	0.21	0.00	0.11
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.00	0.15	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	1.33	0.98	0.99	1.03	1.38	1.82	0.72	1.74	1.11	1.45	1.44	0.94	1.54	1.60	0.81	0.98
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.11	0.18	0.00	0.11	0.13	0.00	0.15	0.00	0.00	0.00	0.00	0.14	0.10	0.00	0.00	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.25	0.14	0.24	0.00	0.15	0.00	0.17	0.00	0.11	0.19	0.00	0.21	0.10	0.12	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.00	0.23	0.00	1.59	0.00	1.06	0.76	0.00	1.97	0.00	1.44	0.29	0.00	0.00	1.46	0.00
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.13	0.11	0.16	0.18	0.48	0.41	0.64	0.21	0.00	0.12	0.13	0.00	0.50	0.24	0.20	0.63
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.39	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.17	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.00	2.13	0.00	0.99	0.00	1.05	0.59	0.00	0.98	0.00	1.18	0.72	0.00	0.00	0.75	2.43
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	1.06	0.48	1.28	0.48	1.04	0.82	1.13	0.26	0.68	0.85	0.86	1.30	0.81	0.85	0.62	0.76
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.26	0.16	0.00	0.00	0.12	0.22	0.22	0.00	0.21	0.14	0.00	0.33	0.29	0.00	0.10	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.25	0.17	0.22	0.15	0.22	0.12	0.20	0.11	0.24	0.00	0.22	0.21	0.22	0.16	0.20	0.34
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	4.55	8.38	4.32	2.98	8.76	1.84	6.68	2.28	3.22	4.86	2.44	3.39	3.21	5.07	1.77	11.24
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.12	0.00	0.00	0.00	0.13	0.13	0.15	0.14	0.11	0.10	0.00	0.00	0.10	0.12	0.11	0.18
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.49	0.42	0.43	0.00	0.37	0.00	0.41	0.22	0.25	0.48	0.30	0.38	0.43	0.35	0.00	0.39
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.12	0.11	0.00	0.12	0.42	0.29	0.49	0.41	0.00	0.26	0.00	0.11	0.36	0.36	0.10	0.64
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	0.00	3.29	0.00	3.30	0.00	2.47	1.48	0.00	1.06	0.00	1.79	0.00	0.00	0.00	1.26	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.00	0.22	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.00	0.16	0.00	0.39	0.00	0.18	0.00	0.00	0.27	0.00	0.00	0.12	0.00	0.00	0.12	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Supplemenatry Table S2.7. Relative abundances of observed genera (%) in samples taken from the solid phase during late time point with treatments including 25% dry matter concentration and different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.23	0.19	0.19	0.20	0.00	0.27	0.11	0.16	0.14	0.00	0.11	0.23	0.20	0.27	0.14	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.53	0.82	0.35	0.28	0.00	0.58	0.00	0.26	0.00	0.16	0.32	0.35	0.66	0.51	0.00	0.26
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	1.50	2.33	1.69	1.28	1.87	2.78	1.38	1.66	0.79	0.84	1.34	2.16	2.91	2.31	1.18	1.58
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	0.68	0.81	0.54	0.50	0.68	1.04	0.28	0.40	0.60	0.24	0.12	0.30	1.00	0.87	0.55	0.30

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	0.34	0.63	0.12	0.21	0.30	0.20	0.00	0.14	0.15	0.15	0.13	0.17	0.28	0.16	0.18	0.20
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	6.60	3.22	6.43	3.07	2.79	3.46	6.64	4.95	4.99	8.33	6.61	7.69	3.70	4.63	2.11	9.90
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	0.36	2.13	6.24	1.79	0.23	1.56	0.71	0.39	3.52	8.93	5.02	0.40	0.99	0.27	0.28	5.94
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	5.04	3.67	2.93	0.42	0.33	1.56	1.02	0.65	3.38	3.29	2.01	5.29	1.20	1.40	0.41	3.70
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	0.84	0.99	0.44	0.46	0.54	0.54	0.49	0.77	0.49	0.46	0.23	1.08	0.53	0.69	0.76	0.77
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	1.40	0.56	0.41	0.94	0.42	1.05	0.56	0.34	0.00	0.00	0.28	1.09	1.15	0.30	0.00	0.24
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	1.58	1.93	1.34	2.47	2.63	2.47	2.23	2.83	0.83	0.56	0.77	0.91	3.12	1.94	1.82	0.90
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	0.29	0.00	0.00	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.36	0.74	0.22	1.14	0.16	0.19	0.27	0.19	0.23	0.34	0.21	0.25	0.13	0.31	0.16	0.32
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.43	0.55	0.43	0.51	1.22	0.44	0.20	0.38	0.15	0.27	0.17	0.41	0.63	0.18	0.68	0.28
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	1.03	11.55	6.68	3.85	9.22	17.12	1.03	2.70	0.86	1.35	0.93	0.79	13.04	0.61	2.45	2.10
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	3.36	0.42	0.15	0.00	0.11	0.00	0.00	0.16	0.00	0.00	0.00	3.23	0.00	4.98	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	5.43	4.37	3.40	1.83	4.71	3.11	1.99	6.92	2.77	2.89	1.92	3.14	4.44	4.91	3.24	2.35
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.27	0.16	0.00	0.26	0.26	0.00	0.18	0.23	0.00	0.10	0.00	0.14	0.00	0.20	0.14	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	6.19	3.76	2.94	1.44	1.84	1.66	3.90	4.41	2.10	3.66	4.48	5.08	1.72	4.68	5.09	2.98
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	0.46	0.48	0.64	0.53	0.78	1.03	0.48	0.64	0.54	0.46	0.29	0.36	1.08	0.87	0.63	0.55
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.09	0.00	0.24	0.67	0.31	0.52	0.33	0.00	0.00	0.00	0.00	1.46	0.33	0.46	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	0.26	0.15	0.15	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.14	0.00	0.00	0.00	0.14
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.41	0.32	0.35	0.57	0.88	0.11	0.97	1.83	0.20	0.14	0.36	0.17	0.13	0.10	0.85	0.17
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.40	0.62	0.00	0.14	0.47	0.11	1.27	0.67	0.71	0.59	0.30	1.01	0.00	0.23	0.50	0.78

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	17.11	13.08	14.85	26.57	23.69	13.60	25.63	20.23	32.75	24.46	31.33	17.89	10.77	16.68	37.34	16.45
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.10	0.11	0.14	0.00	0.00	0.00	0.00	0.00	0.16	0.13	0.00	0.00	0.00	0.00	0.00	0.13
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.10	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.23	0.00	0.00	0.00
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	8.61	4.25	5.23	11.05	4.44	2.88	4.52	8.02	7.20	6.50	11.10	10.97	3.59	3.70	9.53	8.33
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.00	0.00	0.00	0.21	0.11	0.30	0.11	0.26	0.00	0.00	0.00	0.00	0.27	0.16	0.19	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.18	0.30	0.42	0.48	1.10	0.71	0.64	0.65	0.00	0.00	1.38	0.19	0.58	0.64	1.38	0.17
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	0.52	0.65	1.53	0.00	0.00	0.11	0.00	0.00	0.58	1.65	0.52	0.67	0.00	0.00	0.00	0.56
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	1.12	0.84	1.10	2.33	4.74	1.66	6.22	3.65	2.30	1.50	1.18	0.87	1.94	3.82	3.35	2.69
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.00	0.00	0.00	0.00	0.20	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.21	0.25	0.00	0.11	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.67	0.31	0.26	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	1.73	1.92	1.87	2.93	2.69	3.09	3.47	3.93	2.62	1.40	1.64	1.44	3.24	2.90	1.30	2.34
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.21	0.26	0.38	0.32	0.24	0.41	0.20	0.00	0.31	0.34	0.24	0.40	0.40	0.27	0.00	0.33
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.12	0.00	0.12	0.12	0.13	0.17	0.00	0.00	0.00	0.00	0.00	0.15	0.30	0.27	0.00	0.10
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	2.95	3.87	3.25	2.30	4.32	3.56	4.75	3.31	4.19	2.54	2.93	3.22	3.44	5.76	4.12	4.11

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	2.77	2.03	2.15	1.18	3.22	1.79	4.30	3.52	2.02	3.85	2.66	2.94	2.34	3.38	2.52	2.45
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.33	0.27	0.13	0.00	0.16	0.14	0.36	0.30	0.29	0.24	0.16	0.26	0.16	0.25	0.00	0.21
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.00	0.18	0.00	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.16	0.00	0.00	0.00	0.00	0.11	0.13	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	0.24	1.14	0.99	2.04	1.37	2.99	0.92	0.85	0.66	0.41	0.44	0.11	2.74	1.03	0.83	1.54
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.26	0.17	0.14	0.00	0.16	0.20	0.00	0.14	0.00	0.00	0.00	0.18	0.23	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.29	0.26	0.27	0.12	0.14	0.00	0.42	0.38	0.36	0.50	0.30	0.33	0.00	0.17	0.00	0.42
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	8.14	9.71	6.78	4.72	5.49	5.27	6.49	3.54	6.93	3.39	3.83	7.11	5.59	8.13	6.20	5.60
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.13	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.13	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.00	0.13	0.00	0.13	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.12	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	0.37	4.57	8.97	8.82	2.86	7.24	1.45	1.31	1.02	0.80	3.00	0.99	6.34	1.54	1.49	2.63
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	0.30	0.00	0.00	0.81	0.35	0.00	0.76	0.28	1.21	0.82	0.00	0.76	0.00	0.91	0.34	1.53
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	1.78	3.07	2.82	0.11	0.19	0.34	0.61	0.61	2.05	4.58	2.14	1.96	0.76	0.21	0.35	2.41
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.51	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.27	0.00	0.00	0.30	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.18	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.11	0.11	0.11	0.39	0.71	0.46	0.71	0.17	0.15	0.11	0.19	0.00	0.75	0.88	0.34	0.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	0.00	0.15	0.13	2.31	0.00	0.00	0.00	0.00	0.35	1.06	0.00	0.20	0.00	0.00	0.00	2.27
Bacteria	Tenericutes	NA	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	1.10	0.00	0.00	1.30	0.00	0.00	0.42	0.25	0.00	0.41	0.00	0.12
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	3.21	2.42	2.45	3.07	4.02	2.96	6.36	6.69	4.36	3.10	1.98	4.30	4.18	6.23	2.62	3.86
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.39	0.54	0.44	0.35	0.44	0.49	0.40	0.45	0.31	0.31	0.25	0.41	0.64	0.44	0.30	0.34
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	0.30	0.43	0.60	0.17	0.00	0.22	0.19	0.00	0.32	1.64	0.45	0.37	0.25	0.40	0.16	0.33
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.15	0.00	0.00	0.00	0.00	0.00	0.27	0.00	0.24	0.19	0.15	0.00	0.15	0.15	0.16	0.15
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.00	0.00	0.14	0.00	0.00	0.12	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.11	0.19	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.00	0.16	0.16	0.00	0.21	0.16	0.15	0.25	0.00	0.00	0.00	0.13	0.24	0.18	0.13	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.10	0.00	0.00	0.21	0.16	0.26	0.00	0.00	0.00	0.15	0.00	0.11	0.14	0.00	0.00	0.11
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.67	0.76	0.25	0.33	0.17	0.97	0.27	0.20	0.34	0.25	0.29	0.42	0.50	0.59	0.32	0.30
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.12	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	1.18	1.20	1.28	1.40	1.14	1.93	0.91	0.90	0.87	1.02	0.94	0.75	1.29	1.18	1.26	1.16
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.12	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.13	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	0.22	0.18	0.64	0.13	0.11	0.00	0.38	0.15	0.25	0.48	0.91	0.54	0.14	0.00	0.00	0.48

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.15	0.15	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.00	0.12	0.19	0.00	0.00	0.00	0.11	0.00	0.13	0.11	0.00	0.00	0.00	0.11	0.00	0.15
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.11	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.09
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	0.64	0.43	0.26	0.53	0.24	0.82	0.12	0.16	0.19	0.00	0.15	0.17	0.71	0.33	0.16	0.16
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	Unknown_Family	Unknown_Genus	0.64	0.67	0.66	0.21	0.41	0.68	0.19	0.31	0.25	0.32	0.12	0.37	0.72	0.44	0.36	0.27
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	1.43	0.75	0.72	0.42	0.30	0.72	0.54	0.50	0.78	0.57	0.55	0.51	0.82	0.77	0.33	0.58
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	0.87	0.71	0.92	0.79	0.59	0.51	1.22	0.56	0.98	1.35	1.40	0.68	0.49	0.87	0.87	1.15
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.16	0.11	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.20	0.00	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.47	0.22	0.33	0.18	0.18	0.18	0.00	0.13	0.14	0.00	0.00	0.38	0.34	0.19	0.18	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.13	0.11	0.00	0.29	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.21	0.13	0.17	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.49	0.00	0.10	0.00	0.12	0.00	0.00	0.27	0.00	0.00	0.98	0.53	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.12	0.14	0.00	0.21	0.39	0.50	0.56	0.82	0.00	0.00	0.00	0.00	0.68	0.91	0.46	0.00

Domain	Phylum	Class	Order	Family	Genus	25HISA_a	25HISA_b	25HISA_c	25HISA_d	25HI_a	25HI_b	25HI_c	25HI_d	25LISA_a	25LISA_b	25LISA_c	25LISA_d	25LI_a	25LI_b	25LI_c	25LI_d
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.14	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.14	0.00	0.55	0.00	0.00	0.00	0.00	0.00	0.27	0.37	0.00	0.94	0.00	0.49	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.30	0.00	0.00	0.00	0.00	0.00	0.21	0.11	0.55	0.00	0.23	0.22	0.00	0.16	0.00	0.65
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	0.72	0.58	0.88	0.16	0.88	0.61	0.32	1.30	0.58	0.72	0.50	0.72	1.19	0.25	0.43	0.69
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.26	0.20	0.37	0.18	0.21	0.26	0.31	0.33	0.23	0.44	0.36	0.48	0.57	0.46	0.26	0.28
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.46	0.15	0.48	0.10	0.00	0.33	0.00	0.00	0.20	0.45	0.32	0.21	0.39	0.53	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.16	0.17	0.19	0.16	0.13	0.19	0.18	0.20	0.13	0.12	0.00	0.15	0.27	0.18	0.00	0.14
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.25	0.39	0.37	0.00	0.18	0.30	0.45	0.52	0.34	0.39	0.31	0.29	0.44	0.26	0.00	0.40
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.50	0.62	0.80	0.76	1.18	1.47	0.97	1.18	0.55	0.58	0.41	0.62	1.63	0.96	0.60	0.71
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.13	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary Table S2.8. Relative abundances of observed genera (%) in samples taken from the solid phase during late time point with treatments including 35% dry matter concentration and different wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	0.23	0.13	0.13	0.17	0.34	0.30	0.16	0.25	0.35	0.12	0.19	0.27	0.15	0.14	0.13	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	0.65	0.39	0.25	0.37	0.00	0.50	0.15	0.97	0.23	0.21	0.56	0.40	0.00	0.00	0.42	0.00
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	1.72	1.49	1.21	1.78	0.00	1.33	1.44	2.07	2.86	0.70	1.08	1.46	0.00	0.92	1.45	0.76
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	Candidatus_Methanomethylophilus	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Archaea	Euryarchaeota	Thermoplasmata	Thermoplasmatales	Thermoplasmatales_Incertae_Sedis	uncultured	0.26	0.34	0.35	0.48	0.46	1.21	0.79	0.50	1.06	0.36	0.73	0.31	0.57	0.70	0.38	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Unknown_Genus	0.62	0.20	0.16	0.24	0.20	0.35	0.21	0.25	0.11	0.26	0.30	0.16	0.24	0.22	0.51	0.11
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_1	5.17	7.01	4.72	4.06	9.62	5.11	4.86	4.47	3.99	5.05	4.10	7.89	4.28	7.09	5.33	13.36
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_7	4.52	1.02	4.21	0.88	0.79	1.28	1.05	0.91	0.81	4.21	6.92	1.26	2.53	1.05	2.44	8.34
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_YAB2003_group	4.07	8.12	4.41	0.67	1.47	1.37	1.28	1.35	0.58	3.01	3.59	6.46	1.00	1.00	2.95	3.94
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	Unknown_Genus	1.16	0.92	0.91	0.26	0.70	1.06	0.28	0.50	0.36	0.60	1.06	0.64	0.54	0.41	1.12	0.10
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_rumen_bacterium	0.98	0.38	0.51	0.89	0.16	0.72	0.58	1.63	1.50	0.20	0.65	0.71	0.18	0.46	0.98	0.63
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	Unknown_Genus	1.98	1.24	2.36	3.54	1.87	2.53	1.22	1.78	0.86	2.18	3.50	1.30	3.51	0.69	0.92	0.34
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_BS11_gut_group	uncultured_bacterium	0.00	0.00	0.00	0.26	0.00	0.21	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidetes_BD2-2	Unknown_Order	Unknown_Family	Unknown_Genus	0.19	0.17	0.22	0.37	0.22	0.20	0.00	0.00	0.17	0.23	0.28	0.11	0.26	0.00	0.50	0.14
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	PeH15	uncultured_rumen_bacterium	0.24	0.00	0.00	0.25	0.15	0.40	0.96	0.37	1.28	0.37	0.00	0.13	0.21	0.78	0.64	0.45
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_bacterium	6.21	0.56	6.90	2.81	0.28	13.60	2.30	8.16	2.50	2.51	5.90	0.54	9.95	4.04	2.33	1.25
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_S24-7_group	uncultured_rumen_bacterium	0.00	1.04	0.00	0.00	3.55	0.00	0.00	0.00	0.00	0.00	0.00	1.31	0.00	0.13	0.18	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group	4.23	4.23	2.93	3.46	3.67	2.62	5.06	2.43	5.51	3.80	2.01	4.11	1.16	4.83	3.58	3.05
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001	0.00	0.15	0.00	0.22	0.40	0.27	0.29	0.00	0.20	0.12	0.14	0.14	0.14	0.21	0.13	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_Ga6A1_group	4.35	6.35	4.19	1.43	8.20	4.20	7.03	2.80	3.15	4.36	2.95	8.84	1.41	5.88	6.87	4.63
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-004	0.37	0.26	0.15	1.26	0.34	0.69	0.47	0.60	0.83	0.51	0.30	0.16	0.29	0.27	0.53	0.25

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_Incertae_Sedis	Phocaeicola	0.00	0.00	0.00	0.75	0.41	1.02	0.20	1.34	0.44	0.00	0.00	0.00	1.52	0.25	0.16	0.27
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UCG-001	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.14	0.00	0.00	0.17	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_RF16_group	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Unknown_Genus	0.00	0.00	0.00	0.16	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Unknown_Family	Unknown_Genus	0.24	0.00	0.32	0.66	0.14	0.50	1.07	0.16	1.18	0.19	0.38	0.11	1.04	1.99	0.31	0.16
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Unknown_Genus	0.00	0.56	0.00	0.57	0.85	0.00	0.51	0.00	0.31	0.42	0.00	0.14	0.00	0.48	0.70	0.22
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema_2	15.33	22.03	17.22	22.18	20.42	14.18	23.04	10.98	12.48	28.74	12.41	19.18	9.70	20.66	16.27	22.64
Bacteria	SR1_(Absconditabacteria)	uncultured_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	SR1_(Absconditabacteria)	uncultured_rumen_bacterium	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_I	Unknown_Family	Candidatus_Endomicrobium	0.19	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.39	0.13	0.00	0.00	0.13	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Lineage_IV	uncultured_bacterium	Unknown_Genus	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	CPla-4_termite_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	p-1088-a5_gut_group	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	Unknown_Genus	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00
Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	3.18	8.13	7.21	3.28	4.95	2.34	5.03	1.17	3.73	10.60	5.98	8.02	6.59	2.77	12.15	3.05
Bacteria	Verrucomicrobia	Opitutae	Opitutae_vadinHA64	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Synergistes	0.13	0.00	0.00	0.18	0.17	0.36	0.17	0.94	0.19	0.26	0.17	0.22	0.30	0.31	0.00	0.11
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Anaerobiospirillum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio	0.21	0.00	0.25	0.46	0.56	0.86	0.76	1.43	0.80	0.20	0.25	0.19	1.00	0.70	0.61	0.26
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	1.86	1.13	1.35	0.00	0.20	0.00	0.00	0.00	0.00	1.04	1.78	1.33	0.12	0.00	0.43	1.95

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK4A136_group	1.16	0.89	1.31	3.97	2.12	1.95	4.09	3.49	6.54	0.88	1.67	0.81	4.38	4.69	2.36	1.11
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-009	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00	0.13	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_FCS020_group	0.00	0.10	0.00	0.00	0.28	0.18	0.00	0.00	0.40	0.00	0.00	0.00	0.36	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown_Genus	1.16	0.80	1.65	5.17	2.10	2.75	2.53	2.77	3.82	0.89	1.92	0.80	3.75	2.08	1.91	0.74
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_cellulosolvens_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	0.29	0.29	0.25	0.23	0.25	0.19	0.21	0.28	0.31	0.17	0.38	0.17	0.22	0.14	0.20	0.46
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_1	0.00	0.00	0.00	0.15	0.00	0.19	0.00	0.38	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	2.39	2.27	2.56	3.37	4.20	2.91	4.30	4.83	4.73	1.85	1.53	2.66	3.37	4.17	4.85	3.01
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrivibrio	2.37	3.92	2.91	3.35	5.48	3.40	4.74	5.55	3.13	2.00	1.78	3.53	5.00	5.71	3.42	3.41
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_oxidoreducens_group	0.32	0.36	0.41	0.17	0.31	0.18	0.34	0.38	0.17	0.39	0.18	0.57	0.17	0.26	0.30	0.31
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	0.00	0.00	0.18	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-001	0.00	0.00	0.00	0.00	0.75	0.19	0.12	0.25	0.00	0.00	0.00	0.22	0.00	0.20	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes	0.12	0.00	0.00	0.00	0.00	0.62	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ruminantium_group	1.13	0.26	0.56	1.18	0.40	1.39	0.95	1.66	1.92	0.26	0.57	0.11	1.03	0.98	1.02	0.86
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_10	0.20	0.11	0.18	0.00	0.00	0.16	0.00	0.00	0.12	0.00	0.29	0.17	0.00	0.00	0.19	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_ventriosum_group	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.12	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Bacteroides]_pectinophilus_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_1	0.64	0.51	0.57	0.18	0.38	0.00	0.31	0.49	0.24	0.41	0.69	0.47	0.45	0.51	0.40	0.84
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio_2	7.89	5.60	5.42	6.35	6.04	3.98	6.30	4.16	9.89	4.17	4.54	5.34	8.21	6.11	5.44	2.69
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]_gauvreauii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured_bacterium	0.00	0.00	0.00	0.15	0.00	0.00	0.14	0.00	0.18	0.00	0.00	0.00	0.15	0.12	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	0.13	0.13	0.00	0.15	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira	3.84	0.88	5.93	1.19	1.28	8.53	1.34	10.76	1.05	0.52	6.47	0.46	9.66	2.70	1.33	1.51
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_eligens_group	0.00	0.24	0.00	0.85	0.36	0.00	0.21	0.00	0.19	0.00	0.00	0.15	0.00	0.45	0.30	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_NK3A20_group	6.40	2.90	4.18	0.52	0.55	0.82	0.64	0.87	0.24	3.65	6.10	2.39	0.71	0.54	1.81	3.38
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	0.40	0.57	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.50	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UCG-008	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable_genus_10	0.00	0.27	0.18	0.00	0.00	0.24	0.22	0.00	0.29	0.00	0.00	0.00	0.36	0.19	0.24	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_ND3007_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_AC2044_group	0.00	0.00	0.00	1.46	0.20	0.37	0.43	0.57	0.54	0.12	0.11	0.00	0.28	0.14	0.15	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-014	0.22	0.32	0.31	0.26	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.34	0.00	0.00	0.00	0.19
Bacteria	Tenericutes	NA	Unknown_Order	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_6	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.69	0.00	0.00	0.00	0.00	0.24	0.39	0.13
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.20	0.13	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1	3.45	2.65	2.60	7.25	5.14	4.17	6.67	5.90	5.96	1.77	5.16	2.22	7.81	6.32	2.33	2.03
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium]_hallii_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_NK4A214_group	0.38	0.36	0.28	0.54	0.35	0.39	0.38	0.31	0.51	0.24	0.45	0.34	0.39	0.34	0.31	0.24
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas_1	1.34	1.10	1.47	0.13	0.23	0.53	0.00	0.71	0.00	1.03	1.55	1.57	0.34	0.13	0.40	1.69
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	0.00	0.00	0.15	0.16	0.14	0.15	0.14	0.18	0.00	0.00	0.00	0.17	0.17	0.22	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium]_coprostanoligenes_group	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-010	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-002	0.00	0.00	0.15	0.00	0.13	0.14	0.16	0.15	0.15	0.14	0.00	0.00	0.31	0.34	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-005	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-004	0.15	0.00	0.14	0.15	0.16	0.14	0.15	0.12	0.19	0.00	0.27	0.00	0.22	0.18	0.14	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-004	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.13	0.00	0.00	0.00	0.00	0.34	0.14
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae_UCG-001	0.67	0.48	0.32	0.42	0.53	0.50	0.18	1.05	0.49	0.42	0.60	0.73	0.11	0.24	0.61	0.18

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Clostridia	Halanaerobiales	ODP1230B8.23	uncultured_bacterium	0.00	0.00	0.13	0.21	0.00	0.00	0.00	0.00	0.15	0.11	0.11	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.27	0.00	0.00	0.00	0.60
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	1.32	1.12	1.13	2.57	0.95	1.33	0.78	1.85	1.89	1.84	1.50	1.78	0.75	0.79	1.48	0.91
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.26	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_organism	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Armatimonadetes	uncultured	uncultured_bacterium	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Olsenella	0.26	0.74	0.42	0.16	0.38	0.10	0.33	0.23	0.00	0.27	0.70	0.42	0.13	0.11	0.28	1.05
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Senegalimassilia	0.00	0.00	0.00	0.11	0.00	0.00	0.13	0.12	0.23	0.00	0.00	0.00	0.00	0.13	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	0.15	0.13	0.13	0.11	0.12	0.10	0.00	0.32	0.00	0.15	0.24	0.14	0.00	0.12	0.18	0.15
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured_rumen_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.19	0.00	0.00	0.00	0.00	0.00
Bacteria	Verrucomicrobia	OPB35_soil_group	Unknown_Order	Unknown_Family	Unknown_Genus	0.29	0.13	0.12	0.37	0.17	0.67	0.18	0.42	0.37	0.31	0.28	0.28	0.29	0.15	0.33	0.11
Bacteria	Verrucomicrobia	WCHB1-41	uncultured_rumen_bacterium	Unknown_Family	Unknown_Genus	0.56	0.39	0.80	0.32	0.92	0.38	0.18	0.26	0.71	0.53	0.60	0.63	0.17	0.17	0.54	0.22
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0.56	0.56	0.51	1.04	0.53	0.97	0.43	0.66	0.55	1.01	0.79	1.03	0.45	0.53	1.03	0.47
Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Candidatus_Soleaferrea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UCG-012	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_Clostridiales_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	Unknown_Genus	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Allisonella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	0.93	1.18	0.99	1.33	1.12	1.05	0.78	1.82	1.04	1.44	0.98	1.75	0.48	1.29	1.58	1.07
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.12	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
Bacteria	Spirochaetae	Spirochaetes	Spirochaetales	PL-11B10	Unknown_Genus	0.35	0.27	0.18	0.11	0.21	0.26	0.16	0.25	0.14	0.14	0.27	0.31	0.24	0.13	0.21	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_Firmicutes_bacterium	Unknown_Genus	0.13	0.16	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_nodatum_group	0.00	0.29	0.00	0.00	0.19	0.00	0.30	0.00	0.20	0.34	0.00	0.18	0.00	0.27	0.62	1.87
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	0.00	0.00	0.13	0.18	0.49	0.93	0.40	1.17	0.42	0.00	0.00	0.12	0.77	0.31	0.24	0.00
Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	NB1-n	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.11	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_rumen_bacterium	Unknown_Genus	0.00	0.00	0.00	0.12	0.13	0.00	0.00	0.00	0.23	0.33	0.00	0.00	0.00	0.00	0.00	0.58
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	uncultured_bacterium	Unknown_Genus	0.18	0.76	0.31	0.45	0.22	0.00	0.00	0.51	0.00	0.00	0.00	0.48	0.00	0.00	0.00	0.00
Bacteria	Tenericutes	Mollicutes	Mollicutes_RF9	Unknown_Family	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_vadinBB60_group	uncultured_bacterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera	0.00	0.45	0.00	0.00	0.23	0.00	0.00	0.00	0.17	0.42	0.00	0.60	0.00	0.14	0.31	0.62
Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_R-7_group	0.68	0.83	1.09	0.82	0.36	1.02	0.80	0.15	1.13	0.91	1.51	0.89	0.54	0.93	0.54	0.78
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0.32	0.62	0.40	0.43	0.49	0.30	0.35	0.23	0.31	0.33	0.32	0.43	0.29	0.43	0.23	0.46
Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.00	0.00	0.21	0.29	0.00	0.00	0.00	0.00	0.49	0.33	0.64	0.00	0.00	0.00	0.00	1.08
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	[Eubacterium]_saphenum_group	0.15	0.00	0.13	0.22	0.17	0.20	0.12	0.18	0.18	0.11	0.17	0.10	0.26	0.16	0.14	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Mogibacterium	0.44	0.34	0.44	0.38	0.38	0.34	0.32	0.43	0.23	0.33	0.51	0.36	0.46	0.35	0.30	0.28
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_AD3011_group	0.26	0.28	0.51	1.29	1.04	0.91	1.12	0.82	1.49	0.17	0.37	0.47	0.87	0.76	0.39	0.17
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Anaerovorax	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Unknown_Genus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Clostridia	Clostridiales	Family_XIII	Family_XIII_UCG-002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Domain	Phylum	Class	Order	Family	Genus	35HISA_a	35LISA_b	35HISA_c	35HISA_d	35HI_a	35HI_b	35HI_c	35HI_d	35LISA_a	35LISA_b	35LISA_c	35LISA_d	35LI_a	35LI_b	35LI_c	35LI_d
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-007	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UCG-009	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary Table S2.9. Unassigned OTUs (%) of total OTUs at given taxonomic level

Domain	0.00%
Phylum	0.00%
Class	0.31%
Order	0.81%
Family	4.58%
Genus	16.38%

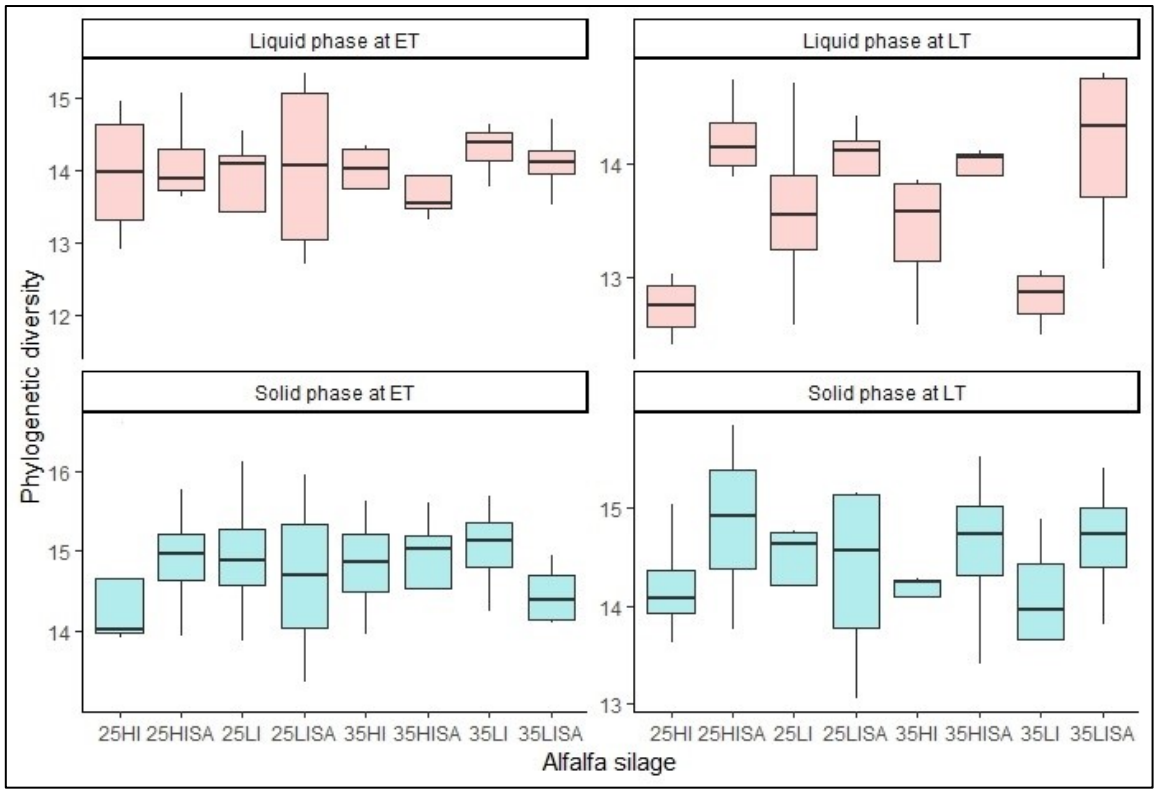
Supplementary Table S2.10. Unassigned OTUs (%) within the phyla.

Phylum	Actino-bacteria	Armatimona-detes	Bacter-oidetes	Chloro-flexi	Cyano-bacteria	Elusi-microbia	Eury-archaeota	Fibro-bacteres	Firmi-cutes	Lenti-sphaerae	Plancto-mycetes	Proteo-bacteria	Spiro-chaetae	SR1_(Abscondita-bacteria)	Syner-gistetes	Teneri-cutes	Verruco-microbia
Class	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	11.54%	0.00%
Order	0.00%	0.00%	0.48%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	11.54%	4.17%
Family	0.00%	0.00%	1.44%	0.00%	33.33%	25.00%	0.00%	0.00%	0.29%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	30.77%	95.83%
Genus	0.00%	100.00%	23.80%	0.00%	33.33%	50.00%	0.00%	0.00%	8.60%	41.67%	0.00%	6.67%	8.97%	100.00%	0.00%	46.15%	100.00%

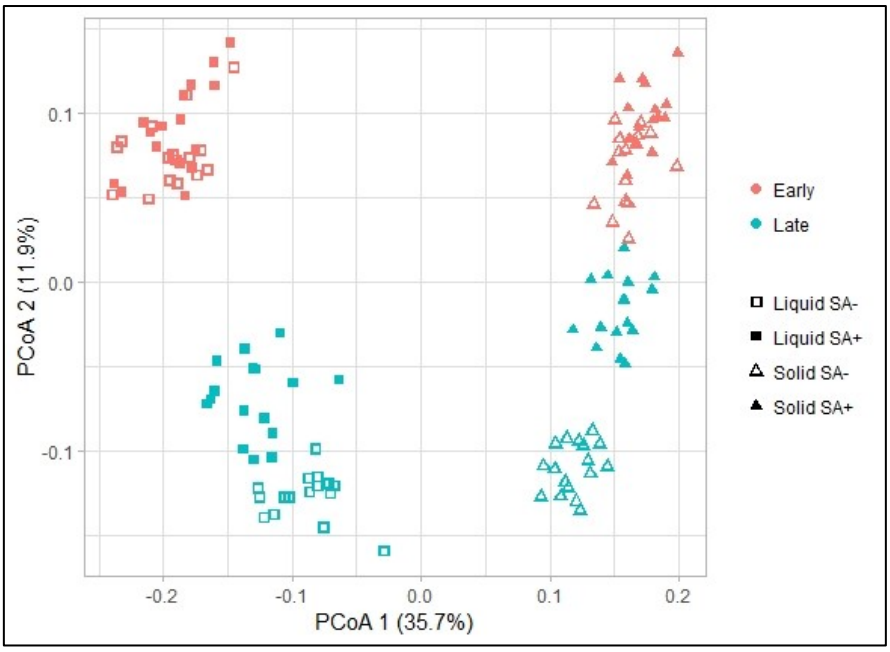
Supplementary Table S3. Determined *P*-values for all tested pre-ensiling treatments[a] and interactions of beta diversity when applying weighted and unweighted UniFrac distance metrics.

			P-value	
Distance metrics	Time point	Treatment	Liquid phase	Solid phase
Weighted UniFrac	Early	DM	0.452	0.706
Weighted UniFrac	Early	WI	0.984	0.848
Weighted UniFrac	Early	SA	0.005	0.011
Weighted UniFrac	Early	DM × WI	0.945	0.912
Weighted UniFrac	Early	DM × SA	0.034	0.093
Weighted UniFrac	Early	WI × SA	0.098	0.185
Weighted UniFrac	Late	DM	0.031	0.139
Weighted UniFrac	Late	WI	0.744	0.561
Weighted UniFrac	Late	SA	0.001	0.001
Weighted UniFrac	Late	DM × WI	0.291	0.459
Weighted UniFrac	Late	DM × SA	0.001	0.001
Weighted UniFrac	Late	WI × SA	0.001	0.001
Unweighted UniFrac	Both	Time point	0.001	0.001
Unweighted UniFrac	Early	DM	0.745	0.099
Unweighted UniFrac	Early	WI	0.940	0.998
Unweighted UniFrac	Early	SA	0.047	0.005
Unweighted UniFrac	Early	DM × WI	0.963	0.841
Unweighted UniFrac	Early	DM × SA	0.294	0.026
Unweighted UniFrac	Early	WI × SA	0.240	0.155
Unweighted UniFrac	Late	DM	0.259	0.027
Unweighted UniFrac	Late	WI	0.692	0.751
Unweighted UniFrac	Late	SA	0.001	0.001
Unweighted UniFrac	Late	DM × WI	0.788	0.304
Unweighted UniFrac	Late	DM × SA	0.001	0.001
Unweighted UniFrac	Late	WI × SA	0.001	0.001
Unweighted UniFrac	Both	Time point	0.001	0.001

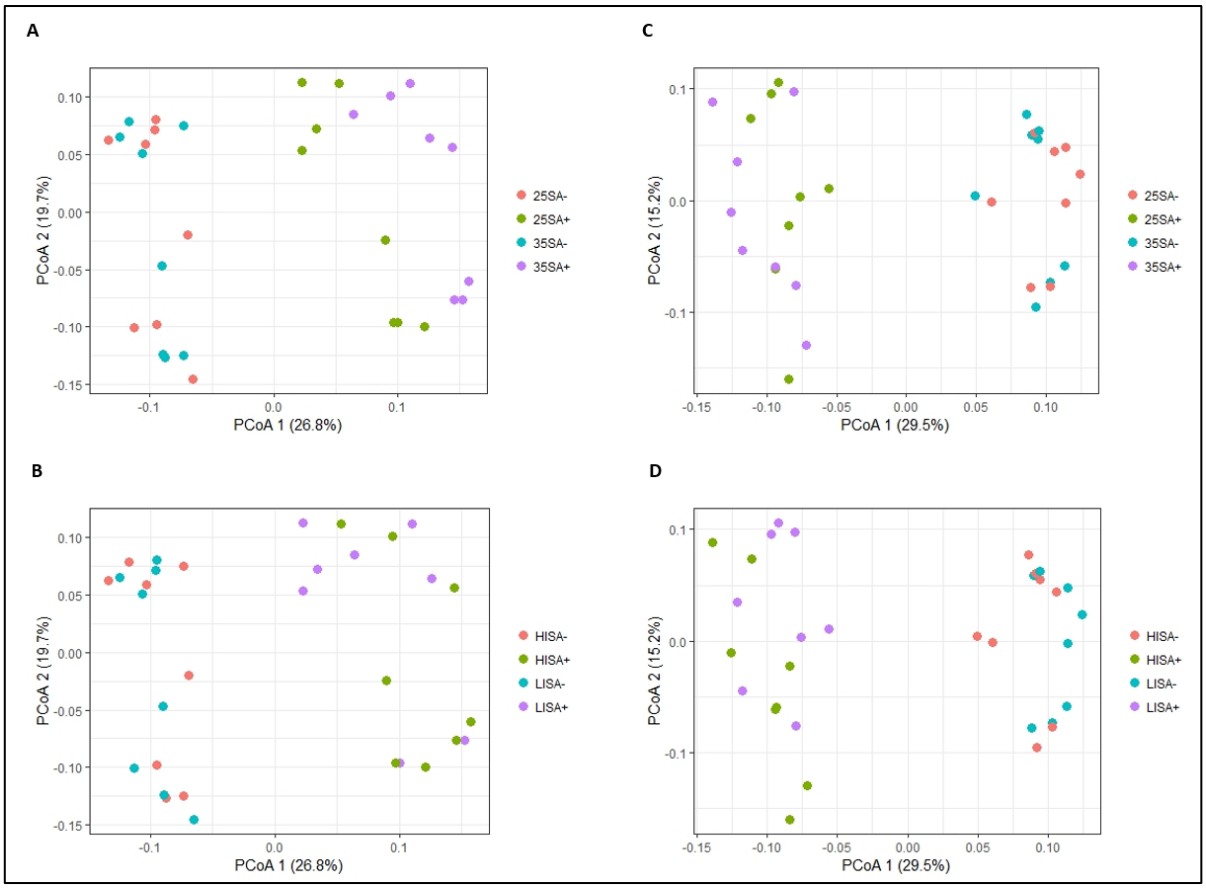
[a]Treatments include different: dry matter (DM) concentrations, i.e. 25 or 35; wilting intensities (WI), i.e. low (LI) or high (HI); and sucrose addition (SA).



Supplementary Figure S1. Alpha diversity calculated with the phylogenetic diversity index for all alfalfa silages in the liquid and solid phase and for the early and late time point, respectively. The boxplots show the 25th, 50th and 75th percentiles, with whiskers showing the extremes of the data. Abbreviations indicate the treatments including different dry matter concentrations (25 or 35), wilting intensities [low (LI) or high (HI)] and sucrose addition (SA).



Supplementary Figure S2. Changes in prokaryotic community composition associated with the time point, phase, and sucrose addition visualized as a principal co-ordinate analysis (PCoA) using unweighted UniFrac distance metrics. Symbol shapes indicate the two phases, i.e., liquid and solid, from which the samples originated, colors indicate the different time points, i.e., early and late, and symbol fillings indicate the sucrose addition, i.e. with (SA+) or without (SA-). The percentage of variation explained is indicated on the respective axes.



Supplementary Figure S3. Changes in prokaryotic community composition associated with the interactions of different pre-ensiling treatments at the late time point, visualized as a principal co-ordinate analysis (PCoA) using unweighted UniFrac distance metrics for A) treatments dry matter (DM) concentration × sucrose addition in liquid phase samples; B) wilting intensity × sucrose addition in liquid phase samples; C) DM concentration × sucrose addition in solid phase samples; D) and wilting intensity × sucrose addition in solid phase samples. Abbreviations indicate the interactions of treatments including different dry matter concentrations (25 or 35), wilting intensities [low (LI) or high (HI)] and sucrose addition [with (SA+) or without (SA-)]. The percentage of variation explained is indicated on the respective axes.

CHAPTER 7

General discussion and conclusions

In the following, discussion key points from Chapters 3 to 6 are debated in an overall context considering protein preservation of lucerne at ensiling and the subsequent effects on *in vitro* rumen fermentation and microbiota composition. To assess the ruminal fermentation in this thesis, lucerne silages were not studied *in vivo*, but *in vitro* using the rumen-simulation technique (Rusitec) system from Czerkawski and Breckenridge (1977). Therefore, the general discussion will also cover extended aspects of feedstuff evaluation by *in vitro* approaches. Finally, an outlook addressing new aspects and possible trends of lucerne ensiling, which in future may promote ruminal N fixation and therefore a more resource-efficient ruminant livestock production, will complement this Chapter.

Ensiling of lucerne

Silages are prone to post-harvesting effects (Higgs et al., 2015) which altogether affect the chemical composition and lead to a great heterogeneity within silages prepared from the same plant species. So far, these effects are widely disregarded (Higgs et al., 2015), but the present research demonstrates that the targeted design of pre-ensiling conditions can beneficially affect the lucerne silage quality and therefore emphasizes the necessity to consider pre-ensiling treatments during silage production.

Wilting in order to increase the dry matter (DM) concentration of lucerne is a common tool to improve silage quality (Kung and Shaver, 2001; Zheng et al., 2017) and further reduces the formation of environmentally harmful silage effluent. The wilting intensity, however, has not yet received much attention and the present work is among the first systematically investigating the effects of different wilting intensities on lucerne silage quality, especially nitrogenous compound (NC) composition. The lower non-protein nitrogen (NPN) concentrations in high-intensity wilted silages confirm the expected true protein (TP) preserving effect, probably by limiting the activity of water-dependent plant-derived proteases (Hoedtke et al., 2010). However, these enzymes were most likely not fully deactivated as the maximum moisture loss was only to 35% DM concentration. Likewise, pH drop in response to sucrose addition has decreased the microbial proteolytic activity (Muck, 1988; Li et al., 2018), but plant-derived proteases remain active under acidic conditions (Heron et al., 1989; Tao et al., 2012). This explains the lower, but still substantial conversion of TP to NPN in the present high-intensity wilted lucerne silages, either with or without sucrose addition. Thus,

refining the concept of high-intensity wilting treatments may increase TP preservation in lucerne silages.

Tannins are phenolic secondary plant metabolites that bind to proteins at pH 3.5–7.0, making them insoluble and less exposed to proteolytic enzymes (Jones and Mangan, 1977). Thus, condensed and hydrolysable tannins of several plants have widely been tested as silage additives and were shown to cause lower ammonia and NPN concentrations as well as reduced protease activities in silages (Salawu et al., 1999b; Tabacco et al., 2006; Li et al., 2018). However, feeding tannin-rich rations is characterized by lower feed intakes (Vasta et al., 2010), which can be ascribed to reduced palatability and lower ruminal degradation rates (Makkar et al., 1995), consequently bearing the risk of insufficient energy and nutrient provision. Moreover, poor intestinal utilization of by-pass protein from tannin-rich legumes is well documented (Salawu et al., 1999a) and likely the consequence of post-ruminally tannin-binding to proteins and host enzymes (Jones and Mangan, 1977).

So in terms of TP preservation, high tannin concentrations are most effective (Li et al., 2018), whereas the risk of reduced feed intake and impaired digestion contradicts the use of high dosages. Therefore, a promising approach to investigate may be the combination of high-intensity wilting with tannin addition before ensiling, because intensive wilting rapidly decelerates, but not completely deactivates proteolytic processes and by adding tannins, the remaining active proteases may be hindered from further breaking down proteins in lucerne silages. Hence, when combining both treatments, the tannin input may be reduced to a level that does not pose the risk of decreased feed intake and digestibility, but still provides increased amounts of TP to the ruminant. In this regard, it is noteworthy that hydrolysable tannins and their metabolites were occasionally found to be toxic to ruminants (Makkar, 2003), which thus may favour the application of condensed tannins to high-intensity wilted lucerne material. The additional inclusion of a water-soluble carbohydrate (WSC) source may still be advisable as high-intensity wilting or tannins themselves will only improve TP preservation, but not the often observed poor lactic acid fermentation in lucerne silages (Salawu et al., 1999b; Li et al., 2018) that was also found for non-sucrose-treated silages in the present research.

For preparing the lucerne silages as reported in this thesis, high wilting intensity was achieved by exposing the plant material to high solar radiation on black plastic. However, weather conditions during harvest may not always apply to this and in this case, maceration of lucerne may help to compensate and still maintain high wilting rates (Savoie, 2001), particularly as forages with large stems respond best to mechanical treatments (Rotz, 1995). Besides the rapid dehydration, maceration breaks the tubular hollow lucerne stems, which otherwise can impede the complete removal of air during ensiling (McAllister et al., 1998).

Thereby, the quicker establishment of an anaerobic environment should be facilitated, also due to an easier compaction of lucerne material in the silos (Savoie, 2001). On the other hand, maceration could counteract the initial idea of preserving TP by rapid inactivation of water-dependent plant proteases. In plant cells, proteases are mainly present in the vacuoles (Callis, 1995), whereas proteins, predominantly Rubisco, are mainly located in chloroplasts (Jones and Mangan, 1977). When the plant cells are ruptured by maceration, the proteases will instantly be mixed with the proteins and therefore a faster proteolysis may take place, which potentially outweigh the beneficial effect of maceration on wilting rate and instead favour TP degradation before ensiling. The aspect of maceration was not included in the present research, but could be done in future to investigate whether it supports high-intensity wilting and in particular, how maceration impacts proteolytic processes before and during ensiling of lucerne.

Assessing the effectiveness of pre-ensiling treatments, with and without maceration, for manipulating lucerne silage quality in future requires the most closely estimation of the silages' chemical composition. Various chemical methods have been developed and refined to most exactly estimate the quality, quantity and ruminal degradability of feedstuff components (Van Soest et al., 1991; Sniffen et al., 1992; Licitra et al., 1996), which is critical to best predict the energy and nutrients available for the host (Higgs et al., 2015). However, uncertainties do remain, e.g. varying ruminal ingesta passage rates (Steingaß and Südekum, 2013), emphasizing that there is still a need to specify the chemical characterization of feedstuffs, particularly the crude protein (CP) fraction.

A commonly used procedure to characterize the CP is the CP fractionation according to the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992), which was also applied for characterizing the present lucerne silages. Thereby, this method partitions the CP into five fractions in terms of their solubility in different chemical solutions, providing indications on the expected extent and rate of ruminal degradation. So far, fraction A, which comprises NPN, is typically assumed to be rapidly and completely degraded in the rumen (Sniffen et al., 1992; Lanzas et al., 2007). However, Choi et al. (2002) suggested that 5-10% of amino acids (AA) flowing to the small intestine were of dietary origin and consequently must have escaped ruminal degradation. Correspondingly, the assumption that NC of fraction A are completely degraded in the rumen is not correct and the ruminal passage rate may be a key factor determining the extent of ruminal NPN degradation (Steingaß and Südekum, 2013). With respect to the observation of Choi et al. (2002), a recent approach from Higgs et al. (2015) revised the CP fractionation of the CNCPS (Sniffen et al., 1992) by separating fraction A into fractions A1 and A2 containing ammonia-nitrogen (N) as well as AA

and peptides, respectively. Thus, this procedure allows an improved estimation of the rumen N balance and the supply with metabolizable protein (Higgs et al., 2015).

As found in the present work, NPN typically comprises largest proportion of total CP in lucerne silages (Broderick, 1995; Guo et al., 2008; Wyss et al., 2017), irrespectively of applied treatment, and a more distinct assessment of this diverse fraction may improve the estimation of the protein value of lucerne silages. Regarding the present data as an example, wilting intensity affected NPN with lower proportions in high-intensity wilted silages, whereas the ammonia-N concentration was not influenced (Chapter 4). Consequently, wilting intensity seemed to affect silages' AA and peptide concentrations, but not ammonia-N and these differences would not have been observed when only 'classical' CP fractionation (Sniffen et al., 1992) had been applied.

Ruminal fermentation of lucerne silages and microbiota composition

When investigating ruminal fermentation of forages, the majority of studies applies very diverse forages, for instance grass vs. legume (Jaurena et al., 2005; Belanche et al., 2013) or corn silage (Boguhn et al., 2013). Such extreme contrasts will most likely lead to observable differences in rumen fermentation patterns or microbial communities. However, the present research analysed the effects of lucerne silages from the same sward, only prepared with different pre-ensiling treatments, on the ruminal fermentation (Chapter 5) and microbiota composition (Chapter 6) and therefore rather slight variations and smaller differences in fermentation profiles and microbial communities should be expected.

The higher TP preservation achieved by pre-ensiling treatments was expected to decrease ammonia-N concentrations in the Rusitec system. In comparison to other forage-based *in vitro* studies (Jaurena et al., 2005; Boguhn et al., 2013; Copani et al., 2015), the present ammonia-N concentrations were on a very high level, irrespective of applied pre-ensiling treatments. Likewise, isovalerate, which is formed during AA degradation (Carro and Miller, 1999), was also greatly concentrated and therefore indicates a higher abundance and activity of NC-degrading rumen microbes, particularly deaminating species like hyper-ammonia producing bacteria (HAB). Thereby, although the combination of high-intensity wilting to 35% DM and sucrose addition (35HISA) resulted in highest TP preservation, ammonia-N concentrations in the Rusitec system were not reduced. The overall lack of rapidly available energy may be the primary cause (Bach et al., 2005), as NPN from lucerne silages was rapidly degraded to ammonia-N, which subsequently could not be utilized for microbial protein synthesis due to the poor energy supply. Likewise, this phenomenon may still apply to 35HISA, where a decelerated proteolysis and consequently slower ammonia release

should have taken place. Besides, the scarce provision of dietary energy may have contributed to the low microbial CP formation that was observed during the Rusitec incubations – although the in Chapter 5 discussed methodical limitations of the here applied microbial CP determination may be the primary reason.

Although not statistically significant, ammonia-N concentrations were numerically lowest at early adaptation time point and among the lowest at late adaptation time point during 35HISA incubation, therefore suggesting that the simultaneous incubation with an appropriate WSC source should result in a more efficient ruminal N utilization. Thereby, the obtained beneficial effects of high-intensity wilting and sucrose addition on lucerne silage composition, namely lower proportions of NPN and ammonia-N and higher lactic acid concentrations, may be more reflected in the rumen fermentation than observed in the present research so far. Likewise, an inclusion of concentrate should decrease the presence of HAB species (Rychlik and Russell, 2000) as it may outweigh the HAB's advantage to yield energy from AA degradation. However, whether this leads to a reduction of total HAB species is questionable as 'new' HAB species (Bento et al., 2015), which are introduced in Chapter 3, can shift their metabolism from AA to carbohydrates and thus stay present in the rumen. So when the host's diet again changes to high provision of NPN along with less readily available energy, a return to enhanced deaminating activity and ultimately high N losses may be the consequence.

For the present *in vitro* research, lucerne silages were incubated solely to study the effects of the applied pre-ensiling treatments on ruminal fermentation characteristics. Based on these findings, the combined incubation of high-intensity wilted lucerne silages, with or without additional treatments, and concentrate may represent a good link for the continuation. Thereby, this may also help to transfer the here obtained knowledge closer to practical conditions, where forage legumes like lucerne silages are combined with other forages and concentrates to meet the animal's energy and nutrient requirements.

The Rusitec system allows a risk-free simulation of high NPN supply to the rumen, which otherwise could result in toxicity under *in vivo* conditions (Helmer and Bartley, 1971; Webb et al., 1972). Webb et al. (1972) defined blood ammonia-N concentrations exceeding 0.7-0.8 mg/100 mL as a rough threshold for ammonia poisoning, but also mentioned a notable animal-individual tolerance towards blood ammonia-N levels. As the ammonia-N concentrations of the rumen and blood are correlated, an estimate of blood ammonia-N concentration can be calculated using the equation (coefficient of determination $R^2 = 0.724$) from Webb et al. (1972):

$$y = -3.6366 + 0.57605 \times pH_{rumen} + 0.00568 \times \text{ammonia-N}_{rumen} - 0.0000362 \times \text{ammonia-N}_{rumen}^2,$$

where y is the blood ammonia-N concentration (mg/100mL), pH_{rumen} is the rumen pH and ammonia-N_{rumen} is the ruminal ammonia-N concentration (mg/100mL). Applying this equation on the average ammonia-N concentration and pH of the present data, the estimated blood ammonia-N concentration would amount for 0.44 mg/100 mL and thus obviously below the critical threshold of Webb et al. (1972). Hereby, three points should be noted, which may limit the meaningfulness of this value. First, this equation was developed to estimate blood ammonia-N levels after intra-ruminal urea administration and may therefore not be fully applicable to the present *in vitro* lucerne silage situation. Secondly, the ratio between the liquid and the solid phase is much smaller *in vivo* than in the Rusitec, which should affect the ammonia-N distribution and consequently its concentration. And thirdly, metabolites are removed from the Rusitec vessels only by dilution and the present dilution rate was rather low. Thus, the here observed ammonia-N concentrations may likely have been higher than *in vivo* – where ammonia-N is additionally absorbed from the rumen – and thereby biases the numbers that are inserted in the equation from Webb et al. (1972).

Despite the variety of fermentation characteristics that can be monitored with the Rusitec model, the important factor of feed intake cannot be assessed. Therefore, the present project also included a preference trial with ten male goats in order to analyse the animals' feed intake and preference over ten days (Schmit, 2017). Two of the three applied pre-ensiling treatments showed an effect on the goats' feed intake being higher for 35% DM lucerne silages than for 25% DM. Likewise, sucrose addition showed an increasing effect on silage consumption, consequently leading to highest feed intakes for 35% DM lucerne silages with sucrose addition, constantly independent of applied wilting intensity that did not influence preference of the goats.

An increase in feed intake with higher DM concentrations is confirmed by various study results that have already been summarized by Campling (1964). However, not the DM concentration per se leads to higher feed intakes, but the associated differences in silage quality. Thereby, the lower acetic acid concentrations of present 35% DM lucerne silages may be causative for higher feed intakes (Eisner et al., 2006). The missing effect of wilting intensity was not expected as Wright et al. (2000) calculated a positive correlation (R^2 = 0.393) between drying rate and grass DM intake. However, there may be differences for lucerne or legumes in general and further factors superimposing the impact of wilting intensity on feed intake. Despite having higher ester concentrations, which were earlier observed to be negatively correlated with short-term DM intake (Gerlach et al., 2013), present sucrose-added silages resulted in higher feed intake. Probably, the elevated NPN and ammonia-N concentrations in lucerne silages without sucrose addition may have outweighed the influence of ester compounds, as the meta-analysis of Eisner et al. (2006)

calculated a reduction in feed intake of 18 g DM per gram increase in soluble N in total N. Thus, although several silage properties allow an estimation of the expected acceptance by ruminants (Eisner et al., 2006), it appears advisable to extend *in vitro*-based experiments for the aspect of feed intake by including preference trials with animals. Otherwise, important facets of lucerne silage assessment may probably not be taken into consideration, e.g. the higher fibre degradability for 25% DM silages found *in vitro* that needs to be weighed against lower DM intakes observed during preference trials.

Besides the investigation of *in vitro* ruminal fermentation characteristics, also microbiological analyses were part of this thesis. When applying culture-independent microbiological analyses, the DNA extraction method is the first critical step that determines the captured DNA yield and its quality, and therefore the absolute microbial numbers and community coverage in the samples (Henderson et al., 2013). The DNA extraction protocol described in Chapter 6 was chosen for the present research, as it provided the best results in terms of DNA quality and yield for assessing bacteria and archaea, the most abundant domains in the rumen (Henderson et al., 2015). This protocol enabled the extraction of high-molecular weight DNA and subsequent use for downstream applications like the here performed quantitative real-time polymerase chain reaction analysis and 16S rRNA gene amplicon sequencing. As preparatory work, several DNA extraction kits, protocols and modifications were tested with pool samples of the experiment (Table 1). Thereby, all protocols comprised a bead-beating step for mechanical cell disruption as chemical lysis alone will not provide sufficient DNA release (Yu and Morrison, 2004). The emerging DNA extracts were analysed for purity and yield by spectrophotometry and integrity of DNA was checked by agarose gel electrophoresis (Burbach et al., 2015). As the spectrophotometric measurement cannot separate nucleic acids into RNA and DNA, an RNase A treatment was also included to ensure an exact DNA quantification.

Table 1. Excerpt of tested procedures for optimization of DNA extraction using a Rusitec vessel fluid-derived pool sample.

DNA extraction kit (Manufacturer)	Modification	Concentration (ng/µl)[1,2]	Purity[1]		Integrity[4]
			OD[3] 260/280	OD 260/230	
Qiamp Fast DNA Stool Mini Kit (Qiagen)	1.4 mm ceramic beads[5], prolonged elution time, repeated elution	14.8	1.67	3.42	NA[6]
Qiamp Fast DNA Stool Mini Kit (Qiagen)	Two-step centrifugation[7], 1.4 mm ceramic beads, doubled	38.5	1.96	2.00	Partly intact
Fast DNA Spin Kit for Soil (MP Biomedicals)	Two-step centrifugation, 2.8 mm ceramic beads[5], 70°C during prolonged elution	15.6	1.78	0.03	Degraded
Fast DNA Spin Kit for Feces (MP Biomedicals)	Two-step centrifugation, 1.4 mm ceramic beads, 70°C during prolonged elution	33.09	2.26	0.07	Partly intact
PowerFecal DNA Isolation Kit (MO Bio)	Two-step centrifugation, 2.8 mm ceramic beads, doubled lysis time	19.80	1.48	0.79	Intact
PowerViral DNA Isolation Kit (MO Bio)	Two-step centrifugation, 1.4 mm ceramic beads, 50°C during elution, repeated elution	59.3	1.60	1.42	Intact
Precellys Soil DNA Kit (Precellys)	Two-step centrifugation, 1.4 mm ceramic beads	12.2	2.00	0.03	NA
First-DNA all-tissue Kit (Gen-IAL)	Two-step centrifugation, 1.4 mm ceramic beads	74.8	1.91	2.26	Intact
First-DNA all-tissue Kit (Gen-IAL)	Two-step centrifugation, 2.8 mm ceramic beads	71.8	2.02	1.86	Intact

[1]Determined using a NanoDrop 8000 spectrophotometer (NanoDrop® Technologies, Thermo Fisher Scientific, Waltham, MA, USA); [2]80µl elution volume; [3]Optical density; [4]Determined by 1% agarose gel electrophoresis with 70 volts for 80 minutes; [5]Used for bead-beating step; [6]Not assessed; [7]Centrifugation at 800 × *g* and 4°C for 15 min and transfer of supernatant and centrifugation at 21,000 × *g* and 4°C for 40 min.

Without a two-step centrifugation of liquid samples, the obtained DNA yield was low for all DNA extraction modifications (Table 1). Thus, the samples were centrifuged at low speed to separate feed particles and the supernatant was then transferred to a fresh tube and centrifuged again at high speed to obtain a microbial pellet. However, during the first centrifugation step, also protozoa are likely separated (Zebeli et al., 2008) and the here applied DNA extraction did therefore not allow the assessment of the protozoal population. The missing coverage of this domain is a shortage of the present research and modifications in the DNA extraction protocol may be considered in the future, particularly as it could help to elucidate the observed *in vitro* fibre degradability patterns. In this context, however, the Rusitec may have a general deficiency for assessing the total rumen microbiome as protozoal survival was often reported to be impaired in the Rusitec system (Wallace and Newbold, 1991; Martínez et al., 2010). Thus, irrespectively of the here applied DNA extraction procedure, this may be a limitation of this simulation model, especially for runs lasting longer than a few days. However, to counteract the potential decline of protozoa, nylon bags with large pore sizes, i.e. 1000 µm, were selected for the present work to ensure access of protozoa to lucerne silages and the effectiveness of large-pored nylon fabric was already confirmed by Carro et al. (1995). Likewise, the here applied low buffer infusion rate in the Rusitec might favour the preservation of protozoa (Abe and Kumeno, 1973), although others (Carro et al., 1995) report that the buffer infusion rate does not affect protozoal numbers.

The present work identified the pre-ensiling addition of sucrose as well as the adaptation time of the Rusitec system to be the main drivers affecting the microbiota composition in both the liquid and the solid phase. Notwithstanding their lacking impacts on the microbial composition, wilting intensity and DM concentration have very likely influenced the microbiome as well, because several effects of these two treatments on short-chain fatty acid (SCFA) concentrations, daily gas production and degradabilities of fibre fractions and organic matter (OM) were observed. These effects may not or only partly be reflected on composition level, as the majority of microbes can express different metabolic pathways, which they first adapt during challenges with new feedstuffs (Kortman et al., 2016) – for instance, as it is proposed for the ‘new’ HAB in Chapter 3. Consequently, it is conceivable that there is a shift in metabolic pathways with effects on metabolic patterns before changes are observable in the microbiota composition. To this end, additional analyses of the transcriptome or proteome, i.e. a multi-omic approach (Deusch et al., 2015), may be advisable to further assess the microbial activity. However, there is a variety of uncertainties in the handling and processing of samples for RNA and protein extraction (McSweeney et al., 2007; Deusch and Seifert, 2015). Therefore, metagenomics could also be a next step to assess both composition and function, which therefore allows a phylogenetic and functional classification

of the rumen microbiome. In this context, it should be considered that cultivation remains important to validate predicted metabolic capabilities (Creevey et al., 2014). Otherwise, properties that are not predicted from the genome remain unknown or alternatively are predicted, but not expressed (Gutleben et al., 2018).

Application of *in vitro* models to study ruminal fermentation

In vitro approaches may closely resemble the *in vivo* situation, but are inexpensive and less labour- and time-consuming compared to *in vivo* trials (Stern et al., 1994), which makes them very attractive for obtaining comprehensive insights into rumen fermentation by testing many treatments with sufficient replication in a short period of time (Hristov et al., 2012).

For assessing the ruminal fermentation of lucerne silages in the present research, the *in vitro* model Rusitec (Czerkawski and Breckenridge, 1977) was used, which constitutes a widely applied system for characterizing ruminal fermentation patterns of several silage types (Jaurena et al., 2005; Merry et al., 2006; Jalč et al., 2009a; Jalč et al., 2009b; Gresner et al., 2015). The Rusitec is a semi-continuous culture system that simulates the rumen by containing both a liquid and a solid fraction with continuous buffer infusion and a diurnal supply of fresh feedstuffs (Czerkawski and Breckenridge, 1977). Thus, this system is suitable for long-term applications, which is emphasized by the observation that Rusitec fermentation profiles and microbial metabolite syntheses were similar to values found *in vivo* (Czerkawski, 1978). Likewise, the meta-analysis of Hristov et al. (2012) observed higher coefficients of variation and variance for nutrient digestibility and SCFA data from non-Rusitec *in vitro* models compared to Rusitec. Moreover, *in vitro* systems like the Hohenheim gas test (Menke et al., 1979) are hampered by the accumulation of metabolites, which inhibits metabolic processes and viability of rumen microbes already after few hours. This, however, can be a particular shortcoming, because alterations in rumen fermentation can develop over days or even weeks rather than hours (Czerkawski and Breckenridge, 1977) and therefore demand for long-term examinations.

Generally, *in vitro* methods can offer a relatively standardized environment and the complete control of its conditions, e.g. ambient temperature or buffer infusion rate, which altogether lead to a high repeatability of results. Regarding rumen-related research, the Rusitec system benefits firstly from removing the microbial variability across individuals (Jami and Mizrahi, 2012) by pooling the inocula from donor animals and secondly from having no host effect on the microbiota (Henderson et al., 2015) during the actual feedstuff incubation. However, this should not mask the fact that a substantial impact of the inoculum itself is always present as recently demonstrated by Belanche et al. (2019), who gave important indications about the

procedure for inoculum collection. They observed a more diverse and active microbial community in ruminal fluid sampled three hours post-feeding from goats compared to ruminal fluid sampled before feeding. Likewise, also the ration of the animals affected the microbiome with a higher diversity and activity, when goats were fed forage plus concentrate, instead of only forage; and this relationship is also confirmed for dairy cows and sheep (Boguhn et al., 2013). Due to a several days-lasting adaptation period, the Rusitec may be less affected by the question of "when to collect liquid and solid content from the rumen". This may be more important for *in vitro* approaches that immediately incubate the obtained ruminal fluid with feedstuffs for investigation purposes, for instance the Hohenheim gas test (Menke et al., 1979). Though, Hohenheim gas test runs include an internal standard to control and correct for biological fluctuations. The influence of the donor animal diet (Boguhn et al., 2013; Belanche et al., 2019), however, seems more severe for Rusitec-based experiments and so far, there are no guidelines or recommendations available. For instance, Lee et al. (2011) collected ruminal content from pasture-grazing cows, Jaurena et al. (2005) only used one dairy cow receiving ad libitum grass silage and 8 kg concentrate per day, whereas the present Rusitec experiment obtained ruminal fluid from three steers fed on maintenance level a ration consisting of grass hay, commercial concentrate and rapeseed meal (70:20:10) to ensure the presence of a diverse microbial population (Belanche et al., 2019). Consequently, not only the microbial activity and composition between the aforementioned inocula is debatable (Belanche et al., 2019), but the general comparability of results suffers from the modest degree of standardization, which is also reflected by further inconsistencies in Rusitec experiments, e.g. different nylon bag pore sizes, particle length of feedstuffs or buffer infusion rates (Jaurena et al., 2005; Jalč et al., 2009a; Lee et al., 2011; Boguhn et al., 2013). In this regard, a strict standardization of the Rusitec model is past-due, particularly because of its frequent application in ruminant nutrition research.

Information on feedstuff acceptance is of outstanding importance to comprehensively assess pre-ensiling treatments, but cannot be provided by any *in vitro* system. To overcome this limitation, the present *in vitro* research was accompanied by preference trials with goats, showing that DM concentration and sucrose addition affected the animals' feed intake (Schmit, 2017). On the other hand, it seems favourable that the confounding effect of diverse feed intakes, which is often observed in animal trials (Dewhurst et al., 2003; Broderick et al., 2017), is excluded *in vitro*. Variations in feed intake will possibly bias metabolite concentrations, for instance ruminal ammonia-N concentrations, and observed differences may not necessarily derive from treatment effects, but actually from the intake frequency and amount of ingested feedstuffs. Besides, no ethical conflicts are faced with the here applied Rusitec model as it allows the non-hazardous simulation of exposing high amounts of NPN

or biogenic amines to the rumen ecosystem, which could cause health disorders *in vivo* (Helmer and Bartley, 1971; Webb et al., 1972; Scherer et al., 2015).

Concerning sample collection, the three-way valve of each Rusitec vessel enables dynamic sampling from the liquid phase over time without stressful rumen cannula opening procedures, which constitute the *in vivo* equivalent and potentially impair feedstuff fermentation through an entry of oxygen into the rumen. On the other hand, the Rusitec's solid phase can only be accessed every 48 hours after feed bag exchange, which automatically means a neglect of the solid fraction harbouring a distinct microbial community (Vaidya et al., 2018), especially a higher abundance and activity of cellulolytic species (Michalet-Doreau et al., 2001). Regarding the determination of OM and fibre degradability (Chapter 5), the temporally restricted access to the solid fraction may be less important. For studying microbial communities (Chapter 6), however, this may be more serious as the microbes likely relocate to the fresh feedbag, which in consequence reduces the observed impact of any treatment on the solid-associated microbial population.

An often stated shortage of most *in vitro* systems is the lack of feedback mechanisms from the host, i.e. no epithelial or immune cells or in case of rumen-oriented research, also the missing rumino-hepatic N circulation, which is often criticized as a potential oversimplification. In case of the current research that aimed to investigate characteristics of intra-ruminal N recycling, the absence of a rumino-hepatic N circulation may indeed be regarded as a benefit, because no ammonia-N is eliminated from the system and thus fully available for the microorganisms and can be put into relation to available dietary energy. Besides, the influence of endogenous protein, being a confounder under *in vivo* conditions (Stern et al., 1994), is eliminated.

In summary, a simplification of the *in vivo* situation is not a disadvantage per se, but depends on the scientific question that is to be answered. Thereby, each aspect of the intended study must be evaluated individually and with respect to this scientific question. Consequently, an appropriate *in vitro* model can be chosen to design a meaningful experiment.

Future perspectives

Sucrose addition prior to ensiling of lucerne resulted in reduced pH, NPN and ammonia-N proportions as well as higher lactic acid concentrations, collectively suggesting an improved silage quality and less proteolysis during ensiling. The addition with sucrose as a pre-ensiling treatment was included in the present study, because lucerne is typically low in WSC and therefore regarded as difficult to ensile (Seale et al., 1986). However, breeding has led to a considerable genetic variability in the WSC concentration of lucerne with cultivars having up

to 179 g/kg DM non-structural carbohydrates (NSC), i.e. fructose, sucrose, glucose, pinitol and starch (Berthiaume et al., 2010). When using high NSC cultivars that provide sufficient amounts of fermentable substrate for lactic acid producing bacteria, similar effects on lucerne silage quality as here obtained for pre-ensiling sucrose addition may be the outcome.

However, it is noteworthy, that Berthiaume et al. (2010) determined NSC concentrations in lucerne cultivars after plants had been cut and directly oven-dried at 55°C, which should have minimized potential respiration losses and enzymatic degradation (Wylam, 1953). It is therefore essential to first investigate whether high NSC amounts are still available at the time of ensiling or if they are lost during wilting period, when lucerne is treated as found in practice. Thus, usage of high NSC lucerne might replace the here applied sucrose addition, particularly when the plant material is wilted with high intensity. As the homogenous addition of a WSC source before ensiling means additional labour and potentially the need for technical equipment, applying high NSC cultivars would also facilitate the work flow of lucerne ensiling. At this juncture, however, the basis of information is too vague to allow an assessment of this potential option and hence needs comprehensive research activity in future.

Regarding proteolysis during ensiling of lucerne, Szumacher-Strabel et al. (2018) recently published interesting results about varying saponin concentrations in lucerne cultivars. Thereby, they calculated a negative correlation of -0.63 between saponin concentration and ammonia-N concentration of lucerne silage, although it is not the saponin concentration per se that reduces the ammonia formation during ensiling, but the available saponin forms that determine their antimicrobial activity. Together with the aforementioned proposal of simultaneously applying high-intensity wilting treatments and lower amounts of tannins to minimize the proteolysis in lucerne silages, the usage of lucerne genotypes with high saponin concentrations might be an approach to pursue.

Most likely, these options may not only optimize TP preservation at silage level, but also likely affect rumen fermentation and improve the N retention from lucerne silages by three mechanisms that are intensively discussed in Chapter 3. First, a lower NPN provision to the rumen should decelerate the ammonia-N release; second, majority of protein should be bound to tannins (Jones and Mangan, 1977) and thereby less ruminally degradable; and third, tannins and saponins should exert direct suppressing effects on NC-degrading rumen microbes (Jones et al., 1994; Wallace et al., 1994; McSweeney et al., 1999; Wang et al., 2000; Min et al., 2005; Zhou et al., 2011; Patra and Yu, 2014). However, also potential impacts of tannins and saponins on feed intake must be investigated as both were earlier found to negatively affect DM intake of ruminants (Benchaar et al., 2008; Vasta et al., 2010). Likewise, a potential reduction of ruminal fibre degradability (Griffiths and Jones, 1977;

McSweeney et al., 2001) and intestinal protein digestibility (Salawu et al., 1999a) by tannins is not desired and must be assessed in future studies.

Conclusions

Investigating the effects of the pre-ensiling treatments DM concentration, wilting intensity and sucrose addition on lucerne silage composition and the subsequent *in vitro* ruminal fermentation as well as microbiota composition was the objective of this thesis.

As outlined in the review article presented in Chapter 3, knowledge on NC-degrading rumen microbes is still sparse. Except for few feed additives, there is only marginal understanding on how to beneficially affect this part of the rumen microbiome by dietary treatments. Likewise, the application of pre-ensiling treatments was neglected so far, but should be considered in future to advance towards an efficient ruminal N utilization in livestock production.

Concerning the prepared lucerne silages, all three pre-ensiling treatments influenced the silage composition and quality. The hypothesized lower NPN proportions in high-intensity wilted lucerne silages could be validated. Likewise, the hypothesis of highest TP preservation by wilting lucerne with high intensity to 35% DM and adding sucrose before ensiling was confirmed by the present results. Besides, the lactic acid fermentation was enhanced by the sucrose addition and thereby particularly improved the silage quality.

For the Rusitec incubation, we hypothesized an improved fermentation, i.e. higher gas productions and fibre degradability, along with reduced protein degradation as well as increased microbial CP production in high-intensity wilted and sucrose-treated lucerne silages. The *in vitro* fermentation, however, revealed overall high ammonia-N and isovalerate concentrations without differences in AA-N concentration and no effects of pre-ensiling treatments on microbial CP formation were obtained. Thus, the hypothesis of reduced *in vitro* protein degradation in high-intensity wilted and sucrose-treated silages was rejected. The higher gas productions and SCFA concentrations during incubation of sucrose-treated silages indicate an elevated ruminal fermentation of carbohydrates and therefore partly validated the hypothesis. Though, the reduced fibre degradability in sucrose-treated silages confounded this statement. Samples were taken from the Rusitec after two and seven days of lucerne silage incubation, i.e. representing an early and late time point, respectively. Hereby, greater fibre degradability due to a microbial adaptation to the lucerne silages over time was hypothesized. However, this hypothesis was rejected as both fibre and OM degradabilities were reduced within course of Rusitec incubation. Until now, the causes

remain not fully understood, but decreased numbers of anaerobic fungi and bacterial genera associated with fibre degradation may have contributed to this development.

The present research also included microbiological examinations of the liquid and solid-associated microorganisms in the Rusitec. Due to the increased *in vitro* concentrations of SCFA for sucrose-treated lucerne silages, higher microbial abundances and diversities in the microbiota deriving from sucrose-treated lucerne silage incubation was hypothesized. Increased quantities of archaea and anaerobic fungi in the liquid and solid phase, respectively, as well as greater phylogenetic diversity indices for microbial communities from sucrose-treated lucerne silage incubation confirmed this hypothesis. Besides, contrasting microbiota compositions between the different pre-ensiling treatments were hypothesized, which was only true for sucrose addition. Here, principal co-ordinate analysis revealed a clear separation between microbial communities from silages with and without sucrose addition. In contrast, no differences in ß-diversity were obtained for DM concentration or wilting intensity.

In conclusion, the here applied pre-ensiling treatments showed promising results in terms of improved lucerne silage quality and higher TP preservation in 35% DM high-intensity wilted lucerne silages that received a sucrose addition before ensiling. These beneficial effects were not completely rediscovered during *in vitro* rumen fermentation, but for instance increased fibre degradability in high-intensity wilted silages encourage the continuation of further research. The microbiological analyses provided valuable insights into the microbial community and helped to explain the observed alterations in rumen fermentation patterns. Presumably, including RNA-targeted methods – therefore also addressing microbial activities instead of solely microbial abundances – will be necessary to better comprehend the modes of action by which the pre-ensiling treatments affect the ruminal microbiome and finally the rumen fermentation.

From a methodical perspective, it can be concluded that regarding each aspect separately – meaning either the outcome of the lucerne ensiling or the *in vitro* ruminal fermentation or the microbiology – is not expedient. In fact, a multidisciplinary approach seems necessary to obtain interrelations and better understand underlying mechanisms, which finally enables a reliable, robust and comprehensive assessment of the pre-ensiling treatments. Therefore, the present research may represent an initiator for future studies with the here obtained findings being initial inputs for refining pre-ensiling treatment-based strategies to improve the N retention from lucerne silage, alone or in combination with further approaches.

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