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Environmental impacts of silage production: Formation, emission and fixation of gases from field to barn

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Abstract

Silages are essential feeds in livestock husbandry. Losses should be minimised to close the nutrient cycle between crop and animal production. Besides, losses are generally associated with emissions of climate and environmental relevant substances, such as greenhouse gases (GHG), e.g. carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), or volatile organic compounds (VOC), e.g. ethanol and ethyl acetate. These gases are emitted during fermentation and aerobic storage, contributing to the negative environmental impact of agricultural animal husbandry.

This thesis addresses the pathways of gas dynamics, i.e. formation, emission and fixation of the gases mentioned above, in order to derive climate impacts and mitigation options through adapted management in silage production.

Study 1 investigated the concentrations of GHGs during the 49-day fermentation of grass and lucerne silage (two varying dry matter contents in each case). The wetter silages showed earlier CO_2 formation due to faster microbial activity. Grass silage had higher CH_4 and N_2O concentrations than lucerne silage during the local maxima (first 4 ensiling days). After a temporary drop in the concentrations, the lucerne silage showed increasing CH_4 concentrations. This was due to malfermentation, i.e. the activity of clostridia. This led to the formation of butyric acid and ammonia. The rising pH value and released hydrogen facilitated methanogenesis.

The other studies investigated GHG and VOC gases' formation, emission and fixation during 30 and 135 days of fermentation (Study 2) and 14 days of aerobic storage (Study 3). Two of the three maize silage treatments were treated with a biological (lactic acid bacteria) or chemical silage additives (organic acids). The biological additives reduced the CH_4 and N_2O, and the chemical reduced the ethanol and increased the N_2O emissions of the fermentation (Study 2). The formation took place mainly in the first 6 ensiling days. The treatments showed decreasing gas emission quantities in the course of ensiling: microbiological fixation was assumed.

Both treated variants showed increased aerobic stability (Study 3), which reduced dry matter losses. This means that lower quantities of harvested forage are required, which can reduce indirect GHG emissions during crop production. This has a positive effect on the climate impact of silage production. At the same time, the treated variants showed increased VOC emissions during aerobic storage. The quantities of ethyl acetate emissions exceeded the original quantities in the material; microbial reformation from ethanol was suspected. Using silage additives can mitigate the negative environmental consequences of poor silage management.

Based on the studies, a structuring of gas formation and fixation phases within the silage production process chain is derived. In addition, recommendations for emissions research and management in practice are formulated in this thesis.

Kurzfassung

Silagen sind wichtige Futtermittel in der Viehhaltung. Verluste sollen minimiert werden, um den Nährstoffkreislauf zwischen Pflanzen- und Tierproduktion zu schließen. Zudem gehen Verluste meist mit Emissionen umweltrelevanter Stoffe, wie Treibhausgase (THG), e.g. Kohlenstoffdioxid (CO_2), Methan (CH_4) und Lachgas (N_2O), oder flüchtige organische Verbindungen (VOC), e.g. Ethanol und Ethylacetat, einher. Diese Gase emittieren während der Fermentation sowie der aeroben Lagerung und beeinflussen die Umweltfolgen der Viehhaltung.

Diese Dissertation befasst sich mit der Bildung, Emission und Fixierung der Gase, um daraus Klimaauswirkungen und Minderungsmöglichkeiten in der Silageproduktion abzuleiten.

Studie 1 untersuchte die Konzentrationen der THG während der 49-tägigen Fermentation von Gras- und Luzernesilage (jeweils zwei variierende Trockenmassegehalte). Die nasseren Silagen zeigten aufgrund schnellerer mikrobieller Aktivität eine frühere CO_2-Bildung. Grassilage wies während der lokalen Maxima (erste 4 Siliertage) höhere CH_4- und N_2O-Konzentrationen als Luzernesilage auf. Nach einem zwischenzeitlichen Abfall der Konzentrationen zeigte die Luzernesilage ansteigende CH_4-Konzentrationen, welche auf eine Fehlgärung, d.h. die Aktivität von Clostridien, zurückzuführen waren. Diese führte zur Buttersäure- und Ammoniakbildung. Die steigenden pH-Werte und der freigesetzte Wasserstoff begünstigten die Methanogenese.

Die weiteren Studien untersuchten die Bildung, Emission und Fixierung der THG und VOC-Gase während der 30- bzw. 135-tägigen Fermentation (Studie 2) und 14-tägigen aeroben Lagerung (Studie 3). Zwei der drei Maissilagevarianten wurden mit einem biologischen (Milchsäurebakterien) bzw. einem chemischen Siliermittel (organische Säuren) behandelt. Das biologische Siliermittel reduzierte die CH_4- und N_2O-, das chemische reduzierte die Ethanol- und erhöhte die N_2O-Emissionen der Fermentation (Studie 2). Die Bildung fand in den ersten 6 Tagen statt. Alle Varianten zeigten abnehmende Gasmengen: eine mikrobielle Fixierung wird vermutet.

Die behandelten Varianten zeigten eine verlängerte aerobe Stabilität (Studie 3) was die Trockenmasseverluste reduziert. So werden geringere Erntemengen benötigt, was zu sinkenden indirekten THG-Emissionen während des Ackerbaus führt. Dies beeinflusst die Klimafolgen der Silageproduktion positiv. So kann der Einsatz von Siliermitteln die negativen Umweltfolgen von schlechtem Silagemanagement reduzieren. Jedoch zeigten die behandelten Varianten erhöhte VOC-Emissionen während der aeroben Lagerung. Die Mengen an Ethylacetatemissionen überschritten die Mengen im Material; eine mikrobielle Neubildung aus Ethanol wird vermutet.

Auf Basis der Studien wird eine Strukturierung der Phasen der Gasbildung und -fixierung innerhalb der Prozesskette der Silageproduktion hergeleitet. Zudem werden in dieser Dissertation Empfehlungen für die Emissionsforschung und das Management in der Praxis formuliert.

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List of Abbreviations

AA	Acetic acid
AAB	Acetic acid bacteria
AEMP	Aerobic emission measurement period
ANOVA	Analysis of variance
ASTA	Aerobic stability
BIO	Variant containing biological additive
C	Carbon
CF	Carbon footprint
CFU	Colony-forming units
CH_4	Methane
CHE	Variant containing chemical additive
CO	Carbon monoxide
CO_2	Carbon dioxide
CO_2eq	CO_2 equivalent
CON	Variant containing no additive
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
DM	Dry matter (concentration)
DML	Dry matter losses
EA	Ethyl acetate
EB	Enterobacteria
FC	Fermentation coefficient
FM	Fresh matter (concentration)
FPCM	Fat- and protein-corrected milk
G	Grass silage
G LW	Grass longer wilted (ca. 24 h wilting)
G SW	Grass shortly wilted (ca. 20 h wilting)
GC	Gas chromatography
GHG	Greenhouse gas(es)
GWP	Global warming potential(s)
H_2	Hydrogen
H_2CO_3	Carbonic acid
$H_3CO_3^+$	Protonated carbonic acid
HACCP	Hazard analysis of critical control points

HCO_3^-	Bicarbonate
IPCC	Intergovernmental panel on climate change
kg_{DM}	Mass of dry matter material in kg
kg_{FM}	Mass of fresh matter material in kg
L	Lucerne silage
L LW	Lucerne longer wilted (ca. 24 h wilting)
L SW	Lucerne shortly wilted (ca. 20 h wilting)
LA	Lactic acid
LAB	Lactic acid bacteria
LAB_{he}	Heterofermentative lactic acid bacteria
LAB_{ho}	Homofermentative lactic acid bacteria
LCA	Life cycle assessment
LW	Longer wilted (ca. 24 h wilting)
MO	Microorganism(s)
N	Nitrogen
N_2	Elemental nitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_3–N	Ammonia–nitrogen
NH_4^+	Ammonium
NO	Nitric oxide (nitrogen monoxide)
NO_2	Nitrogen dioxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_x	Oxides of nitrogen / Nitrogen oxides
nXP	Nutzbares Rohprotein am Duodenum (utilisable crude protein at the duodenum)
O_2	Oxygen
O_3	Ozone
PAS	Photoacoustic spectroscopy
pK_a	Acid dissociation constant
r_S	Spearman's correlation coefficient
SA	Silage additive(s)
SD	Standard deviation

SW	Shortly wilted (ca. 20 h wilting)
t_{si}	Silage temperature
VOC	Volatile organic compound(s)
WSC	Water-soluble carbohydrates

1 Introduction

1.1 General introduction and thesis' scope

The growing world population and advancing anthropogenic climate change put modern agriculture under pressure. On the one hand, food security and changing dietary habits lead to a rising demand for plant- and animal-based food (FAO, 2022; van Dijk et al., 2021). On the other hand, agriculture and livestock farming are increasingly the focus of research and discussion regarding environmental impacts (Clark and Tilman, 2017; Nabuurs et al., 2023; Poore and Nemecek, 2018). Several aspects affect these issues, among others: land-use change, energy and fossil fuel demand, manure management, enteric fermentation, and feed supply (e.g. Beauchemin et al., 2008; Deeken et al., 2023; Kupper et al., 2020; Nabuurs et al., 2023; Wilkinson and Garnsworthy, 2021). This thesis contributes to the latter and in particular to silage production.

High-quality feed production is globally essential, especially for farm animal nutrition (Spiekers, 2012; Wilkinson and Muck, 2019) or plant-based biogas production (Hijazi et al., 2016; Teixeira Franco et al., 2016). One-time crop yields during harvest periods must be conserved resource-efficiently to supply feed over the year. Silage, i.e. the fermentation of forage, is one conservation procedure, and it is estimated that global silage consumption by dairy cattle exceeds 665 million tons of fresh matter (FM) per year (Xu et al., 2021). Nevertheless, crop production and the process chain of silage provision emits climate- and environment-relevant gases based on machinery or material use (e.g. Bacenetti and Fusi, 2015; Wilkinson and Garnsworthy, 2021) or microbial processes (e.g. Chen et al., 2021; Krommweh et al., 2020; Shan et al., 2023). In this context, Chen et al. (2021) stated: '*In recent years, much endeavor has been devoted to mitigate greenhouse gases emissions from livestock production and manures treatment. However, little attention has been paid on CO_2 emission during ensiling.*' Carbon dioxide (CO_2) emissions from plants or microbial metabolism can be considered climate-neutral. Still, the statement also applies to the required research concerning other climate- and environment-relevant gas emissions during silage production. Furthermore, indirectly connected greenhouse gas (GHG) emissions, including CO_2 from fossil fuel combustion, should be considered during crop or silage additive (SA) production. Assessing and improving silage production's climate and environmental impact is essential to enhance the carbon footprint (CF) of animal-based food, like meat or milk.

This thesis focuses on the formation, emission and fixation of climate- and environment-relevant emissions, i.e. GHG and volatile organic compounds (VOC), of grass, lucerne and maize silage during the fermentation and the aerobic feed-out phase. Moreover, SA use's CF will be assessed. The findings address open research questions and evaluate management effects from crop production and harvest management on the field to feed submission in the barn.

1.2 Thesis outline

This thesis comprises six sections (Fig. 1.1). Section 1 introduces the thesis' scope. Section 2 describes the basics of silage production and emission research, focusing on silage's ecological relevance, phases of silage production and effects of microorganisms (MO) and management for silage emissions. The section concludes with the derivation of the research questions.

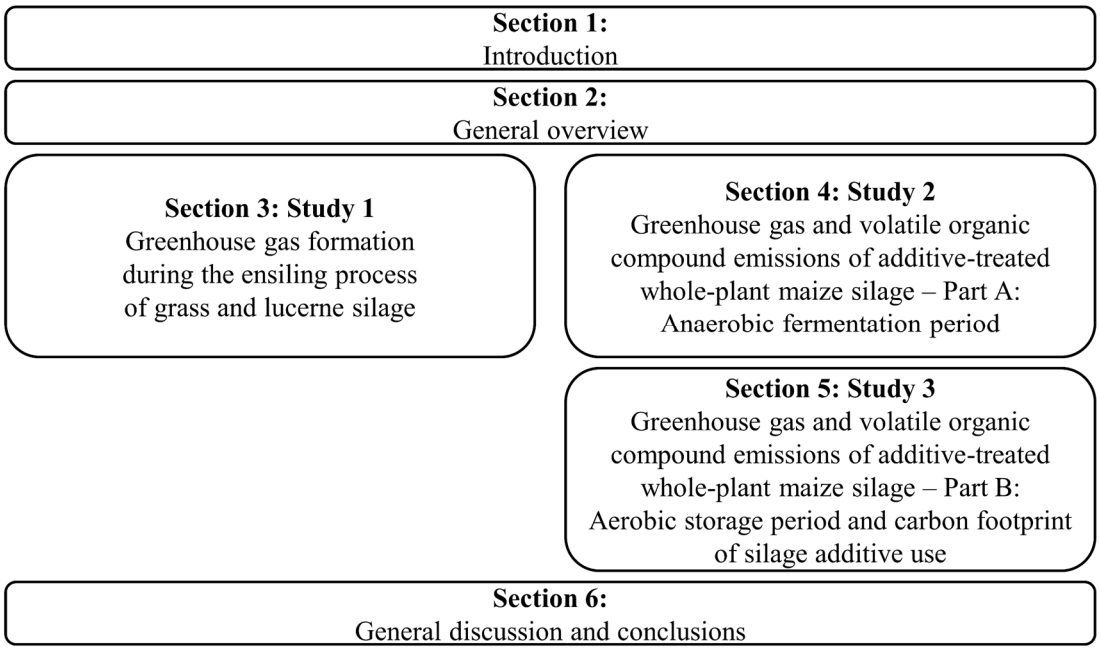

Fig. 1.1 Structure of the thesis.

Sections 3 to 5 comprise self-standing research articles. Study 1 (Section 3) focused on CO_2, methane (CH_4) and nitrous oxide (N_2O) formation during grass and lucerne fermentation (69 days). The forage was ensiled with varying dry matter concentrations (DM). Malfermentation by clostridial butyrate formation affected GHG and ammonia (NH_3) formation.

In Studies 2 and 3, the GHG and VOC formation during maize silage fermentation (30–135 days; Section 4) and aerobic feed-out phase (Section 5) was examined. To the author's knowledge, this is the first trial with continuous silage material for both periods. Study 2 also addresses the fixation of gases during fermentation. SA use affected the gas formation and enabled emission mitigation. Study 3 comprises an assessment of silage's emission mitigation by SA use and their CF. This empiric approach was conducted for the first time in scientific literature.

Section 6 answers the research questions based on the above studies and literature review. In addition, a new structure of phases within the process chain of silage provision is derived with a focus on gas dynamics. The classification allows assigning which gases are formed or fixed at which time and by which pathways. Further, recommendations for (commercial) silage management will be derived and the relevance of the environmental impact of silage production will be discussed. The conclusions round off the thesis.

2 General overview

2.1 Agricultural resource flux and the role of conservation and silage

Ruminants allow the use of agricultural land that is unsuitable for arable farming, and therefore direct food production, for the production of animal feed and thus animal-based food. However, the heterogeneity of feed availability due to the varying seasons requires the conservation of forage for whole-year animal nutrition (Staudacher, 2012; Wilkinson and Davies, 2013; Wilkinson and Rinne, 2018). Livestock husbandry tends to a decreasing number of farms with increasing herd sizes. This affects feed consumption, for instance, in the dairy industry: Rotz et al. (2024) calculated a nearly constant amount (decrease of -2.6%) of forage consumed in the USA between 1971 and 2020. However, conserved forage consumption changed by +17% and grazed forage by -71%. Various agricultural conservation techniques are possible (Jungbluth et al., 2017b). One conservation procedure is mitigating deterioration due to water extraction to undercut the water availability of microbial activity. For instance, this procedure is used to dry grass or legumes by on-field sunlight radiation or technical dryers to produce hay. Conservation procedures without water extraction include the ones with chemical conservation and the ones with airtight storage. For the latter, procedures can be split up into the ones with deterioration mitigation by CO_2-atmosphere and those with pH-changes based on fermentation, often called silage.

Hay (drying) and silage (fermentation) are considered the most essential feeds for ruminant nutrition (Ávila and Carvalho, 2020; Wilkinson et al., 2003). In addition, silage can be used for plant-based energy production utilising CH_4 formation in biogas plants. However, besides several aspects, the trend towards increasing herd sizes and challenging harvest conditions due to climate change favoured the change towards silage (Pahlow et al., 2003; Rotz et al., 2024; Wilkinson et al., 2003; Wilkinson and Rinne, 2018). The diets of US dairy cows comprise up to 60% of silage (Vyas and Adesogan, 2023). This led to a quantity increase of silage in the past decades. Weinberg and Ashbell (2003) report a global silage production of 200×10^6 t_{DM} per year. Assuming average DM losses (DML) of about 20.95% between field and trough (Wilkinson, 2015), this equals 241.9×10^6 t_{DM} of harvested forage; this aligns with the magnitude stated in Section 1.

Silage is embedded in the nitrogen (N) and carbon (C) flux for on-farm feed provision. It starts with crop production, includes the process chain of silage provision and is complemented by silage digestion. The latter can be significantly influenced by silage quality, affecting, among others, animals' dry matter intake or health (Bandla et al., 2024; Brüning et al., 2018; Driehuis and Oude Elferink, 2000; Queiroz et al., 2018; Roß, 2014; Wilkinson, 1999). The slurry and manure can be used as fertiliser for crop production, closing the cyclical flux of resources. Several pathways of losses and emissions may apply in the various stages, as shown in Fig. 2.1.

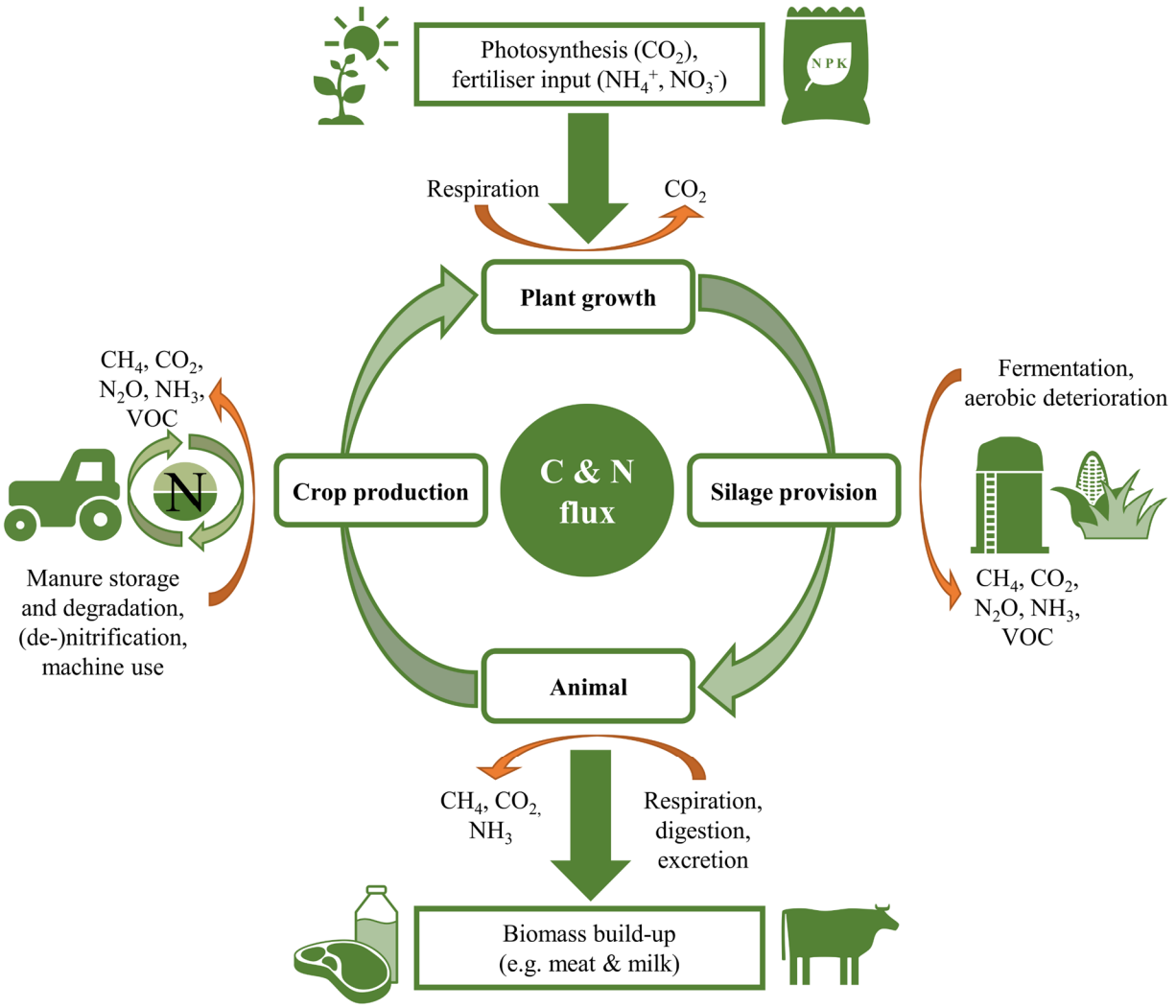

Fig. 2.1 Simplified carbon (C) and nitrogen (N) fluxes in arable forage production systems for animal-based food production. As shown in the top elements, the extra nutrient input is via photosynthesis (CO_2) and artificial fertilisers (NH_4^+ and NO_3^-). In addition to gas emissions (orange arrows) and the removal of end products (e.g. milk and meat, bottom elements), additional nutrient discharge occurs via the removal of liquid and solid manure, silage effluent or leaching and erosion processes (not included).
CH_4 = Methane, CO_2 = Carbon dioxide, N = Nitrogen, N_2O = Nitrous oxide, NH_3 = Ammonia, NH_4^+ = Ammonium, NO_3^- = Nitrate, VOC = Volatile organic compounds.

The losses during this flux are generally considered climate- or environment-relevant. The formation of GHG, like CH_4 or N_2O, applies at various stages. CH_4 is mainly formed during anaerobic digestion or by manure and slurry, N_2O, as part of the N cycle in the soil (Umweltbundesamt, 2018). CO_2 is also a GHG, but the climate-relevance depends on the emission source. CO_2 from fossil fuel combustion is climate-relevant. In contrast, CO_2 emissions based on the metabolism of plants and MO are climate-neutral since initial biomass build-up during crop growth is based on CO_2 sequestration during photosynthesis (Fehrenbach and Bürck, 2022). In accordance with the data provided by the Intergovernmental Panel on Climate Change (IPCC), GHG have varying global warming potentials (GWP): CO_2 = 1, CH_4 = 25 and N_2O = 298 (Forster et al., 2007). These are required to calculate the CO_2 equivalent (CO_2eq) emissions.

Furthermore, the metabolism of prokaryotes and eukaryotes leads to the synthesis of organic matter. Some products, e.g. the VOC, may change into the gaseous phase emitting to the atmosphere. These lead to environmental pollution (aerosol formation), and the photochemical reaction of VOC and oxides of nitrogen (NO_x) may form climate-relevant ozone (O_3) (Howard et al., 2010a). Besides GHG and VOC emissions, NH_3 may be formed during manure or slurry degradation based on urease activity (Umweltbundesamt, 2018).

Livestock farming is increasingly being investigated for its impact on global sustainability, partly due to the emissions mentioned above. Assessing the CF or lifecycle assessment of plant- and animal-based food mostly favours plant-based foods (Aleksandrowicz et al., 2016; Nonhebel, 2006; Poore and Nemecek, 2018). CF for animal-based food can be derived (FAO, 2022); for instance, the CF of cattle meat is considered 30.3 (kg CO_2) kg^{-1}, and the one of fresh cow milk 1.0 (kg CO_2) kg^{-1}. However, detailed models show that the CF of milk varies widely between countries and production systems from 0.74 to 5.99 (kg CO_2eq) per kg of fat- and protein-corrected milk (FPCM) (Mazzetto et al., 2022). In detail, the provision of feed contributes 3%–31% of this CF, showing high ratios for farms with indoor husbandry for (nearly) all year (Mazzetto et al., 2022). In these systems, the feed can be off-farm feeds such as, among others, concentrates or co-products of other (industrial) processes. On-farm feeds include pasture, hay, milled grain or silages (Cortés et al., 2021; van Boxmeer et al., 2021).

One of the main objectives in silage production – to supply a high-quality product efficiently – is minimising DML (Adesogan, 2014; Ávila and Carvalho, 2020; Wróbel et al., 2023). Parameters like the concentrations of digestible energy, proteins and trace elements, as well as impeccable hygiene, i.e. the absence of contaminating substances such as mycotoxins or pathogens (Gallo et al., 2023; Queiroz et al., 2018), are part of the quality assessment. Silages of low quality can reduce feed intake and feed values or increase feed disposal rates up to total losses. Losses can be divided into unavoidable and avoidable losses (Borreani et al., 2018; Köhler et al., 2013). The former is based on biochemical metabolism pathways required for oxygen (O_2) depletion or fermentation, e.g. the activity of lactic acid bacteria (LAB) (Borreani et al., 2018; Muck, 1988). The latter is due to wastage of material or substrate breakdown in (an-)aerobic metabolism of (undesired) MO (Ávila and Carvalho, 2020; Wilkinson, 2015; Wróbel et al., 2023). In general, most of the heterofermentative or aerobic respiration pathways in the metabolism of eukaryotes or prokaryotes lead to DML and the formation of products with a low feed value. Losses may increase due to challenging harvesting conditions, wrong machine settings, human errors or adverse forage material characteristics. DML were considered relevant for feed conservation efficiency for animal nutrition for several decades. This connection remains relevant

in future livestock farming to produce resource-efficient animal-based food. Nevertheless, DML are connected to gas – primarily CO_2 – emissions (Chen et al., 2021; Daniel and Nussio, 2015; Milimonka et al., 2019). Thus, parameters like management decisions, silo and material characteristics or MO activity influence not only the DML (Pahlow and Muck, 2009) but also the emissions of silage about climate and environmental relevant substrates. In general, DML should be minimised to follow the United Nations sustainable development goal 12, 'Sustainable consumption and production' to reduce losses along the supply chains of human food production (cf. Nabuurs et al., 2023; United Nations Environment Programme, 2018).

The course of the ensiling process and the quality of the final product can be influenced by SA, which are usually applied during harvesting or storage. The form of SA varies and includes, among others (cf. Kung et al., 2003), targeted inoculation with specific MO, the application of (organic) acids or substrates for nutrient supplementation, e.g. SA containing molasses or nitrite (NO_2^-). Thus, biological and/or chemical components are used. At the same time, they can be categorised according to their direction of action (Kalzendorf and Staudacher, 2012). Two of the most important directions are influencing the fermentation (direction of action 1) and increasing the aerobic stability (ASTA) (direction of action 2). The former is especially suitable for crops with a low fermentation coefficient (FC), for instance, grass or lucerne silage with low DM and water-soluble carbohydrates (WSC) concentrations, high protein and crude ash concentrations, and a high load of undesirable MO, such as clostridia. Direction of action 2 reduces aerobic deterioration caused by acetic acid bacteria (AAB), yeasts and moulds. This is achieved, for example, by the microbiological formation of acetic acid (AA) (CH_3COOH) or the application of organic acids with suitable acid dissociation constant (pKa) values (Kung et al., 2003; Woolford, 1975). Acids are often used in the form of salts to reduce corrosion and work safety hazards. Combination products and subsequent SA applications, e.g. to the silo face, are possible. The effect of SA on silage emission has scarcely been investigated (Cai et al., 1997; Chen et al., 2021; Daniel et al., 2015). SA of the direction of action 1 are most suitable in situations of malfermentation risks (Study 1) but may not affect aerobic DML. Studies 2 and 3 and the general discussion (Section 6) focus mainly on SA of the direction of action 2 unless otherwise stated.

2.2 The process chain of silage provision and quality management

In this thesis, the following definitions apply to clarify the stages of silage management (Fig. 2.2). The silage storage period comprises the anaerobic phase of fermentation and the aerobic phase of feed-out. Beyond this, the ensiling process also includes the period of silo packing, i.e. filling and compaction (Pahlow et al., 2003). The process chain of silage provision, also comprises the harvest and transport of the forage, the silo packing, the silage removal and mixing

and the submission of the animal diet. Finally, silage production includes crop production, from sowing the crop to feeding the silage in the trough. This is followed by the final fate of the silage, i.e. digestion. Crop production provides the forage material for the process chain. Management decisions in each phase affect the subsequent phases regarding DML and resource retention efficiency.

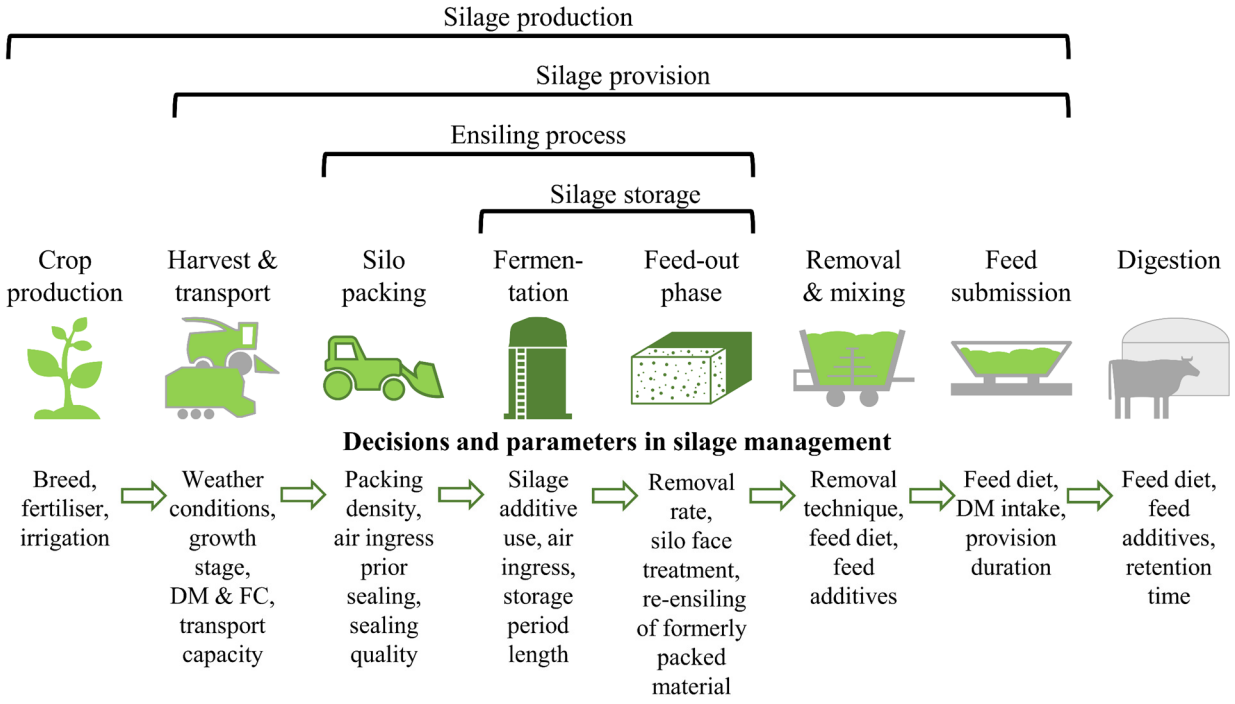

Fig. 2.2 Phases of the process chain of silage production and selected decisions and parameters influencing the management and emissions (partially based on Cooper and Hutley, 2010; Pahlow et al., 2003; Rooke and Hatfield, 2003; Wagner, 2005).
DM = Dry matter (concentration), FC = Fermentation coefficient.

Management during silage production affects DML, emissions and the characteristics of the final product. Decisions within a process can be categorised into two groups (Daydé et al., 2014). Strategic decisions are long-term actions that usually involve increased effort and serve to define the process conditions or objectives. Operational decisions are short-run actions during the production process to reach the goals.

Previous studies addressed quality management in producing feedstuffs and silage (Borreani et al., 2018; Kung and Neylon, 2002; Lindgren, 1999; Pickert et al., 2019; Wagner, 2005). Due to the interdisciplinary complexity, quality management must be implemented in all phases (Wagner, 2005), and the focus should be on process-orientated decision-making steps. Previous studies used the 'Hazard Analysis of Critical Control Points' (HACCP) to assess the essential decision-making parameters to ensure that the product fulfils the quality demands (Lindgren, 1999; Wagner, 2005). However, the HACCP method requires clearly defined limits for quantifiable parameters in order to decide whether the current state puts the target parameters of the end product at risk (cf. National

Advisory Committee on Microbiological Criteria for Foods, 1998; Wagner, 2005). In most cases, the aim is high quality and perfect hygiene. In the case of this thesis, low DML and reduced emissions are the objectives. In the context of silage emissions, it is to be feared that the complexity and limited research into the matter affect the quantification of generally applicable limit values at this point (cf. Lindgren, 1999). Thus, the chance of implementation of general HACCP limits is vague considering the several open questions: When do which emissions occur? Which pathways in which periods are of the most importance? Which material and management factors affect the emissions? Can later management compensate for wrong decisions? Answers are needed to specify if HACCP can be formulated and help to assess and improve silage management.

2.3 Phases of the ensiling process

The most common definitions of phases structuring a successful ensiling process are shown in Table 2.1. These are defined by O_2 availability and MO metabolism rather than chronology. The phases differ in length and intensity, and the transition between the phases may be temporal-spatially blurred (Pahlow et al., 2003). The literature shows that MO activity determines whether it leads to a positive course of the ensiling process or impairments, such as increased DML and emission quantities or the formation of harmful substances, e.g. mycotoxins or butyric acid ($CH_3CH_2CH_2CO_2H$). The abundance and activity of various MO groups and species varies widely between the phases (Table 2.2).

Table 2.1 Overview of published models for structuring the phases of the ensiling process.

Conditions	Merry and Davies (1999)	Pahlow et al. (2003) [A]	Cooper and Hutley (2010)	Seglar (2003)
aerobic	Phase P1 Initial phase	Phase P1 Filling	Phase P1 Aerobic fermentation	Phase P1 Aerobic metabolism
anaerobic	Phase P2 Fermentation	Phase P2 Main fermentation	Phase P2 Heterofermentation (EB)	Phase P2 Heterofermentation (EB)
anaerobic	/	/	Phase P3 Homofermentation (LAB)	Phase P3 Homofermentation (thermophile LAB)
anaerobic	/	/	/	Phase P4 Homofermentation (mesophile LAB)
anaerobic	/	Phase P3 Stable storage	Phase P4 Stable storage	Phase P5 Stable storage
aerobic	Phase P3 Feed-out phase	Phase P4 Feed-out phase	Phase P5 Feed-out phase	Phase P6 Feed-out phase

EB = Enterobacteria, LAB = Lactic acid bacteria.

[A] High-impact publication, however, the structure is probably based on Barnett (1954) (cited by Pahlow et al., 2003).

Phase P1 lasts for the period of silo filling until the O_2 in the silo is respired. After chopping, the increase of particulate surface area, damaged cell structures, and subsequently, the provision of WSC facilitates the metabolism of aerobic MO. WSC are respired, and enzymatical activity provides further substrates due to hydrolysis and proteolysis (Pahlow et al., 2003; Rooke and Hatfield, 2003; Schroeder, 2004). In addition, the aerobic respiration of the plant material continues during this first aerobic phase. Previous studies showed Phase P1 lasted for about 3 h in the laboratory (Li et al., 2017) and 6 h in commercial silos (Wang and Burris, 1960). However, O_2 availability shows temporal-spatial differences throughout the silo, and therefore varying microbial metabolism (Emery and Mosier, 2015; Weiß et al., 2020). Silage temperature can increase due to enterobacterial and plant material activity (McCullough, 1984, cited by Schroeder, 2004; Seglar, 2003). The respiratory pathways metabolise O_2 to CO_2. Thus, the gas volume within the silo is constant, and the gas composition turns anaerobic. DML in this phase is considered minimal (Muck, 1988).

Table 2.2 Common microorganisms during the phases of the ensiling process (modified, based on Ávila and Carvalho, 2020; Seglar, 2003).

Group of microorganisms	Phase P1 Aerobic metabolism	Phases P2–P3 Fermentation	Phase P5 Stable	Phase P6 Feed-out
LAB	Present Low population	Present Population ↑	Present	Present Population ↓
AAB	Present Very low population	Present Population ↓	Absent	Present Population ↑
Enterobacteria	Present	Present Population ↓	Absent	Absent
Clostridia	Present [A]	Present (Spores) [B,C]	Present (Spores) [B]	Present [D]
Yeasts	Present	Present Low population	Present Low population	Present Population ↑
Moulds	Present	Present (Spores) [B] Population ↓	Present (Spores) [B] Population ↓	Present Population ↑

AAB = Acetic acid bacteria, LAB = Lactic acid bacteria.

[A] Based on contamination.

[B] Survive in the form of spores.

[C] High population increase and activity in case of malfermentation.

[D] Development in anaerobic niches possible.

Phase P2 starts with total O_2 depletion within the closed silo; the fermentation begins. The anaerobic phases differ in the varying models (Table 2.1); subsequent descriptions align with Seglar (2003) focussing on maize silage. Heterofermentation converts hexoses and pentoses to short-chain volatile fatty acids like acetate, lactate and propionate (Table 2.3). Furthermore, ethanol (CH_3CH_2OH), CO_2, and other products are formed, and high ratios of DM and energy losses are applied. Heterofermentation by enterobacteria (EB), yeasts, LAB or propionic acid bacteria is considered inefficient, i.e. the pH drops slowly in times of high DML (Day and

Liscansky, 1987; Seglar, 2003; Wróbel et al., 2023). The various MO are inhibited one by one when the pH decreases according to their acidic pH tolerance. EB and LAB remain active, showing hetero- and homofermentative metabolism until pH drops below pH 5 (Seglar, 2003). After this point, EB activity decreases (above pH > 4.5–5.0) (Pahlow et al., 2003; Spoelstra, 1985), and homofermentation increases lactic acid (LA) ($CH_3CH(OH)COOH$) production and reduces DML. Phase P2 is considered to last about 24–72 h (Seglar, 2003).

Table 2.3 Acidification and fermentation efficiencies of main fermentation pathways of silage bacteria (Rooke and Hatfield, 2003).

Organism	Pathway	Substrate	Product	Recovery [%]	
				Energy	DM
LAB	Homofermentative	Glucose	2 Lactate	96.9	100
LAB	Heterofermentative	Glucose	1 Lactate + 1 Acetate	79.6	83
LAB	Heterofermentative	Glucose	1 Lactate + 1 Ethanol	97.2	83
Yeasts	/	Glucose	2 Ethanol	97.4	51
Clostridia	/	Glucose	1 Butyrate	77.9	66
Enterobacteria	/	2 Glucose	2 Lactate + 1 Acetate + 1 Ethanol	88.9	83

DM = Dry matter, LAB = Lactic acid bacteria.

Phase P3 lasts about 24 hours (Seglar, 2003) and can be seen as a transition period. The low pH favours the activity of acid-tolerant, homofermentative LAB (LAB_{ho}). LAB_{ho} ferment hexoses to LA efficiently, resulting in energy losses of only 3.1% (Table 2.3) (Rooke and Hatfield, 2003). However, increased silage temperatures resulting from the previous phases lead to the activity of specific thermophile LAB species and strains fostered by these temperatures (Seglar, 2003).

The transition to Phase P4 is fluid due to the change in the LAB_{ho} community towards mesophilic species. Considerable amounts of LA are formed, leading to a pH decrease until the potential activity of (almost) all MO is inhibited (Seglar, 2003), but many, e.g. yeasts, bacilli or clostridia, persist in endospore dormancy (Pahlow et al., 2003). The total length of all phases up to this point is given as 10–21 d, which can be shortened by SA (Schroeder, 2004; Seglar, 2003).

Phase P5 is a stable phase stretching until the end of anaerobic storage unless leakages allow O_2 penetration (Seglar, 2003). In the latter, aerobic respiration can lead to substantial deterioration. The length of Phases P2–P5 may depend, among others, on the MO community or SA use (cf. Arriola et al., 2021) but also on the plant type (cf. Whittenbury et al., 1967) or silage characteristics like the crude protein, NH_3–N and crude ash concentrations affecting the buffer capacity (cf. Kung et al., 2018; Kung and Shaver, 2001). If anaerobic conditions remain, little changes occur. Acid-tolerant enzymes cause hydrolysis of carbohydrates or proteolysis to NH_3 (Pahlow et al., 2003). LAB counts are reduced, but the conversion of lactate into AA is possible

by heterofermentative LAB (LAB_{he}) (Oude Elferink et al., 2001; Rooke and Hatfield, 2003). Some acidic-tolerant yeast strains may convert excess sugars to ethanol (Rooke and Hatfield, 2003).

Phase P6 is the last phase of the ensiling process when the silo is opened and aerobic conditions are restored. The silage shall be fed quickly to the animals or biogas plant before ASTA – i.e. the silage temperature is at a maximum of 2 K above ambient temperature (Shan et al., 2021a; Wilkinson and Davies, 2013) – is at risk. ASTA is the duration of stable silage temperature which is influenced by – despite fluctuating ambient temperatures – physical silo characteristics (e.g. silo geometry and thermal insulation, silage mass, DM and porosity) and aerobic MO activity. The latter is the product of active MO counts and (substrate and) O_2 supply rate. The facultative or obligate aerobic MO, in particular LAB, AAB, EB, bacilli, yeasts and moulds, reactivate their respiratory metabolism (Borreani et al., 2018; Merry and Davies, 1999; Wróbel et al., 2023). LAB are the most abundant MO for several days (Drouin et al., 2021; Vigne, 2022; Yin et al., 2023). The amount of O_2 supplied is mainly determined by the silo type, geometry, sealing and silage porosity (cf. Leurs, 2006). The various silo types, such as bunker silos, tower silos, silo bags or silage bales, can be designed in varying geometries, i.e. dimensions or shapes (e.g. vertical or sloping walls of the bunker silos). The latter influences the temporally and spatially varying amounts of O_2 that penetrate into the different layers of silage material behind the silage face. Silages with high concentrations of DM, LA and WSC, low concentrations of AA and high porosity are particularly prone to aerobic deterioration (Borreani et al., 2018; Merry and Davies, 1999; Seglar, 2003). AAB and yeasts initiate deterioration by metabolising the remaining WSC and LA to, among other things, heat and CO_2 (Merry and Davies, 1999; Pahlow et al., 2003). Subsequently, moulds displace yeasts, resulting in additional heat production, CO_2 emissions, DML and toxin production (Merry and Davies, 1999). This reduces the feed value to the point where the feed should be discarded entirely.

Except for the description of aerobic deterioration, the phases presented assume a successful, high-quality ensiling process. In some cases, malfermentation may apply due to the activity of clostridia. Besides glucose metabolism, LA is converted into butyric and AA with high DML (Day and Liscansky, 1987; Pahlow et al., 2003; Wróbel et al., 2023). Clostridial activity occurs especially in contaminated plant material with low DM and high protein and crude ash concentrations. These parameters increase the buffer capacity, so the pH remains above the limit values for clostridial activity ($pH > 4.2–4.5$) (Pahlow et al., 2003) in phases P2–P4.

2.4 History of silage emission research

The first approaches to silage gas research date back to the year 1868 (cf. Reid et al., 1984). Further research addressed the formation of toxic gases like nitrogen dioxide (NO_2) (Peterson et

al., 1958; Wang and Burris, 1960). The so-called ‘silo-fillers disease’ led to injuries and deaths in people working with silos based on the formation of nitric acid (HNO_3) in the lungs after inhalation of brown or yellow-orange fumes containing NO_2 (Grayson, 1956; Pahlow et al., 2003). NO_2 results from microbial nitric oxide (NO) formation, which passes over to NO_2 when it comes into contact with air (Pahlow et al., 2003). The formation of NO was traced back to the nitrate (NO_3^-) degradation by EB in ongoing research (Pahlow et al., 2003; Spoelstra, 1985). Although N_2O formation was known, it was not linked to any influence on climate change (e.g. Reid et al., 1984). The synergy of silage emission and silage microbiome research showed mutual interactions. Subsequent studies investigated the development of anaerobic and aerobic conditions within the silo. These focussed on the escape of CO_2 and the penetration of O_2 at the silage face to derive findings on aerobic deterioration (Ashbell and Weinberg, 1992; Honig, 1991; Weinberg and Ashbell, 1994). This led to the development of models for determining silage deterioration (Pitt et al., 1991; Pitt and Muck, 1993). In the new millennium, the environmental consequences of silage production came into focus (Howard et al., 2010b; Howard et al., 2010a; cf. Zhao et al., 2021). Here, the emission behaviour of VOC, e.g. ethanol, methanol (CH_3OH) and ethyl acetate (EA) ($CH_3CO_2CH_2CH_3$), was investigated with material from the silage face (Hafner et al., 2010; Montes et al., 2010) and contextualised to the farms (Bonifacio et al., 2017; Malkina et al., 2011). Other scientists linked silage emissions to the context of gas formation during ruminal digestion (Gerlach et al., 2018). The use of additional measurement technology enabled the more precise differentiation of gas components and corrected earlier possible measurement errors (cf. Wang and Burris, 1960; Zhao et al., 2021, 2016). Later on, results regarding the gas formation at the start (Li et al., 2017) or the total duration (Daniel et al., 2015; Daniel and Nussio, 2015) of fermentation were published. Varying plant materials were used (Daniel and Nussio, 2015; Li et al., 2017; Schmidt et al., 2011), and the influence of biological SA was investigated (Daniel et al., 2015). The effect of chemical SA on emission behaviour has not yet been investigated. For the first time, the total amount of GHG emissions during the ensiling process was determined; a large proportion of the gases formed is CO_2 (Schmidt et al., 2012). Some studies reported gas concentrations and quantities of accumulated gas before gas sampling (Schmidt et al., 2011; Zhao et al., 2021, 2016). Thus, the exact time and length of gas formation remained uncertain. The innovative application of additional sensors helped reveal profound findings in aerobic deterioration and correlations between microbial respiration and changes in the silage characteristics, e.g. pH, temperature, or CO_2 formation (Jungbluth et al., 2017a; Shan et al., 2021a; Shan et al., 2019; Sun et al., 2015). Modern sensors were also used to determine the fermentation efficiency of silage-relevant LAB (Shan et al., 2021b). Furthermore, silage gas formation was directly connected with MO

community analysis (Chen et al., 2021). While most of the investigations were conducted on a laboratory scale using silage material from laboratory or commercial silos, some initial studies have carried out measurements directly at commercially used silos (Krommweh et al., 2020; Shan et al., 2023; Zhao et al., 2021). Most recently, researchers have reported that silage material and its MO can fix CO_2 and N biochemically, e.g. via the Wood-Ljungdahl-pathway (Schmidt et al., 2023; Schmidt et al., 2018; Vigne et al., 2019). This has led to the hope that silage could serve as a CO_2 sink. In this subject, Schmidt and Vigne (2023) said: '*We believe this is the beginning of a new branch of silage science. By now, we have more questions than answers.*'

2.5 Open research questions

The overview shows that gas dynamics in silages are currently being examined, but several questions remain in this field of science. These determined the scope and impetus of this thesis. The studies comprised were conducted to directly address the questions 1–5. The studies' results and discussion combined with additional literature review derived the additional questions 6–7:

1 **When are which climate- and environment-relevant gases and gas quantities formed during fermentation?**

Former research conducted comprehensive gas analytics within the first hours of fermentation (Li et al., 2017; Spoelstra, 1985; Wang and Burris, 1960) or collected samples of accumulated gases after several ensiling days (Schmidt et al., 2011; Zhao et al., 2021, 2016). Other approaches focused on silage-related broth (Shan et al., 2021b). Extensive sampling and analysis of GHG and VOC in silos during the whole fermentation process is necessary to complete knowledge of phases in gas dynamics. Developing previous methodology (Knicky et al., 2014) should help answer this question.

2 **To what extent do the factors of plant type, DM, and management (e.g. packing density and use of SA) affect gas formation? What emissions occur under more challenging conditions, such as malfermentation and increased risk of aerobic deterioration?**

Growing challenges – e.g. unfavourable harvest periods or complications during the harvesting process – in commercial silage management can increase the risk of adverse material characteristics, like too low or high DM or packing densities. Furthermore, management choices like the use of SA may apply. All aspects affect gas dynamics during the ensiling process. Particularly harsh circumstances can lead to malfermentation based on clostridial activity or extensive heating in the feed-out phase. The design of the studies addresses these situations, showing gas dynamic impacts to derive recommendations.

3 Which emission quantities apply in the fermentation and which in the aerobic feed-out phase? Which ratio is derived for continuous silage material? When should action be taken to reduce emissions?

Former studies focused on specific periods of the ensiling process, i.e. the fermentation or the aerobic feed-out phase, with varying measurement periods. Therefore, comparing emission quantities between the studies may be misleading. To the author's knowledge, the design of Studies 2 and 3 will address this required long-time measurement over both phases for the first time in scientific research.

4 Does silage fix gases in modified trial set-ups using gas-proof materials? When are which quantities fixed? Can silage be a CO_2 sink?

Brazilian researchers reported that silages absorb CO_2 and N during fermentation due to biochemical pathways, but trial set-ups contained CO_2 diffusion-permeable materials (Bueno et al., 2020; Restelatto et al., 2019; Schmidt et al., 2018). Modified trials are necessary to prove or refute these claims. Furthermore, the quantification of gas fixation should be compared to the actual gas formation of silages to balance overall gas dynamics.

5 If the use of SA mitigates silage emissions during fermentation and/or the feed-out phase, what's the balance of silage-related mitigations and the CF of SA themselves?

SA affect the fermentation and ASTA. The gas formation during fermentation can be increased (Daniel et al., 2015), but it decreases during feed-out due to reduced degradation. However, SA's production and application are connected to climate-relevant emissions (Milimonka et al., 2019). By now, no empirical study has compared their CF and their CO_2eq mitigation potential to the author's knowledge. Quantifying emissions enables this balance based on trials and literature data.

6 Which gases are formed, emitted or fixed by which MO in which pathways? What phases of gas dynamics can be deduced?

The previous categorisation of the ensiling process into 3 to 6 phases (see Table 2.1) may not be precise enough to apply to the phases of gas dynamics. The trial results and literature research can be combined to derive modified phases of gas dynamics, including the microbial and biochemical pathways involved.

7 Which recommendations can be formulated for silage research and commercial silage management to reduce environmental impacts?

By now, most silage emission research has been conducted in lab-scale trials. New approaches for further research can be derived. However, recommendations for commercial silage are necessary to mitigate emissions in global silage management.

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3 Study 1:
Greenhouse gas formation during the ensiling process of grass and lucerne silage

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Abstract

Silage is an essential global feedstuff and an emitter of greenhouse gases. However, few studies have examined the formation of carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) during the ensiling process. This study aimed to record the course of gas concentrations in forage during the ensiling process and determine the temporal variation in the (microbiological) formation processes. Grass and lucerne, each with two different dry matter (DM) concentrations (four variants, each n = 3), were ensiled in laboratory-scale barrels (120 L). Gas samples were taken from the headspace of the barrels and analysed using a gas chromatograph. The measurement period included the first 49 days of the ensiling process and the measurement interval was 0.5–48.0 h. For all variants, a rapid increase in CO_2 concentration and a one-time N_2O concentration peak was observed between ensiling hours 36 and 96. Lower DM concentration led to significantly faster CO_2 production ($p < 0.05$). Lucerne forage and higher DM concentrations led to significantly increased N_2O concentrations ($p < 0.05$). The extensive measurements demonstrated that butyric acid formation by clostridia contributes to CH_4 formation; thus, lucerne silage had a significantly higher concentration from ensiling day 13 ($p < 0.05$). Therefore, malfermentation actively contributes to the formation of greenhouse gases. The method described here provides further insights into greenhouse gas formation during the ensiling process and can thus help to improve ensiling research and management.

Keywords

Fermentation, Carbon dioxide, Methane, Nitrous oxide, Enterobacteria, Clostridia

Graphical abstract

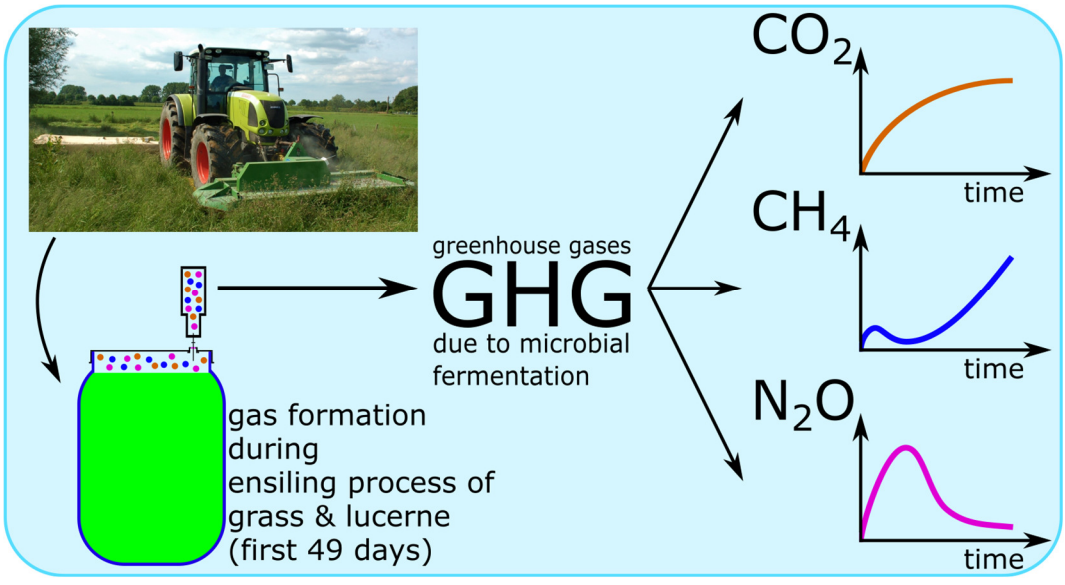


Fig. 3.1 Graphical abstract of Study 1.

3.1 Introduction

The quantification of climate-relevant emissions from agriculture is increasingly important, particularly in climate change research (Gerber et al., 2013; Grossi et al., 2019; Myhre et al., 2014). In livestock husbandry, emissions from the husbandry process itself (Mostafa et al., 2020; Philippe et al., 2011; Schmithausen et al., 2018a, b) as well as the downstream process chain (Amon et al., 2006; Kupper et al., 2020; Rodhe et al., 2015), e. g. slurry management, have been thoroughly investigated. However, emission behaviour in the upstream part of the process chain, such as during feed production, is not well studied.

To ensure that livestock are adequately fed throughout the year, one-time harvest yields must be preserved. The aim is to ensure that the final feed product suffers the smallest possible losses in quantity and quality. Consequently, feed preservation methods are used to conserve natural resources. Silage is one of the most important feeds used in global livestock production (Weinberg and Ashbell, 2003; Wilkins et al., 1999; Wilkinson and Muck, 2019), especially for ruminants. In addition, silage is used to feed pigs (Ebertz et al., 2020; Lengling et al., 2020) and for biogas production (Jacobs et al., 2017; Weiland, 2010). It is now known that silage emits greenhouse gases (GHGs) (Krommweh et al., 2020; Schmidt et al., 2011, 2012; Zhao et al., 2016) with various global warming potentials (GWPs) (Myhre et al., 2014): carbon dioxide (CO_2; GWP = 1), methane (CH_4; GWP = 28) and nitrous oxide (N_2O; GWP = 265). CO_2 emissions are considered climate-neutral because of their biological origin, but forage production does require the use of fossil carbon reserves (e.g. fertilisers or fuels). Additionally, carbon emissions during the ensiling process or the aerobic feed-out phase (mostly due to yeast metabolism) are associated with feed and energy losses. Poor silage quality due to, for example, butyric acid production by clostridia [high risk in silages with low dry matter concentrations (DM)] can induce the degradation of proteins and amino acids to ammonia (Ohshima and McDonald, 1978). These effects are considered negative as they can exacerbate the loss of feed quality and negatively affect animal nutrition. Importantly, the conversion of forage into climate-relevant gases and the environmental impact of this process are increasingly understood to play a role in ongoing climate change. Therefore, the development of successful and efficient feed conservation processes is relevant to both animal nutrition and environmental protection. CH_4 and N_2O emissions are not considered climate-neutral because of their higher GWPs; thus, the conversion of high-value feed substances into these climate-relevant gases is a particularly important issue.

Much of the previous research has focused on silage emissions during the aerobic feed-out phase (Gerlach et al., 2018; Hafner et al., 2010; Krommweh et al., 2020; Malkina et al., 2011; Montes et al., 2010), with the focus in part on yeast activity and aerobic stability (Jungbluth et al.,

2017; Shan et al., 2021a, b; Sun et al., 2015). Some GHGs are emitted once after formation during the ensiling process (Krommweh et al., 2020). Studies on GHG concentrations during the ensiling process date back several decades (Peterson et al., 1958; Wang and Burris, 1960; Weinberg and Ashbell, 1994) or do not include measurements of CO_2, CH_4 and N_2O (Franco, 2016; Peterson et al., 1958; Wang and Burris, 1960; Weinberg and Ashbell, 1994; Zhao et al., 2016). The earlier studies (Peterson et al., 1958; Wang and Burris, 1960) had methodological limitations in this context because the gas analysis technique used (mass spectrometry) was not capable of differentiating precisely between CO_2 and N_2O (Zhao et al., 2016). Furthermore, the earlier conducted studies were mostly aimed at assessing gas emissions from silage from the perspective of occupational safety (e.g. silo filler's disease caused by nitrogen dioxide) or in relation to the effects on animal nutrition. The current focus is largely on the environmental consequences of these gas emissions, especially in modern political discussions (e.g. farm-to-fork). A large proportion of the studies have been conducted using maize (*Zea mays*) silage (Peterson et al., 1958; Schmidt et al., 2012; Wang and Burris, 1960; Weinberg and Ashbell, 1994; Zhao et al., 2016), whereas studies using grass (Krommweh et al., 2020; Wang and Burris, 1960) or lucerne (*Medicago sativa*) silage (Franco, 2016; Krommweh et al., 2020; Peterson et al., 1958) are scarce. Since the anaerobic ensiling process involves several phases (Pahlow et al., 2003), the composition and metabolic activity of microorganisms change, especially during the early days of the process. Consequently, the formation and concentration of gases from the silage can also change. Although earlier studies (Peterson et al., 1958; Wang and Burris, 1960) primarily used measurement intervals of 6 h within the first 66 ensiling hours, more recent studies (Bueno et al., 2020; Schmidt et al., 2011, 2012, 2013) have mainly examined gas concentrations on single ensiling days (days 5–61). Only Franco (2016) has investigated silage gases over shorter measurement intervals (down to 0.5 h) within the first 209 ensiling hours; however, in this PhD dissertation, not all GHGs were investigated and concentration courses were not provided.

Consequently, there is still a research gap to be filled, i.e. a study of the formation of individual GHGs at different stages within the ensiling process. In the present study, detailed measurements of the gases produced were taken using precise measurement technology over short measurement intervals and a long measurement period. To this end, two different forages, namely grass and lucerne, with varying DM concentrations (following shorter and longer wilting periods) were investigated. Both DM concentrations were kept at low levels to simulate poor environmental conditions during harvesting and provoke poor silage quality. Measuring the course of the gas concentrations should provide conclusions on the (microbiological) formation processes and therefore the quality of the ensiling process itself.

To summarise, the objectives of this investigation were as follows: (1) to examine whether the method is suitable for analysing climate-relevant gases in silage at a laboratory scale; (2) to record the course of concentrations of three GHGs (CO_2, CH_4 and N_2O) from two forage types (grass and lucerne) ensiled with different DM concentrations (shorter and longer wilting periods) using short sampling intervals over a long period of the ensiling process; (3) to determine the temporal changes in microbiological gas formation during the ensiling process in relation to the chemical composition of the silage.

3.2 Material and methods

3.2.1 Forage material and silage variants

Grass and lucerne grown at Campus Frankenforst of the Rheinische Friedrich-Wilhelms-Universität Bonn (Königswinter, Germany; 50°42′50.1″ N, 7°12′24.9″ E) were used as forage for the experiment. The crops were fertilised and managed under common practice conditions. The forage (for chemical composition, see Section 3.3.1) was cut on the evening of the 9 May (second cut) and kept overnight on the pasture for wilting. After collection with a loading wagon (theoretical cutting length: 55 mm) at noon the following day (ca. 20 h of wilting), half of each forage material was ensiled. The other half was spread on a black film in direct sunlight for an additional 4 h (at about 17°C, i.e. ambient air temperature, under a clear sky) and regularly turned to ensure higher DM concentrations. Consequently, four forage material variants were produced (for details see Table 3.1): grass shortly wilted (G SW), lucerne shortly wilted (L SW), grass longer wilted (G LW) and lucerne longer wilted (L LW).

Table 3.1 Characteristics of silage variants within laboratory-scale barrels (120-L volume) according to wilting period and forage material.

		Silage variants [A]			
	Unit	**G SW**	**L SW**	**G LW**	**L LW**
Number of barrels		3	3	3	3
Forage material		Grass	Lucerne	Grass	Lucerne
Wilting duration		Shortly (ca. 20 h)		Longer (ca. 24 h)	
Temperature range during wilting	°C	0.8–16.8		0.8–17.9	
Temperature sum during wilting [B]	°C	151.3		219.9	
Fresh material per barrel (mean)	kg FM	85.0	81.1	72.9	63.2
Dry matter concentration (mean)	%	21.5	19.5	26.2	22.7
Silage density (mean)	(kg DM) m^{-3}	152.3	131.7	159.1	119.5

FM = fresh matter, DM = dry matter.

A Variants: grass shortly wilted (G SW), lucerne shortly wilted (L SW), grass longer wilted (G LW) and lucerne longer wilted (L LW).

B Sum of hourly mean values.

After wilting, the plant materials were added to twelve 120-L barrels (high-density polyethylene) for ensiling (n = 3 for each forage variant). The fresh material was filled in layers and each layer was compacted with a hydraulic press to ensure uniform compaction within each barrel (Jungbluth et al., 2016). The density of the material was determined using the volume and weight of the barrel as well as the DM concentration (see Table 3.1). The plastic barrels were sealed with a modified lid, which was pressed onto the corresponding barrel using a clamping ring. A rubber septum was inserted into the plastic lids, which allowed gas sampling during the ensiling process (see Section 3.2.3). The lids prevented the penetration of ambient air but allowed the (formed) gases inside the barrel to escape above a certain overpressure within the barrel.

After the ensiling procedure was complete, the barrels were stored indoors to ensure constant ambient air temperatures (23.2 ± 1.4°C). Finally, the barrels were opened on day 149 to collect material samples for laboratory analysis (see Sections 3.2.2 and 3.3.1).

During wilting, the outdoor air temperatures were collected from the German weather service's climate data centre (station ID 603; opendata.dwd.de, 2021); the weather station itself was positioned in Bonn-Roleber (Germany, 50 44′06.4″ N 7 11′ 35.2″ E). During forage storage, the ambient air temperature was measured using NTC thermistor sensors (TinyTag Plus 2 Logger TGP-4500; Gemini Data Loggers Ltd, Chichester, UK). The temperature measurement interval was 15 min.

3.2.2 Laboratory analysis of the silage material

Material samples were collected from the fresh material (on the day of ensiling, before filling barrels with SW variants) and the ensiled material (149th ensiling day). All samples were stored immediately at -20°C until they were analysed, which is necessary to avoid further microbial activity and material composition changes before analysis. Because various types of technical equipment were required for analysis, the samples were sent to different laboratories for testing.

The first laboratory (Institute of Animal Science, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany) analysed the crude ash (see Section 3.3.1) and crude protein concentration of samples according to specific numbered methods in the German Handbook of Agricultural Research and Analytic Methods (VDLUFA, 2012). Crude ash was determined by ashing the silage samples at 550°C (method number 8.1). Crude protein concentration was determined using the Dumas combustion method (method number 4.1.2; using a FP328, Leco 8.1; Leco Instrumente, Mönchengladbach, Germany) in which the sample is burnt at 1,000°C, nitrogen oxides are reduced, and other combustion products are removed. The remaining molecular nitrogen was detected using a thermal conductivity detector and the data were used to calculate the crude protein

concentration. These measurement methods have been well established in previous studies (Brüning et al., 2018; Gerlach et al., 2014, 2018).

The second laboratory (Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Universität zu Berlin, Berlin, Germany) analysed the pH and concentrations of lactic acid, acetic acid, butyric acid and its (higher) homologues (in a total sum of n- and iso-butyric acids, C_4 molecules; n- and iso-valeric acids, C_5 molecules; and n-caproic acid, C_6 molecules), and ammonia–N (NH_3–N). DM was corrected for losses of volatiles during drying (Weiß et al., 2020) according to the method of Weißbach and Strubelt (2008). After pre-treatment, lactic acid levels were determined using a liquid chromatography method (via refractive index detection; LC-20 AB, Shimadzu Deutschland, Duisburg, Germany). The other acids were detected using a gas chromatograph (including a flame ionisation detector; GC-2010, Shimadzu Deutschland, Duisburg, Germany) with a free fatty acid phase column (Permabond FFAP 0.25 µm; Macherey-Nagel, Düren, Germany). NH_3–N levels were determined colourimetrically using a continuous flow analyser (San++, Skalar Analytical, Breda, Netherlands). This laboratory methodology has been described in detail in previous studies (Brüning et al., 2018; Gerlach et al., 2018; Weiß et al., 2020; Weiß and Sommer, 2012).

3.2.3 Sampling and analysis of fermentation gases

During the early part of the ensiling period (i.e. the first 49 days), gas samples were taken manually at regular intervals from the headspace of the silage barrels. Samples were collected using a double cannula connected to a vacuumed glass vial (20-mL volume; Jungbluth et al., 2016; Schmithausen et al., 2018b). The intervals at which gas samples were collected from the barrels varied during the ensiling process: in the first 12 h, the sampling interval was 30 min; from 12 to 48 h, the sampling interval was 2 h; and from ensiling days 3–49, the sampling interval was 2 days. No further gas samples were taken after the 49th ensiling day. The number of gas samples per barrel was 82, with 984 samples taken in total.

Subsequently, the gas samples were analysed using a gas chromatograph (equipped with an electron capture detector and a flame ionisation detector; model 8610C, SRI Instruments, Torrance, CA, USA) according to an established analytical procedure (Krommweh et al., 2020; Schmithausen et al., 2016). CO_2, CH_4 and N_2O were analysed with detection limits of 50.00, 0.08 and 0.01 ppm, respectively. If the concentrations were outside the measuring range of the gas chromatograph, the samples were diluted (1:101 with ambient air) and the corresponding original concentration was calculated.

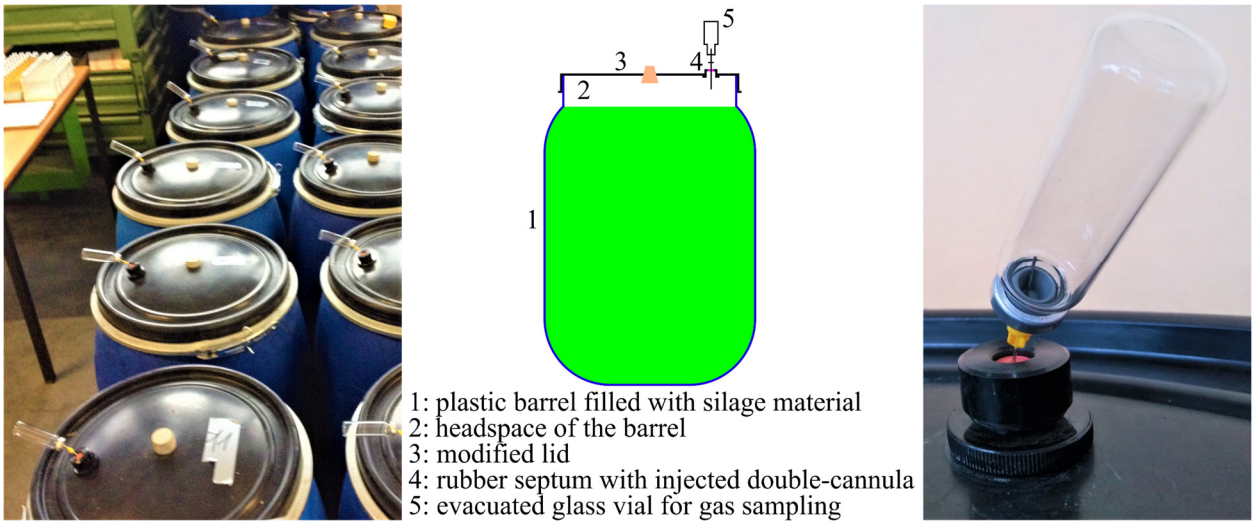


Fig. 3.2 Silage barrels with injected double cannulas and glass vials for gas sampling. Left: parallel gas sampling. Middle: schematic sketch of the barrels and the gas sampling. Right: rubber septum, injected double cannula and vial.

3.2.4 Data processing and statistics

The compositions of the different silage variants were compared using one-way analysis of variance (ANOVA). The gas concentrations within the silage barrels during ensiling were compared using a mixed ANOVA. In all analyses, Tukey's-HSD test was used as a post hoc test if the homogeneity of variance was given; if not, a Games-Howell test was used. For all statistical analysis, p values < 0.05 were considered significant. IBM SPSS Statistics (Version 26.0) was used to conduct statistical analysis, whereas Microsoft Excel was used to perform descriptive data analysis.

3.3 Results and discussion

3.3.1 Composition of the silage

The fresh forage and the ensiled material had low DM concentrations (see Table 3.2) below or at the lower limit of the recommended range, which is 25%–35% DM for grass silage (Kung et al., 2018) and 30%–35% DM for lucerne silage (Kung et al., 2018; Seglar, 2003). Nevertheless, the difference in DM concentration between the SW and LW variants was significant ($p < 0.05$). The L SW variant was under the target value for the crude protein concentration of lucerne silages (however, incorrect laboratory results were possible due to NH_3–N out-gassing, as explained in the last paragraph of this Section), whereas the other variants exceed the target values, i.e. < 170 g (kg DM)$^{-1}$ for grass silage (Spiekers, 2012) and ~ 200 g (kg DM)$^{-1}$ for lucerne silage (Seglar, 2003). These characteristics are due to the early cutting time of lucerne, a leafy second grass cut, the fertiliser management process and the low external air temperature during wilting (see Table 3.1).

Table 3.2 Chemical compositions of fresh and ensiled materials [A] for the tested silage variants [B].

Variant	pH	Dry matter	Crude ash	Crude protein	Lactic acid	Acetic acid	Butyric acid [C]	Ammonia nitrogen	Ammonia nitrogen
		[g (kg FM)$^{-1}$]	[g (kg DM)$^{-1}$]						[g (kg total N)$^{-1}$]
G_{fresh}	6.4	258	131	172	2.2	16.2	0.5	3.3	119
L_{fresh}	6.9	237	118	217	2.0	17.7	0.7	4.2	121
G SW [D]	**4.9**^{a} ± 0.2	**215**^{b} ± 3	**131**^{a} ± 0.3	**208**^{b} ± 8.1	**47.2**^{c} ± 11.0	**11.3**^{a} ± 1.0	**41.6**^{a} ± 7.4	**6.9**^{a} ± 0.5	**206**^{a} ± 12
L SW [D]	**6.2**^{b} ± 0.0	**195**^{a} ± 3	**179**^{b} ± 3.5	**176**^{a} ± 4.0	**1.9**^{b} ± 0.1	**38.6**^{b} ± 2.3	**83.5**^{b} ± 7.2	**22.2**^{b} ± 1.2	**788**^{c} ± 66
G LW [D]	**4.8**^{a} ± 0.1	**262**^{d} ± 1	**127**^{a} ± 4.9	**225**^{c} ± 1.7	**64.6**^{c} ± 7.4	**9.6**^{a} ± 1.7	**26.5**^{a} ± 0.7	**5.8**^{a} ± 0.1	**160**^{a} ± 5
L LW [D]	**7.1**^{c} ± 0.2	**227**^{c} ± 1	**185**^{b} ± 9.0	**246**^{c} ± 8.9	**0.0**^{a} ± 0.0	**29.9**^{b} ± 4.0	**69.0**^{b} ± 7.6	**22.8**^{b} ± 0.3	**580**^{b} ± 22

FM = fresh matter, DM = dry matter, N = nitrogen.

Means with different superscript lowercase letters within a column differ significantly (Tukey's-HSD or Games-Howell tests, $p < 0.05$).

[A] Fresh material was sampled on the ensiling day before the SW variants were ensiled; ensiled material was sampled on the 149th ensiling day.

[B] Variants: grass shortly wilted (G SW), lucerne shortly wilted (L SW), grass longer wilted (G LW) and lucerne longer wilted (L LW).

[C] Sum of n- and iso-butyric acids (C_4 molecules), n- and iso-valeric acids (C_5 molecules) and n-caproic acid (C_6 molecules).

[D] Means ± standard deviation.

High crude protein, NH_3–N and crude ash concentrations in the silage have buffering properties and can inhibit a rapid decrease in pH at the beginning of ensiling (Kung et al., 2018; Kung and Shaver, 2001). Both grass variants had pH values at the upper end of the recommended values, i.e. 4.0–5.0; however, especially with low DM concentrations, pH values should be at the low end of this target range (Spiekers, 2012). L SW and L LW variants had pH values that were too high given that the maximum target value is pH 4.5 (Kung et al., 2018; Seglar, 2003). These values can be explained by high NH_3–N [partially in the form of dissolved ammonium–N (NH_4^+-N), alkaline substances], low lactic acid and high butyric acid concentrations in the lucerne silage. The butyric acid concentration was greater than the given maximum value of 3 g $(kg\ DM)^{-1}$ (Spiekers, 2012); thus, it can be considered an indicator of the undesirable activity of clostridia (Rooke and Hatfield, 2003), which can metabolise lactic acid to butyric acid, hydrogen (H_2) and CO_2 during anaerobic (saccharolytic) metabolism. Clostridia are particularly active in silage with pH values $>$ 4.2–4.5 (Pahlow et al., 2003) and DM concentrations $<$ 30%–35% (Kung et al., 2018). The LW variants tested here tended to have low butyric acid concentrations ($p < 0.1$ within G variants; $p < 0.05$ within L variants).

All tested materials showed higher NH_3–N concentrations than those measured in previous analyses (Hartinger et al., 2019; Kung and Shaver, 2001; Wyss et al., 2017). Previously, Weiß (2001) reported NH_3–N concentrations of up to 30% in a grass-legume silage mix with added clostridia spores. Wet silages seem to have higher NH_3–N concentrations (Hartinger et al., 2019; Kung and Shaver, 2001) and low wilting intensity also favours high NH_3–N concentrations (Hartinger et al., 2019). Concentrations of NH_3–N at $>$ 12% of total N indicate protein degradation by enterobacteria (Pahlow et al., 2003; Spoelstra, 1987) and clostridial activity (Kung and Shaver, 2001; Pahlow and Hünting, 2012). Enterobacteria, which are particularly active during the early stage of ensiling (Heron et al., 1993), can convert nitrate first to nitrite and then to NH_3 and N_2O during denitrification at high pH values ($>$ 4.5). Nitrate is known to have an inhibitory effect on clostridia (Kaiser and Weiβ, 2007; Weiβ, 2001; Wilkinson, 1999), but this was apparently insufficient in the tested materials. Proteolytic clostridia metabolise various proteins and amino acids to NH_3 (Ohshima and McDonald, 1978; Weiβ, 2001), among other compounds, during the second stage of anaerobic (clostridial) malfermentation (deamination, decarboxylation and Stickland reactions) (Kaiser and Weiβ, 2007; Rooke and Hatfield, 2003; Weiβ, 2001). However, clostridial activity has the greatest impact (Kaiser and Weiβ, 2007); it causes the formation of ammonia, butyric acid and (higher) homologues (Weiβ, 2001) (Table 3.2).

The combination of low lactic acid, high butyric acid and high NH_3–N concentrations indicates high clostridial activity (Kaiser and Weiβ, 2007; Weiβ, 2001), in which the saccharolytic clostridia

that are active at > 4.2 pH (Pahlow et al., 2003) precede the proteolytic clostridia (Weiβ, 2001) that are active at > 5.0 pH (Pahlow et al., 2003), during the malfermentation phase (lactic acid degradation). The conversion of high-value proteins and amino acids to NH_3–N is known to reduce feed quality (Kung and Shaver, 2001; Wilkinson, 1999) and lead to gaseous emissions, especially when the material has high pH values (i.e. when the equilibrium between volatile ammonia and nonvolatile ammonium shifts towards ammonia). These emissions could be relevant during material analysis, e.g. potential NH_3–N emissions before or during the analysis of crude protein in the L SW variant could affect the calculation of relative NH_3–N concentrations, and for determination of environmental pollution. Further research is therefore recommended because NH_3 emissions were not assessed during this investigation.

3.3.2 Gas formation during the ensiling process

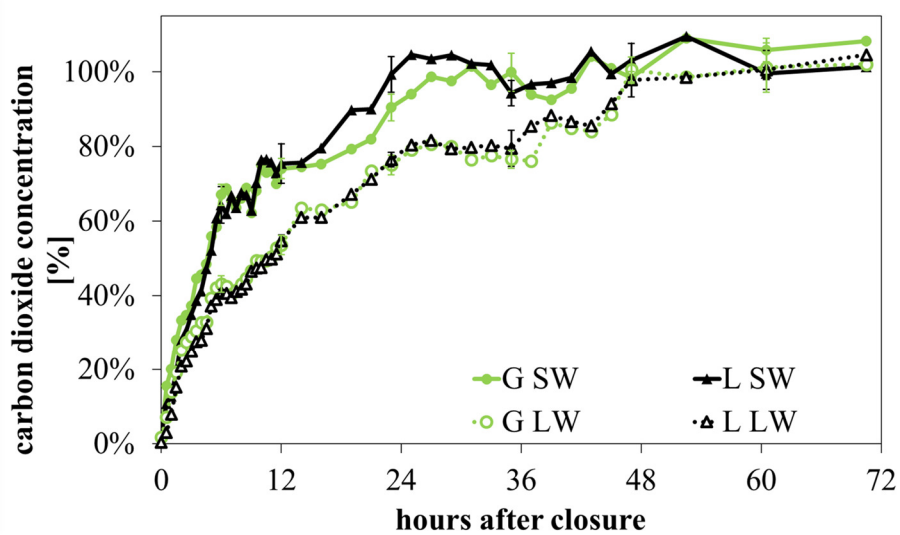
3.3.2.1 CO_2

A rapid increase in CO_2 concentration was detected in all silage barrels (see Fig. 3.3). The G and L variants did not differ in terms of these concentrations. However, lower DM concentrations in the SW variants led to a significantly faster increase in CO_2 concentrations at the start of ensiling, i.e. between ensiling hours 4.5 to 35.0 ($p < 0.05$; exception for ensiling hour 19). CO_2 concentrations above 100% may have resulted from the analysis of diluted gas samples (see Section 3.2.3); thus, a modified methodology (e.g. dilution with pure nitrogen or a smaller dilution ratio) should be considered in future investigations.

The course of the measured CO_2 concentrations is in agreement with data published in earlier literature, which show a rapid increase in CO_2 concentrations in silage (Li et al., 2017; Wang and Burris, 1960). Wang and Burris (1960) showed a degressive course in the measured CO_2 concentrations of maize silage (field tower silos), which is consistent with the results of the present study (see Fig. 3.3) and with those reported by Li et al. (2017). In contrast, Wang and Burris (1960) recorded a linear increase in a feed mix containing soybean and Sudan grass (42.6% CO_2 after 60 h). However, the measured concentrations can vary depending on the gas tightness of the silos and the remaining air inside.

Li et al. (2017) showed that CO_2 formation starts once the silo is closed but that most of the CO_2 is formed by lactic acid bacteria after anaerobic conditions have been reached. Gomes et al. (2019) found that lower DM concentrations lead to higher gas formation, which explains the rapid increase in CO_2 concentrations in the SW variants observed in the present study between ensiling hours 4.5 to 35.0 (see Fig. 3.3). Higher water availability affects microbial activity, regardless of the plant material. Faster gas formation can be interpreted as a rapid start to ensiling when it is (primarily) due to lactic acid bacteria. In the present study, all variants were characterised by wet

plant material that provokes malfermentation. Thus, even the low DM concentrations are not recommended and the target values should be used in practice (see Section 3.3.1).



G SW	b	b	b	b	a	a	a
L SW	b	b	b	b	a	a	a
G LW	b	a	a	a	a	a	a
L LW	a	a	a	a	a	a	a

Fig. 3.3 Mean carbon dioxide concentration (%) within the headspace of the silage barrels (n = 3) containing each silage variant during the ensiling process.
Error bars indicate standard deviations. Significant differences ($p < 0.05$) among the four variants at selected time points are indicated by different lowercase letters.
Variants: grass shortly wilted (G SW), lucerne shortly wilted (L SW), grass longer wilted (G LW) and lucerne longer wilted (L LW).

During CO_2 formation, the pressure inside the silage storage containers increases (Daniel et al., 2016; Daniel and Nussio, 2015b; Li et al., 2017) and the gases escape, which results in measurable (GHG) emissions (Schmidt et al., 2011, 2012). CO_2 concentrations are correlated with the microbial activity and positive pressure in the silage barrel (Daniel et al., 2016; Li et al., 2017). After completing the main fermentation phase (Pahlow et al., 2003), a pressure drop can be detected that leads to negative pressure in the silage containers (Schmidt et al., 2018). This could be attributable to the dissolution of CO_2 in the liquid phase (Li et al., 2017) or microbial activity (possibly the Wood-Ljungdahl pathway) (Schmidt et al., 2018; Vigne et al., 2019). Qualitative observations in the current study, i.e. lids slightly curved inwards, suggested negative pressure within the silage barrels containing G variants. In contrast, barrels with L variants showed overpressure during the complete anaerobic storage phase, i.e. the lid was curved outwards; moreover, when the clamping rings on the barrel were opened on ensiling day 149, gas was observed to escape immediately. This indicates that the lucerne silage never reached the stable anaerobic storage phase (Pahlow et al., 2003), which was confirmed by the butyric acid formation results (see Section 3.3.1). Consequently, steady but varying gas formation may have occurred

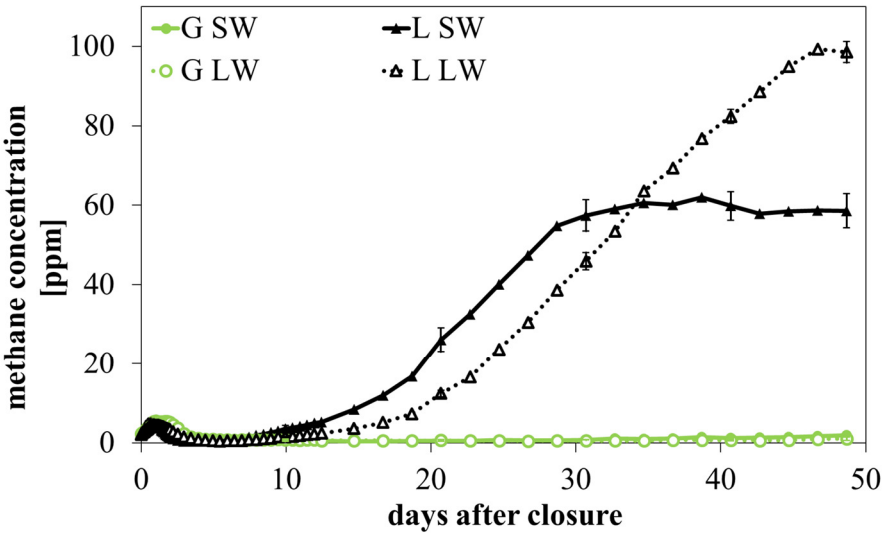
from the L variants over the storage period, which resulted in an outward gas flow. However, gas formation can vary widely (Daniel et al., 2015; Daniel and Nussio, 2015a; Gomes et al., 2019); therefore, it is difficult to predict the emission quantities produced in these trials.

Nevertheless, continuous gas formation is relevant for evaluating the measured CH_4 and N_2O concentrations (see Sections 3.3.2.2 and 3.3.2.3). Furthermore, emission measurements should be performed for both high- and low-quality silages in future studies.

3.3.2.2 CH_4

The four experimental silage variants had an initial CH_4 concentration peak that varied from 4.6 ± 0.2 to 5.8 ± 0.3 ppm between ensiling hours 16.2 ± 4.8 and 39.2 ± 3.1 (Figs. 3.4 and 3.6). The G SW variant showed a significantly higher CH_4 concentration than the G LW variant between ensiling hours 6 and 27 ($p < 0.05$) but lower concentrations between ensiling hours 40 and 106. A similar trend applied to the L SW and L LW variants for ensiling hours 7 to 21 and 31 to 94, respectively. After reaching these peaks, the CH_4 concentrations in all variants decreased to < 1 ppm. The CH_4 concentrations in the G barrels were constant after ensiling day 6 (0.7 ± 0.4 ppm), whereas the CH_4 concentrations from lucerne silage substantially increased after ensiling day 12 resulting in a significant difference between the G and L variants ($p < 0.05$). Additionally, the L SW variant had significantly higher CH_4 concentrations than the L LW variant for ensiling days 9.5–10.5, 11.5–12.5 and 21.0–30.0 but lower concentrations after ensiling day 38 ($p < 0.05$).

Studies of CH_4 formation within forage, especially in silage, are scarce. Emery and Mosier (2015) studied CH_4 concentrations at the laboratory-scale using gas-tight plastic containers with aerobically stored nonforage switchgrass and corn stover. During the storage phase (59 days), these researchers were generally able to measure CH_4 concentrations at 2–15 ppm (although single peaks were up to 2,100 ppm); low moisture concentrations resulted in the highest CH_4 concentrations. The current findings are only partially consistent with those of Emery and Mosier (2015), i.e. higher CH_4 concentrations were detected in the SW variants during the first ensiling day (Figs. 3.4 and 3.6); however, the SW variants had lower CH_4 concentrations in the second phase of the initial peak. The earlier formation of CO_2 in the SW variants (see Section 3.3.2.1) led to the release of CH_4 via outward gas mass flow. In one study, Schmidt et al. (2011) measured CH_4 concentrations at 2 ppm in sugarcane silage on ensiling days 5, 33 and 61; in another study, they detected 7 ppm CH_4 in maize silage on ensiling days 5 and 15 (Schmidt et al., 2012). Krommweh et al. (2020) measured CH_4 concentrations of 3.2–9.6 ppm within grass silage bales and of 10.2–24.4 ppm within lucerne silage bales at the time of silage opening. However, Gerlach et al. (2018) did not detect CH_4 emissions during the feed-out phase of maize and lucerne silage.



G SW	a	a	a	a	a
L SW	b	c	c	b	b
G LW	a	a	a	a	a
L LW	a	b	b	c	c

Fig. 3.4 Mean methane concentration (ppm) within the headspace of the silage barrels (n = 3) containing each silage variant during the ensiling process.
Error bars indicate standard deviations. Significant differences ($p < 0.05$) among the four variants at selected time points are indicated by different lowercase letters.
Variants: grass shortly wilted (G SW), lucerne shortly wilted (L SW), grass longer wilted (G LW) and lucerne longer wilted (L LW).

To our knowledge, a detailed course of CH_4 concentrations during the ensiling process has yet to be reported. Consequently, the curves shown in Figs. 3.4 and 3.6 represent new information on the time course of CH_4 formation. Nevertheless, further investigations with various forage types will be necessary.

An increase in CH_4 concentrations (see Fig. 3.4) has yet to be detected during the ensiling process. One earlier postulated explanation (Pahlow et al., 2003; Spoelstra, 1983; see Section 3.3.1) is as follows: during lactate degradation to butyric and acetic acid, clostridia can form H_2 that is converted to CH_4 during anaerobic methanogenesis. In addition, archaea can form CH_4 (as well as CO_2 and other compounds) from H_2 and acetic acid (Aumüller-Gruber et al., 2013). Given the current lack of research related to CH_4 production, it is unclear which microorganisms within the silage contribute to methanogenesis. Likewise, it is uncertain whether the population of methanogenic microorganisms differs among different forage materials (Emery and Mosier, 2015; Yenjai et al., 2012) and among different stages of the anaerobic storage phase. However, the production of biogas has shown that obligate anaerobic methane-forming organisms are active when pH values are neutral (Aumüller-Gruber et al., 2013). Thus, the organisms likely benefit from the provision of H_2 and the rising pH values during the course of lactic acid degradation and

NH_3 or NH_4^+ formation, respectively, in lucerne variants (see Section 3.3.1). Consequently, increasing CH_4 concentrations may be useful for indicating clostridia activity. Compared with the L LW variant, the L SW variant showed an increase in CH_4 concentration at an earlier stage of the ensiling process (Fig. 3.4). The higher water availability seems to affect the onset of microbial activity (see Section 3.3.2.1). However, the L LW variant subsequently shows higher CH_4 concentrations than the L SW variant. This is in line with higher pH values and lower lactic acid concentrations (Table 3.2), i.e. indicators of higher clostridial activity, as well as previous reports in the literature (Emery and Mosier, 2015).

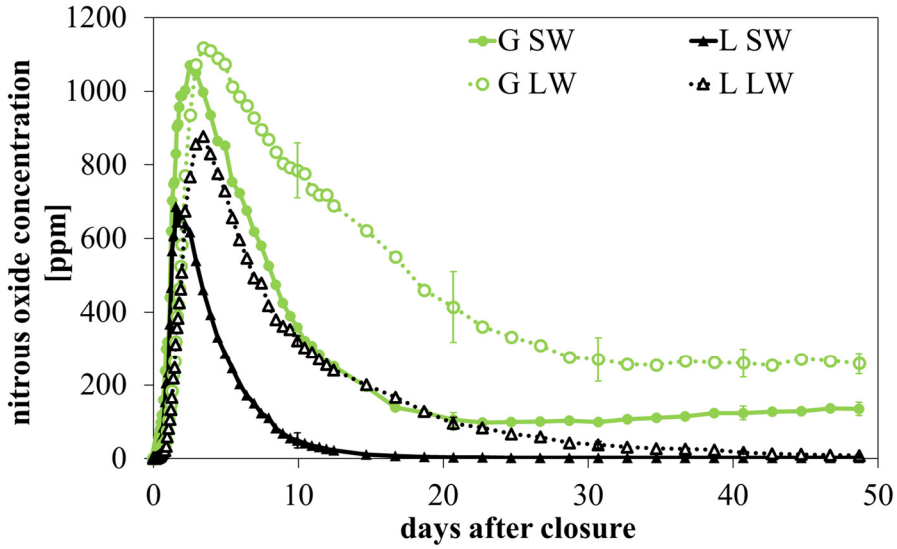
Gerlach et al. (2018) stated that '*fermented forages seem to be an unlikely source of CH_4 emissions*', which is contradicted by the current results, at least for lucerne silage with poor ensiling quality. At present, it is not possible to determine whether CH_4 is formed at climate-relevant levels. Further investigations with different forage types and (induced) clostridial activity must therefore be conducted.

3.3.2.3 N_2O

The time course of N_2O concentrations in the silage barrels revealed that N_2O levels increased for all variants in the first hours of the ensiling process, peaked between ensiling hours 38.3 ± 4.2 and 94.5 ± 6.9, and then subsequently regressively decreased (Fig. 3.5). The grass silage produced higher N_2O concentrations than the lucerne silage from ensiling day 3 onwards. Thus, with a few exceptional individual gas samples, significant differences between the G SW and L SW variants and between the G LW and L LW variants, respectively, were consistently detected ($p < 0.05$). Additionally, higher DM concentrations in individual forage led to higher N_2O concentrations but delayed N_2O peaks. The barrels containing the LW variants had significantly lower N_2O concentrations than those containing the SW variants between ensiling hours 16 to 45, but N_2O levels were higher in the former from ensiling days 3.5–16.0 ($p < 0.05$). After the peaks had been reached, the N_2O concentrations of L variants decreased to < 10 ppm until ensiling day 47. In the G variants, N_2O concentrations remained at higher levels; thus, from ensiling day 29 onwards, a significant difference in N_2O concentrations was detected between the G and L variants ($p < 0.05$).

Zhao et al. (2016) measured N_2O concentrations in maize silage (gas sampling during the first week of ensiling in a laboratory-scale silage experiment) at 1,806–1,836 ppm. Wang and Burris (1960) measured N_2O concentrations in a field silo (maize silage) at 10,000–43,500 ppm within the first 66 h of ensiling. The substantially higher values reported by Wang and Burris (1960) have already been discussed by Zhao et al. (2016) with one possible explanation: the identical mass of CO_2 and N_2O could have led to uncertain N_2O values in the course of mass spectroscopy and subsequent differentiation. The current results tend to confirm N_2O levels stated by Zhao et al.

(2016). In the emission studies from a Brazilian working group, detected N_2O concentrations were 1–937 ppb in emitted gas samples from various silages (Schmidt et al., 2011, 2012). Furthermore, Franco (2016) detected emission rates of 374 mg (kg DM)$^{-1}$ up to the 120th hour of ensiling for lucerne silage, although no further emissions occurred after this point. Additionally, Gerlach et al. (2018) reported that lucerne silage with pH values of ~ 5.8 emitted N_2O during the feed-out phase.



G SW	b	a	b	c	b
L SW	a	a	a	a	a
G LW	c	b	b	d	c
L LW	b	a	a	b	a

Fig. 3.5 Mean nitrous oxide concentration (ppm) within the headspace of the silage barrels (n = 3) containing each silage variant during the ensiling process.
Error bars indicate standard deviations. Significant differences ($p < 0.05$) among the four variants at selected time points are indicated by different lowercase letters.
Variants: grass shortly wilted (G SW), lucerne shortly wilted (L SW), grass longer wilted (G LW) and lucerne longer wilted (L LW).

Lower DM concentrations led to earlier gas formation (see Sections 3.3.2.1 and 3.3.2.2). After a specific point within the first four days of ensiling (N_2O peaks; Fig. 3.5), no further (relevant) amounts of N_2O were produced. The formation by enterobacteria ends as soon as the nitrate and respective nitrite contents in the materials have been entirely converted or when the enterobacteria are inhibited by decreasing pH values (see Section 3.3.1). Unfortunately, pH measurements and nitrate concentrations were not recorded for this period. Consequently, it is unclear whether additional N_2O was formed from the LW variants or whether the higher concentrations were due to other unknown effects. N_2O concentrations decreased more quickly in the SW variants than in LW, which might have been due to the higher bacterial activity and earlier CO_2 formation in the former (see Sections 3.3.2.1 and 3.3.2.2). After fermentation was complete, the grass silage did not produce any additional gas; thus, the produced amounts of N_2O remained in the barrels.

For the L variants, it can be assumed that the outward gas flow entirely released the produced N_2O (see Section 3.3.2.1). Nevertheless, enterobacteria may have remained active and formed N_2O from other sources because the pH of the lucerne silage was above the critical activity limit of enterobacteria, i.e. > 4.5–5.0 (Gerlach et al., 2018; Spoelstra, 1985). However, considering the increased CH_4 concentrations in the barrels (see Section 3.3.2.2), it can be assumed that relevant amounts of N_2O were not produced (Fig. 3.5). Franco (2016) showed that lucerne silage emitted significantly higher amounts of CO_2 and N_2O than were emitted by maize silage. Nonetheless, further studies will be necessary to determine the emission behaviour during the ensiling process for different forage types under various conditions and ensiling management practices.

3.3.2.4 CH_4 and N_2O concentrations within the first four ensiling days

Fig. 3.6 shows that, for each silage variant, CH_4 formation occurred before N_2O formation. The time interval between the concentration peaks of the two gases was longer in the LW variants: 34.7 ± 13.3 h for G SW, 22.2 ± 5.6 h for L SW, 55.3 ± 5.0 h for G LW and 66.0 ± 2.0 h for L LW, respectively.

At this stage, a final conclusion cannot be made regarding which microorganisms and metabolic processes are involved in gas formation at the beginning of ensiling. One potential explanation follows, although other (biochemical) formation processes are also possible (e.g. degradation of cell components). It is possible that facultative anaerobic enterobacteria enzymatically convert formate (HCO_2^-) into CO_2 and H_2 during the first hours (Pahlow et al., 2003). The increase in CH_4 concentration ended when CO_2 concentrations exceeded 88.9% for G SW, 81.4% for L SW, 84.1% for G LW and 74.4% for L LW. The formed H_2 can be used for methanogenesis because of the anaerobic conditions and formation of small anaerobic pockets within aerobic plant material (Emery and Mosier, 2015; Yenjai et al., 2012). Additionally, the residual respiration of the forage and the increasing activity of the lactic acid bacteria lead to complete anaerobic conditions and a decrease in pH levels. The former condition leads to increased formation of N_2O by enterobacteria (see Sections 3.3.1 and 3.3.2.3). This process releases oxygen that this is rapidly respired. The latter condition first inhibits methanogenic microorganisms, which are typically active at higher pH levels, e.g. > 6.8 pH for archaea (Aumüller-Gruber et al., 2013), and then inhibits the enterobacteria, which are active at > 4.5 pH (see Section 3.3.1). Consequently, the first CH_4 peak and the N_2O peak can be attributed (indirectly) to the metabolism of the enterobacteria.

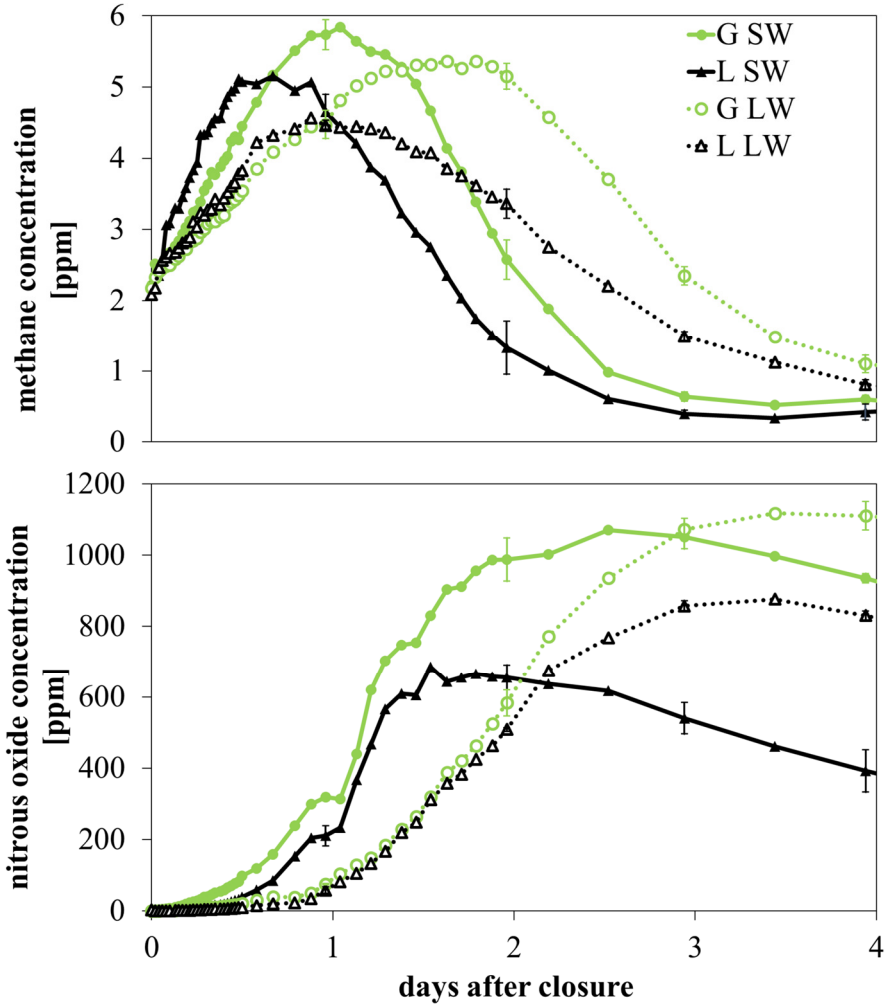


Fig. 3.6 Mean methane and nitrous oxide concentrations (ppm) within the headspace of silage barrels (n = 3) containing each silage variant within the first 4 days of the ensiling process.
Error bars indicate standard deviations at selected time points.
Variants: grass shortly wilted (G SW), lucerne shortly wilted (L SW), grass longer wilted (G LW) and lucerne longer wilted (L LW).

The higher measured gas concentrations suggest that the activity of enterobacteria is higher in the G variants. Unfortunately, the concentrations of formate and nitrate in the fresh material and microbial population were not analysed. Consequently, it is not possible to conclude whether additional substrate or a bigger microbial population led to increased gas formation in the grass variants. The similar CO_2 formation results for the two forages (see Section 3.3.2.1) suggest that more CH_4 and N_2O were formed in the grass variants. Earlier release of the gases via CO_2 mass flow seems unlikely. However, it is unclear why the time interval between the gas peaks was longer in the LW variants. A more detailed investigation of gas concentrations and emission quantities could potentially clarify when the gases are released; thus, further studies evaluating GHG formation during this period should be conducted.

3.3.3 Examination of the methodological procedure

In this study, 82 gas samples per silage barrel were taken and analysed using a gas chromatograph; overall, 984 gas samples were analysed. To our knowledge (see Section 3.1), this is the first study to investigate such an extended ensiling period (49 days) using such short sampling intervals (as frequent as 0.5 h). Furthermore, this study used the largest common laboratory-scale containers available (120-L volume), whereas previous studies mainly used much smaller silos (maximum volume: 20 L) (Bueno et al., 2020; Franco, 2016; Schmidt et al., 2011, 2012).

The gas samples were taken in the headspace of the standing laboratory-scale barrels above the silage material. Although the methods used do not provide an answer to the open question (Zhao et al., 2016) of how relevant gas measurements taken at the laboratory-scale are on a practical scale, the plastic barrels used here are established laboratory silos (Jungbluth et al., 2017; Sun et al., 2015) and the gas sampling technique is comparable to that used in earlier studies (Bueno et al., 2020; Restelatto et al., 2019; Schmidt et al., 2011, 2012). In practice bunker silos, gas formation during the ensiling process is observed through inflated silo film or gas escape at ground level (for instance, reddish-brown gas clouds for nitrous gases containing nitrogen dioxide). CO_2 and N_2O have a higher molecular weight than air and can accumulate within the silage barrels. The gas measurements show a N_2O peak in the first ensiling days (no discolouration was visible), but gas concentrations may have been higher in the deeper layers of the barrel. However, gas formation (mainly CO_2) from the material led to an outward gas flow (see Section 3.3.2.1), which escapes between the barrel and the lid. The gas sampling point (see Section 3.2.3) was located at the edge of the lid; thus, the escaping gases, which contained quantities of N_2O, flowed past the measuring point. Given the lower molecular weight of CH_4, the concentration of this gas (see Section 3.3.2.2) could be measured continuously. Consequently, it is assumed that the measurement results are transferable to practice silos with a comparable ensiling process.

The measurement of GHGs using a gas chromatograph is an established method that is practically suitable for use in animal houses, even when gas concentrations are low (Schmithausen et al., 2016, 2018b). However, this methodology is not applicable for measurements in practice. Therefore, additional studies are required to examine the potential use of rapid testing systems with lower measurement accuracy to determine the appropriateness of the ensiling process in practice.

In the present study, the measurements taken using the tested materials under the specific conditions showed that gas concentrations within silage vary considerably over time. On the one hand, the experiment indicated that CH_4 formation preceded N_2O formation by several hours

during the first days of the ensiling process (see Section 3.3.2.3). On the other hand, the measured values show CH_4 formation after ensiling day 12 in silages with increased butyric acid concentrations (see Section 3.3.2.1). To our knowledge, this was the first practical measurement to show this phenomenon. Therefore, the methodology described here could provide further insights into the metabolic activity of microbiota during the ensiling process.

Unfortunately, the quantification of GHG emissions was not possible in this study because the objective was instead to record detailed concentration courses over time. Schmidt et al. (2012, 2011) concluded that GHG emissions from the ensiling process play a subordinate role compared with the emissions from cattle and dairy farming processes. This statement could be re-examined considering the varying gas concentrations observed during the ensiling process as well as calculated emission quantities. Any projections of GHG emission quantities based on one-time measurements of gas concentrations (Bueno et al., 2020; Schmidt et al., 2011, 2012) could underestimate or overestimate the emissions from silage (especially in cases with poor ensiling quality). Further studies should also include assessment of NH_3 concentrations or emissions as well as more extensive material analyses.

Finally, it can be concluded that, in addition to chemical analyses of silage material, repeated gas analyses, as described in the present study, could contribute to improving our understanding of the ensiling process and ensiling quality. Therefore, new studies addressing open research questions and comparing various types of forage are recommended.

3.3.4 Implications for ensiling management research

In practice, silages are too often of poor quality; hence, it is necessary to investigate these situations appropriately. In some regions of the world, weather fluctuations and relatively short harvest periods (due to precipitation) can impair harvest conditions, harvest security and fermentation capacity (Persson and Höglind, 2014). In addition, the demand for high-quality silage will increase globally over time (Wilkinson, 1999; Wilkinson and Muck, 2019). However, if high-efficiency harvesting machinery is not available, it is difficult to use shorter harvesting periods effectively. For this reason, fresh material with low DM concentration was used here to provoke malfermentation and simulate adverse harvest conditions. However, differences in the gas courses of the silage variants show that DM concentrations noticeably affect microbiological gas formation. Additional research should therefore involve experiments with varying DM concentrations.

According to current knowledge, malfermentation leads to five negative outcomes: (1) decreased feed intake (Spiekers, 2012), (2) reduced feed quality (especially energy and protein losses) (Wilkinson, 1999), (3) increased feed quantity demand to fulfil the nutritional needs of the

animals, (4) higher levels of feed disposal, and (5) increased direct GHG emissions during the anaerobic storage period. The first four factors lead to a rise in indirect emission quantities and climate impacts from animal feeding and biogas production, respectively, because silage quantities must be increased to produce the final outputs. This situation may also be applicable to the ensiling of other forages (e.g. maize or whole-plant grain silage), although further experimental studies are required to confirm this. Future studies must also examine whether significant GHG emissions occur in this case.

Especially in modern times, various methods should be used to investigate and analyse the environmental impact of ensiling for livestock feed or biogas production and the optimisation of these methods should be attempted. The use of ensiling additives to control the ensiling process may be one viable option. The addition of chemical compounds or microorganisms (mostly homofermentative or heterofermentative lactic acid bacteria) could positively influence the ensiling process, which could improve ensiling quality even under unfavourable conditions. However, the production and application of these substances is associated with increased effort for which the climate impact cannot currently be quantified. A future comparison could involve the reduction in GHG emissions as result of minimising fermentation losses vs. the additional effort required to include silage additives.

This study investigated the GHG emissions of different feeds with varying DM concentrations. DM concentrations especially were found to affect the timing of microbial formation processes (see Section 3.3.2). Thus, studies must be conducted to determine the effects of various DM concentrations and investigate larger DM differences between silage variants. In addition, DM concentrations can influence other silage parameters, such as the possible packing density of the silo, and can therefore influence aerobic stability. As stated above, several additional studies are required to improve our understanding of the multidisciplinary natural and man-made silage process chains.

3.4 Conclusion

Based on the execution and results of the experiment, the sampling methodology was suitable to measure the varying gas concentration courses within the silos and conclude the gas formation. This method can be used for future fundamental research concerning different silage variants for laboratory-scale measurements. However, further research should quantify gas emissions and analyse microbial populations for more detailed insights. The short measurement intervals (down to 0.5 h) demonstrated that gas formation occurs within short periods, especially during the first four ensiling days. Lower dry matter concentrations favoured an earlier onset of CO_2, CH_4 and N_2O formation. Besides, the produced gases affect each other; the ongoing formation of CO_2 forces

the other gases out of the silos. Thus, dry matter concentration plays a significant role for the measured concentration courses and should be considered in future studies. For lucerne variants, lactate degradation and butyrate formation by clostridia (malfermentation) led to the production of CH_4 at rising pH values from ensiling day 12. This phenomenon is reported here for the first time. Thus, malfermentation impairs silage quality (reduced feed value or increased disposal quantities) and actively contributes to GHG formation. An optimal ensiling process, obtained using the best possible management practices, would therefore be desirable from the perspective of animal nutrition and environmental protection. However, further research is needed to determine GHG emission quantities and the effects of various elements such as versatile forage types, DM concentration, silage additives and environmental factors.

3.5 Supplementary information

List of abbreviations

ANOVA	Analysis of variance
DM	Dry matter
FM	Fresh matter
G	Grass
G LW	Grass longer wilted (ca. 24 h wilting)
G SW	Grass shortly wilted (ca. 20 h wilting)
GHG	Greenhouse gas
GWP	Global warming potential
L	Lucerne
LW	Longer wilted (ca. 24 h wilting)
L LW	Lucerne longer wilted (ca. 24 h wilting)
L SW	Lucerne shortly wilted (ca. 20 h wilting)
SW	Shortly wilted (ca. 20 h wilting)

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Author contributions

Alexander J. Schmithausen and Hauke F. Deeken are equal first authors of this work.

Alexander J. Schmithausen: Conceptualisation, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – Review & Editing, Visualisation, Supervision, Project administration, and Funding acquisition. Hauke F. Deeken: Validation, Formal analysis, Data curation, Writing – original draft Preparation, Writing – Review & Editing, and Visualisation. Katrin Gerlach: Conceptualisation, Validation, Investigation, Resources, and Writing – Review & Editing. Manfred Trimborn: Conceptualisation, Methodology, Validation, Formal analysis, Investigation, Resources, and Writing – Review & Editing, Supervision. Kirsten Weiß: Validation, Investigation, Resources, and Writing – Review & Editing. Wolfgang Büscher: Resources, Writing – Review & Editing, Project administration, and Funding Acquisition. Gerd-Christian Maack: Validation, Writing – Review & Editing, and Visualisation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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4 Study 2:

Greenhouse gas and volatile organic compound emissions of additive-treated whole-plant maize silage: part A—anaerobic fermentation period

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Abstract

Background: Silage emits climate- and environment-relevant gases during fermentation and feed-out periods. This trial aimed to determine the unknown carbon dioxide (CO_2), methane, nitrous oxide, ethanol, and ethyl acetate emissions of constant maize silage material over both periods. The results will be published in two consecutive articles (Part A: anaerobic fermentation period, Part B: aerobic storage period).

Methods: The untreated control (CON) was compared with the chemical additive treatment (CHE; 0.5 g sodium benzoate and 0.3 g potassium sorbate per kg fresh matter) and the biological additive treatment (BIO; 10^8 colony-forming units (CFU) *Lentilactobacillus buchneri* and 10^7 CFU *Lactiplantibacillus plantarum* per kg fresh matter). Barrel silos (n = 4) were connected to gas bags to quantify gas formation during anaerobic fermentation (30 or 135 ensiling days). Glass jar silos (n = 12) were used for laboratory silage analysis.

Results: CHE produced significantly ($p < 0.05$) less gas (6.7 ± 0.3 L per kg dry matter ensiled material (kg_{DM}) until ensiling day 14.0 ± 0.0) and ethanol (8.6 ± 1.5 mg kg_{DM}^{-1}) than CON did (8.5 ± 0.2 L kg_{DM}^{-1} until ensiling day 19.5 ± 6.4; 12.2 ± 1.5 (mg ethanol) kg_{DM}^{-1}). BIO indicates prolonged gas formation (9.1 ± 0.9 L kg_{DM}^{-1} until ensiling day 61.3 ± 51.9; 12.0 ± 2.1 mg kg_{DM}^{-1}). CO_2 is the main component of the gas formed. All treatments formed methane and nitrous oxide in small quantities. CON emitted significantly more CO_2eq emissions than BIO and less than CHE ($p < 0.05$). Additives had no effect on ethyl acetate gas emissions. For BIO, ethanol concentrations in the material ($r_S = 0.609$, $p < 0.05$) and gas quantities ($r_S = 0.691$, $p < 0.05$) correlate with ethyl acetate gas quantities. All the treatments exhibited decreasing gas and CO_2 quantities, and the dry matter mass increased between ensiling days 14 and 30 ($-0.810 \leq r_S \leq 0.442$; $p < 0.05$ to $p = 0.20$).

Conclusion: Silage generates climate- and environmental-relevant gases during fermentation and silage additives affect this pattern. Gas formation exceeds the fixation potential, and the carbon footprint of silage fermentation is negative.

Keywords

Carbon dioxide, Carbon footprint, Corn silage, Ethanol, Ethyl acetate, Lactic acid bacteria, Methane, Nitrous oxide, Silage additives

Graphical abstract

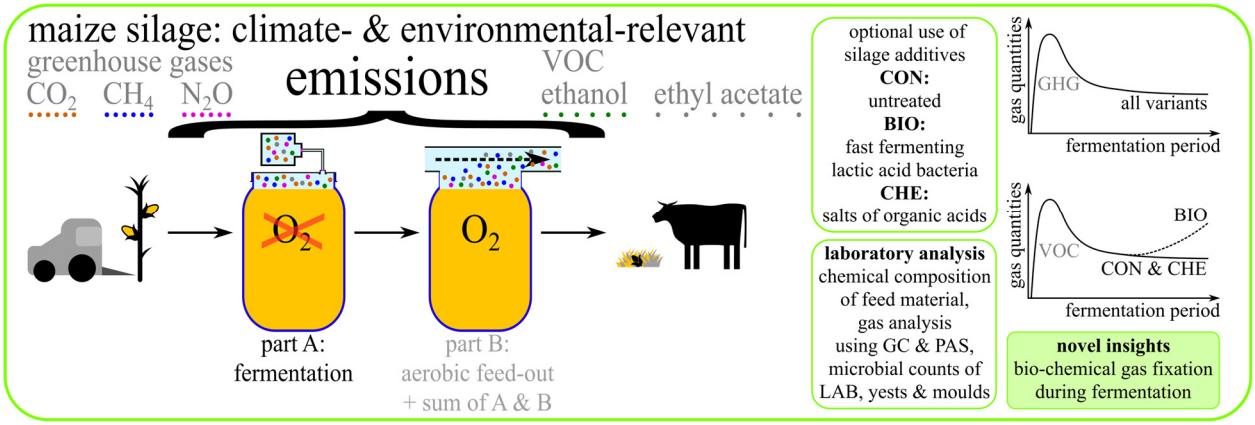

Fig. 4.1 Graphical abstract of Study 2.

4.1 Introduction

Silage is an essential global feedstuff, with the opportunity to conserve one-time crop yields. The supply of high-quality feed is crucial to feed ruminants resource-efficiently throughout the year. The same applies to biogas plants. The ensiling process includes, among others, the anaerobic main fermentation and aerobic feed-out phase [1]. One of the main objectives is to minimise dry matter (DM), energy, and quality losses to maintain the resource cycle and the nutritional value of harvested plant material in the best possible manner. DM losses in silage are generally accompanied by gaseous emissions [2, 3, 4, 5] or effluent losses.

Losses are partially unavoidable for high-quality silage fermentation, e.g. heterofermentative metabolism of lactic acid bacteria (LAB) [6, 7], but include avoidable losses, too. The latter consists of exceeding activity of undesirable microbes, such as enterobacteria, yeasts, or moulds during anaerobic fermentation or aerobic storage. Several authors provided overviews [7, 8, 9] concerning losses and management effects, e.g. silage additive (SA) use, packing density, or aerobic stability (ASTA). Köhler et al. [10] reported losses of -5 to -15% for farm-scale maize silos during anaerobic fermentation. According to Wilkinson [9], the expected total loss of maize silage production was -20.6% from field to trough. SA, such as LAB inoculants and organic acids or their salts, can influence microbial metabolism and losses in various ways. This article focuses on the specific group of SA that achieves a prolongation of ASTA through increased acetic acid (AA) production or antimicrobial properties [1, 6, 7, 11, 12].

Silage production leads to the emission of climate-relevant greenhouse gases (GHG) [2, 4, 5, 13, 14] with various global warming potentials (GWP) and other climate- and environment-relevant gases, e.g. volatile organic compounds (VOC), which are precursors of ground-level ozone formation [15, 16, 17]. The inoculation of ensiling material with heterofermentative LAB can increase DM losses [6] and gas formation during the anaerobic fermentation period [5] due to AA and carbon dioxide (CO_2) production [18]. Chemical additives can decrease DM losses [7] and the formation of VOC during anaerobic fermentation [19, 20]. Both additives can improve ASTA and, therefore, reduce respiratory emissions during the feed-out phase [6, 7]. Ethanol can be used as an indicator of VOC formation patterns, since alcohols contribute to the majority of VOC in silage [15, 16, 21]. Ethyl acetate (EA) is reported to have antibacterial and antifungal properties and may affect microbial metabolism [22, 23]. Furthermore, the high vapour pressure of EA could lead to increased volatilisation into the gaseous phase [20].

According to Schmidt et al. [13], most of the gas produced during anaerobic fermentation is CO_2. The same applies to the aerobic feed-out phase based on respiration pathways. CO_2 can be considered climate-neutral. In the carbon (C) cycle of agricultural resources, photosynthesis converts CO_2 to biomass, which will be converted back to CO_2 in later stages. While photosynthesis is considered a CO_2 sink, the other stages are CO_2 sources. If biomass is degraded to CO_2 during silage storage, those energy-rich C-molecules are unavailable in the later stages of the cycle. Therefore, DM losses during silage storage affect the C retention efficiency of the resource cycle. As far as the authors are aware, quantification of GHG and VOC emissions from anaerobic fermentation to feed-out of constant silage material is lacking in the scientific literature. Former trials examined either emissions of ensiling material during anaerobic fermentation or of ensiled material during the feed-out period. Total quantities could be used to compare the emissions during silage storage with those during the other stages of the cycle or with alternative methods of conserving animal feed. Moreover, a comparison between the carbon footprint (CF) of SA and their effect on silage emissions can be made. Therefore, the CO_2 emissions from silage storage are not classified as climate-relevant emissions but rather as emissions of climate-relevant gases.

Schmidt et al. [13] estimated that silage emissions during anaerobic fermentation are lower than those during animal husbandry. However, others demanded more research to assess the relevance of all silage production stages for VOC – and the same applies to GHG – emissions [15]. Henriksson et al. [24] stated: '*In-depth knowledge of GHG emissions associated with silage production is, therefore, crucial in mitigating GHG emissions on farm level*'. This applies in modern times, to assess the CF of various agricultural food products [25]. However, some studies

have reported the opposite behaviour, i.e. a gas fixation and DM increase during anaerobic fermentation, based on unclear biological or chemophysical processes [14, 26].

Previous silage emission research has shown that the activity of microorganisms leads to ongoing gas production and an outwards-directed gas flow from silos [2, 27]. Brazilian working groups assessed gas production by measuring positive pressure in silos [4] or collecting gases in a beaker [28, 29]. Knicky et al. [30] used gas bags to collect silage emissions. Most recently, Krueger et al. [14] published a calculation model to estimate CO_2 emissions during the fermentation process of ensiled maize. An American working group established a model to calculate the emission quantities of ethanol during the ensiling process [31]. However, no working group has conducted trials to verify the calculated data. Shan et al. [32] investigated the ethanol gas emissions of silage-related LAB in broth. Earlier research revealed several measurement and procedural inaccuracies [33, 34]. In one of the most recent studies, gas was sampled in the silo headspace regularly within the first 49 days of anaerobic fermentation [2]. Furthermore, gaseous substances formed during the ensiling process are emitted once the silo is opened [21, 35, 36, 37].

The quantity of emissions generated during the ensiling period is affected by microbiological activity [5]. In addition, factors, such as plant species [2, 4], the wilting period and DM concentration of the harvested material [2, 38], a delayed sealing time [20, 39, 40], and the use of SA [5, 20, 29], influence metabolite formation. SA are usually considered to ensure high silage qualities and improved ASTA [7, 8, 12]. However, the effect of heterofermentative LAB inoculation depends on the length of the fermentation process [5, 6].

Moreover, recent research has examined the negative pressures within silos [2, 41] and the ability of silage to absorb gas. An overview is given by Schmidt and Vigne [42]. Maize silage was observed to absorb supplied CO_2 and nitrogen (N_2) gas [43]. Empirical data from the Brazilian working group [26, 44, 45] were strengthened by a model for CO_2 absorption and DM build-up [14]. This model has yet to be validated. Schmidt and Vigne [42] expressed the optimistic question: '*Can silage absorb more carbon than it emits during fermentation?*' This question still has to be answered.

Within this trial, the quantification of emission masses during anaerobic fermentation was determined considering the optional use of SA. The objectives of this article are (1) to examine whether former gas concentration measurements in the silo headspace [2] are combinable with gas quantity collection [28, 29, 30] to quantify the gas quantities formed; (2) to calculate the GHG, ethanol and ethyl acetate emissions of untreated or treated (biological inoculants or chemical additives) maize silage during varying anaerobic storage periods (duration 30 or 135 days); (3) to assess the temporal changes in gas formation and fixation during the ensiling process; and (4) to

determine the chemical and microbiological parameters of the silage (as indicators of ensiling quality) and the emission quantities of climate- and environment-relevant gases.

4.2 Methods

4.2.1 Principles of the overarching trial and the two consecutive articles

A trial was conducted to determine the emissions of CO_2, nitrous oxide (N_2O), methane (CH_4), ethanol, and EA as indicators of VOC emissions from constant maize silage during anaerobic and aerobic storage. Constant material means that the forage was filled into silos, where it remained intact and unchanged for the entire trial duration (both storage periods). Forage material treatments were supplemented with SA to affect microbial metabolism. Heterofermentative LAB may lead to a trade-off between increased CO_2 formation during anaerobic fermentation and decreased respiratory losses during the feed-out phase. The impact of chemical SA on VOC gas formation during anaerobic fermentation has yet to be examined. To the authors' knowledge, this trial is the first to determine the emission quantities of constant silage material during all phases of silage storage. The results are to be presented in two consecutive research articles. This article (Part A) describes two sub-experiments (see Fig. 4.2). Experiment A1 focuses on gas formation and fixation during the anaerobic fermentation period using barrel silos. Furthermore, Part A includes the analysis of chemical and microbial composition of the treatments ensiled in glass jars used in parallel (Experiment A2). The second article (Part B) addresses the emissions during two aerobic feed-out periods and the sum emissions during anaerobic fermentation and feed-out. Furthermore, the second article provides a first step toward balancing SA's CF and effects on emission quantities during silage storage.

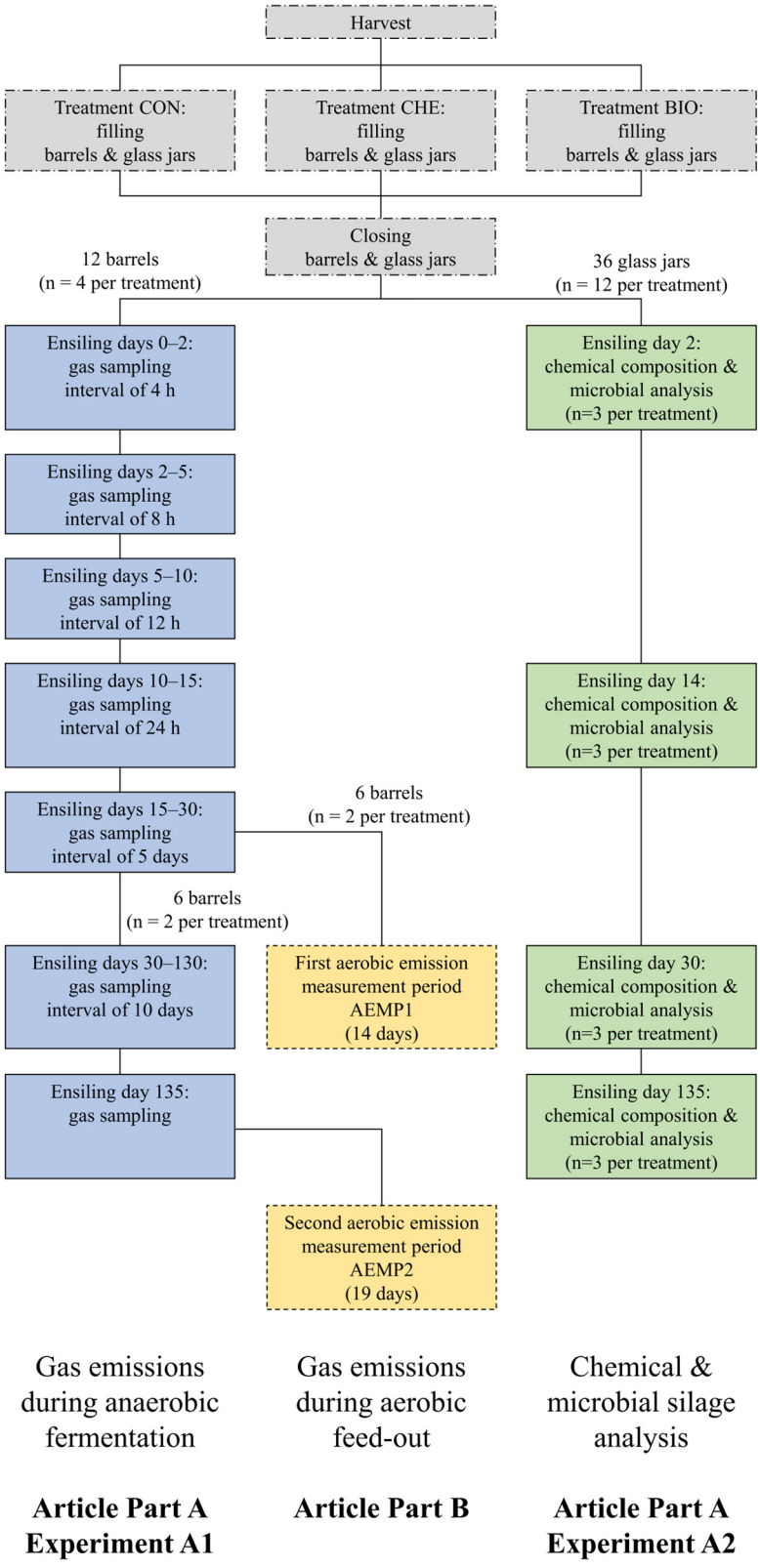

Fig. 4.2 Procedure of the overarching trial and the two consecutive articles. The processing of the treatments (grey boxes) is followed by the gas emission measurements (Article Part A, Experiment A1; blue boxes) and the analyses of chemical and microbial composition (Article Part A, Experiment A2; green boxes) during anaerobic fermentation. After 30 and 135 days of anaerobic storage, two aerobic emission measurement periods (AEMP) follow (Article Part B; yellow boxes).
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

4.2.2 Forage material, silage treatments, and ensiling management

Whole-plant maize (*Zea mays*; variant SY Werena, Syngenta Agro GmbH, Frankfurt am Main, Germany) grown at the Campus Frankenforst of the University of Bonn (Königswinter, Germany; 50° 42′ 50.1″ N, 7° 12′ 24.9″ E) was harvested on 6th October 2021 using a forage harvester with a corncracker (Claas Jaguar 940, Claas KGaA mbH, Harsewinkel, Germany). Chopped material was collected randomly. The theoretical cutting length was set to 11 mm. However, 7% of the particles were ≥ 15 mm, 28% were 10–15 mm, 34% were 6–10 mm, 22% were 3–6 mm, and 9% were < 3 mm long.

The forage material was split into equal parts to prepare the three silage treatments. The control treatment (CON) was not supplemented with a silage additive. The chemical additive treatment (CHE) was supplemented with the additive Kofasil Stabil (Addcon GmbH, Bitterfeld-Wolfen, Germany) to improve the ASTA. The resulting dosage was 0.5 g of sodium benzoate per kg fresh matter (kg_{FM}) and 0.3 g of potassium sorbate per kg_{FM}. The biological additive treatment (BIO) was supplemented with 1.0×10^8 colony-forming units (CFU) of *Lentilactobacillus buchneri* per kg_{FM} and 1.0×10^7 CFU of *Lactiplantibacillus plantarum* per kg_{FM} of silage. In detail, the original concentrations of the bacteria in the additive (SILA-BAC RAPID REACT Maize Combi, Pioneer Hi-Bred International Inc., Johnston, Iowa, USA) were obtained as follows: *Llb. buchneri* ATCC PTA-2494 7.0×10^{10} CFU g^{-1}, *Llb. buchneri* NRRL B-50733 3.0×10^{10} CFU g^{-1}, *Lpb. plantarum* DSM 18112 5.0×10^9 CFU g^{-1}, and *Lpb. plantarum* ATCC 55942 5.0×10^9 CFU g^{-1}. LAB are considered bacterial strains for short ensiling periods. Both additives were applied according to the manufacturers' dosage recommendations using a cleaned backpack sprayer, while the material was mixed regularly.

In Experiment A1, the silage treatments ($n = 4$) were packed into high-density polyethene barrels (34.8 L maximum volume). The barrel-specific silage material was retained within the barrels throughout the anaerobic fermentation (Experiment A1) and aerobic measurement period (Article Part B). Consequently, constant material was employed for both emission trials. The filling was performed in layers to ensure even filling and packing density. In detail, silage was loaded onto perforated plastic intermediate plates (diameter of 27.5 cm; Fig. 4.3), which were positioned on small stands into plastic barrels and had 63 drill holes (diameter of 10 mm). Underneath these intermediate plates was a 3 L volume of gas space (hereafter referred to as floor space) to ensure adequate ventilation of the silage during the following aerobic emission measurements (Article Part B). The silage used to fill each barrel had a mass of 10,206.6 ± 7.6 g_{FM} for CON, 10,200.3 ± 1.3 g_{FM} for BIO and 10,198.9 ± 0.9 g_{FM} for CHE; a volume of 28.8 L for all silos; and a resulting packing density of 150.60 ± 0.08 kg_{DM} m^{-3}. During packing, a temperature

logger (Testo 174 T, Testo SE Co. KGaA, Titisee-Neustadt, Germany) was positioned in the centre of the silage material. The silage was filled in such a volume that a gas space (3 L in volume, hereafter referred to as head space) remained above it when the barrel cover was put on and closed with a clamping ring. Both the barrel (at the level of the floor space) and the cover were equipped with two hose connections each (Section 4.2.4).

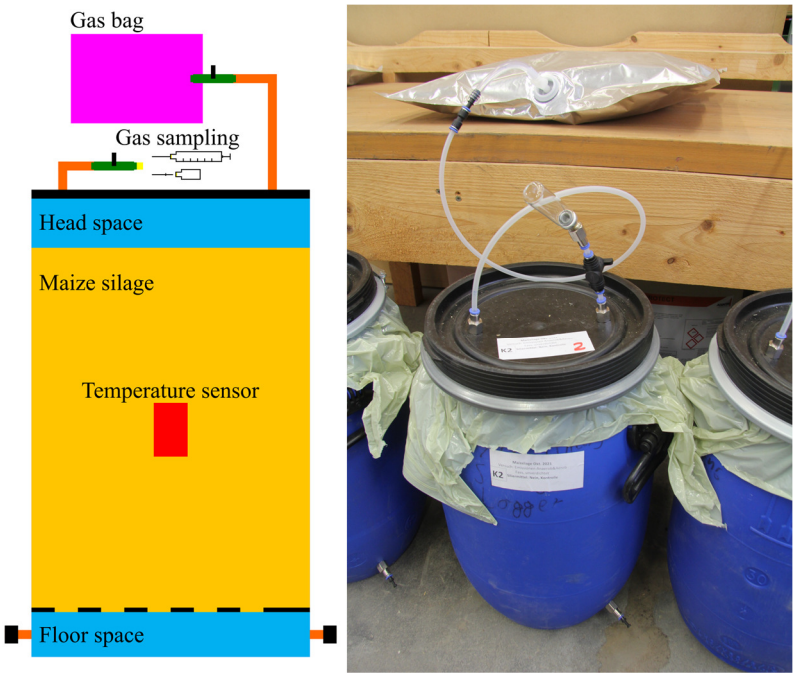

Fig. 4.3 Maize silage barrels and set-up used in the anaerobic emission measurements.

On the harvest date, the silage barrels of the CON treatment were filled first. Afterwards, the CHE forage and the BIO forage were treated, and the silos were packed. All 12 silage barrels were closed and sealed simultaneously. This should ensure (a) that the silage is exposed to oxygen for the same length of time and (b) that the ensiling process starts simultaneously.

The barrels were transported to the Institute of Agricultural Engineering (University of Bonn) and stored indoors (for ambient temperatures, see Section 4.3.1). During anaerobic storage, the barrels were regularly checked, and emission measurements were carried out (Section 4.2.4). After 30 days of ensiling, 6 barrels (2 of each treatment) were opened for aerobic emission measurements (Fig. 4.2 and Article Part B). Anaerobic emission measurements were taken with the remaining 6 barrels until the second aerobic measurement period started on day 135.

In Experiment A2, in parallel with the barrel preparation, the same silage treatment material was added to glass jars (maximum volume 1.8 L; J. Weck GmbH u. Co. KG, Wehr-Öflingen, Germany). The jars were filled with 0.602 kg_{FM} up to a volume of 1.65 litres. This corresponds to a packing density of 155.1 kg_{DM} m^{-3} and is therefore at a similar level as the barrel silos. The 36 jars

($n = 12$ per treatment) provided the material for the chemical and microbiological analyses (Section 4.2.3), so the silage barrels in Experiment A1 were unaffected during the ensiling process.

4.2.3 Laboratory analysis of the silage material

Material samples were collected before the CON barrels were packed for chemical and microbiological analysis on the harvest date. In Experiment A2, the ensiled material was collected for analysis on ensiling days 2, 14, 30, and 135 (Fig. 4.2). Samples for the chemical analyses were stored at -18°C immediately after sampling; samples for microbiological analyses were stored at 4°C and analysed on the same day.

Silage barrels and glass jars were weighed during the anaerobic fermentation period on ensiling days 0, 2, 14, 30, and 135. The silo masses were used to calculate the DM losses of the silage during the fermentation process. Two balances were used to balance the barrel (range 0–35,100 g, readability of 0.10 g; BBK 422-35 LA, Mettler Toledo, Germany) and the glass jar weights (range 0–2,410 g, readability of 0.01 g, linearity ± 0.05 g; KB 2400-2N, Kern & Sohn GmbH, Balingen, Germany). Crude ash, sugar, starch, crude fibre, crude protein, utilisable crude protein at the duodenum, and metabolizable energy concentrations were analysed according to the German Handbook of Agricultural Research and Analytic Methods [46].

Organic acids, alcohols and esters, pH, and water-soluble carbohydrates (WSC) were analysed in aqueous silage extracts after mixing 50 g of frozen silage material with toluene (1 mL) and distilled water (300 mL) [47]. Subsequently, various analyses were performed after filtration (MN 615 filter paper; Machery-Nagel, Düren, Germany) and microfiltration (0.45 µm pore size, Minisart RC, Sartorius, Göttingen, Germany) on the following day. Lactic acid (LA) was detected using high-performance liquid chromatography (refractive index detection; LC-20 AB, Shimadzu Deutschland, Duisburg, Germany; [48]). Volatile organic acids, alcohols, and ethyl esters (including EA) were determined using gas chromatography (GC) with a free fatty acid phase column (Permabond FFAP 0.25 µm, Macherey-Nagel, Düren, Germany) or an optima wax column (Macherey-Nagel, Düren, Germany), respectively, and a flame ionisation detector (GC-2010, Shimadzu, Deutschland, Duisburg, Germany) [47]. The detection limit for all parameters with the free fatty acid phase column was 0.01% of FM, and that with the optima wax column was 0.001% of FM. WSC were analysed by the anthrone method [49] using a continuous flow analyser (Scan++, Skalar Analytical, Breda, The Netherlands). The pH was analysed potentiometrically using a calibrated pH electrode. The DM concentration was corrected based on Weißbach and Strubelt [50].

The microbial analysis procedure was conducted by two different laboratories. The first analysed the fresh material samples according to the methods of VDLUFA [46] for aerobic and

mesophilic bacteria, moulds, Dematiaceae, and yeasts (methods 28.1.2 and 28.1.3). In the first step, 20 g of silage was suspended in 180 mL of solution (pH 7.0, 0.58 g L^{-1} NaH_2PO_4, 2.5 g L^{-1} Na_2HPO_4, 4.0 g L^{-1} NaCl, 1.0 g L^{-1} peptone, and 0.3 mL L^{-1} Tween 80) and treated with a paddle blender. From this solution, subsequent dilutions were prepared in phosphate buffer (pH 7.0, 0.58 g L^{-1} $NaH_2PO_4 \times 2\ H_2O$, 2.5 g L^{-1} $Na_2HPO_4 \times 2\ H_2O$, and 4.0 g L^{-1} NaCl), and the appropriate dilutions were used for microbial analysis. For bacteria, tryptose/TTC agar (pH 7.3, 20.0 g tryptose, 1.0 g L^{-1} glucose, 5.0 g L^{-1} NaCl, 15.0 g L^{-1} agar, and 10 mg L^{-1} 2,3,5-triphenyltetrazolium chloride) was used, and the plates were incubated for 2 days at 30°C. For fungi, rose-bengal chloramphenicol agar supplemented with Tergitol (pH 7.2, 5.0 g peptone, 10.0 g L^{-1} glucose, 1.0 g L^{-1} K_2PO_4, 0.5 g L^{-1} $MgSO_4$, 0.05 mg L^{-1} Rose-Bengal, 15.5 g L^{-1} agar, 0.1 ml L^{-1} Tergitol, and 20 mg L^{-1} chlortetracyclin-HCl) was incubated for 3 days at 25°C. For enumeration of mesophilic LAB (method 28.3.3, [51]), pour-plates of de Man, Rogosa, and Sharpe agar (68.2 g L^{-1}; type 1.10660, Merck, Darmstadt, Germany) – to provide micro-aerophilic conditions – with an overlay were prepared from the dilutions used for determination of aerobic, mesophilic bacteria, and fungi and incubated at 30°C for 5 days. The second laboratory analysed the samples collected on ensiling days 2, 14, 30, and 135. For this purpose, 30 g of silage was suspended and homogenised in ¼-strength Ringer solution (0.05 g L^{-1} $NaHCO_3$, 0.06 g L^{-1} $CaCl_2$, 0.105 g L^{-1} KCl, 2.25 g L^{-1} NaCl; Merck, Darmstadt, Germany). This suspension was used for the analysis of total bacterial counts on plate-count agar (pH 7.0, 1.0 g L^{-1} glucose, 2.5 g L^{-1} yeast extract, 5.0 g L^{-1} enzymatic digest of casein, 15 g L^{-1} agar; Merck, Darmstadt, Germany) after 2 days of incubation at 30°C; LAB counts on de Man, Rogosa, and Sharpe agar (Merck, Darmstadt, Germany) after 3 days of incubation at 30°C under anaerobic conditions; and yeasts and moulds on yeast extract glucose chloramphenicol agar (pH 6.6, 0.1 g L^{-1} chloramphenicol, 5.0 g L^{-1} yeast extract, 14.9 g L^{-1} agar, and 20.0 g L^{-1} glucose; Merck, Darmstadt, Germany) after 3 days of incubation at 25°C.

Furthermore, silage quality in Experiment A2 was assessed using the V-Score according to the procedure described by the Society of Utilisation of Self Supplied Feeds (2009) [52] and applied by Tian et al. [53]. V-Scores of $Y > 80$ were considered favourable, $80 \geq Y \geq 60$ average and $60 > Y$ bad silage quality [54].

In Experiment A1, silage quality was assessed using the silage scoring system of the German Agricultural Society [55, 56] after opening the barrels. This methodology helps to assess the quality of various silage parameters, such as smell, structure, colour, and mould. A more detailed

qualitative observation supplemented this scoring system to assess mould and yeast contamination by two trained persons. For this purpose, a scale from 0 (very good) to 5 (very bad) was used, which included the following values: 0.0 = no mould/yeast spots; 0.5 = occasional mould/yeast spots, approx. < 5% of the surface; 1.0 = occasional mould/yeast spots, approx. 5% of the surface; 2 = small mould/yeast nests, approx. 15% of the silo face; 2.5 = small mould/yeast nests, approx. 20% of the silo face; and 3.5 = multiplied mould/yeast nests, approx. 30% of the silo face.

4.2.4 Measurement of silage emissions

During the anaerobic storage period, measurements of silage emissions occurred regularly in Experiment A1 (Fig. 4.2), hereafter referred to as measurement time points.

Each silo barrel had four hose connectors (Section 4.2.2). Two connectors (at the level of the floor space) were closed during the ensiling process to ensure anaerobic conditions.

One of the connectors in the cover was attached to a short hose (all hoses in the experimental set-up were made of polytetrafluoroethylene unless otherwise stated), to which a ball valve and a rubber septum were mounted. The ball valve was only opened for gas sampling. For this purpose, a laboratory syringe (50 mL volume) was inserted into the rubber septum, and 50 mL of gas was removed and pumped back into the barrel twice. This procedure was used to ensure homogeneous mixing of the gas in the barrel headspace. A double needle was subsequently inserted, and four vacuumed glass vials (20 mL volume each) were filled one after the other. This procedure was performed for all the barrels in the anaerobic storage phase, i.e. for 12 barrels between ensiling days 0 and 30 and 6 barrels between ensiling days 30 and 135, respectively. A similar methodology was used by Schmithausen et al. [2].

The other connector in the cover was connected to a gas sampling bag (nominal volume 25 L), which collected the gases formed by the silage during the ensiling process [30]. For the bag connection, polyurethane and polytetrafluoroethylene hoses were fitted using connectors. The system of a barrel and a gas bag can be regarded as a zero-pressure system, as inflation of the bags captured the formed gas. After gas sampling at ensiling hour 36, each gas bag was exchanged for an empty, new gas bag due to the high gas formation of the silage material.

After gas sampling, the gas bags were carefully clamped in a calliper with flat pads until the bags were under tension to a certain degree. The filling volume of the bags could be measured using the deflection of the calliper and a preliminary calibrated scale. Afterwards, all hose connectors were checked for gas tightness, and the bags were stored until the subsequent measurements. To ensure the comparability of this procedure, all the measurements were taken by only two trained persons. Furthermore, this procedure was carried out indoors to avoid any change

in the gas volume due to temperature (Section 4.3.1). After 36 h of ensiling, the first and second gas bag volumes were added to determine the total gas formation quantity per silo.

The gas samples in the vials were used to analyse the composition of the gas mixture. One of the vials was used to analyse the greenhouse gas concentrations using a gas chromatograph (electron capture detector and a flame ionisation detector; model 8610C, SRI Instruments, Torrance, CA, USA). Due to the high CO_2 concentrations in the barrel headspace, a diluted sample (diluted 1:101 with room air) was analysed (detection limit of 50.00 ppm). The initial CO_2 concentration was calculated using the CO_2 concentration in the room air. Subsequently, the undiluted gas sample was used to analyse the concentrations of CH_4 (detection limit of 0.08 ppm) and N_2O (detection limit of 0.01 ppm). The subsequent values considered the amount of gas taken for CO_2 analysis. This procedure of Experiment A1 is similar to the methodology used in the previous trials [2, 35].

Another vial was used to analyse the concentrations of VOCs. For this purpose, the sample air was diluted with room air (dilution 1:153) and then analysed using infrared photoacoustic spectroscopy (PAS; Multi-Gas Analyser INNOVA 1312; LumaSense Technologies SA, Ballerup, Denmark). Cross and water compensation were turned on for measurement [21, 35]. The initial concentration was calculated again based on the results of the room air analysis. The accuracy of the Multi-Gas Analyser INNOVA 1312 was 3% of the gas concentration, and the detection limits, based on the calibration chart of the manufacturer, were 0.1 ppm for ethanol and 0.02 ppm for EA. Furthermore, the analyser was used to measure the CO_2 concentration. These data are not shown but should be considered in terms of cross-compensation (Section 4.4.4).

Each gas bag was sampled after removing it from the barrel.

The remaining two vials per barrel and measurement time point were stored in the laboratory for use in case of erroneous measurements. In Experiment A1, 2,280 vials were filled; 1,681 gas samples were analysed using GC, and 926 using PAS.

4.2.5 Calculation of gas emissions during anaerobic storage

Due to the accumulation of gases formed in the zero-pressure system (barrel plus gas bag), cumulative gas emissions were calculated for each measurement time point in Experiment A1.

For this purpose, homogeneous gas dispersion within the total gas space of each zero-pressure system was assumed. The total gas space was calculated using Equation 4.1:

Equation 4.1 Calculation of the total gas space of the zero-pressure systems.

$$V_{gas} = V_{bag} + V_{headspace} + V_{gas\ pores} + V_{floorspace} \qquad (4.1)$$

where V_{gas} is the volume of the total gas space [L]; V_{bag} is the volume of the gas bag [L]; $V_{headspace}$ is the volume of the headspace [L] (3 L volume); $V_{gas\ pores}$ is the volume of the gas pores in the packed silage material [L] (Equations 4.2 and 4.3); and $V_{floorspace}$ is the volume of the floorspace [L] (3 L volume).

The volume of the gas pores was calculated using Equations 4.2 and 4.3 [57]:

Equation 4.2 General calculation of the gas space of the gas pores.

$$V_{gas\ pores} = V_{silage} \times porosity_{silage} \qquad (4.2)$$

Equation 4.3 Specific calculation of the gas space of the gas pores (based on [54]).

$$V_{gas\ pores} = V_{silage} \times (1.733 \times DM - 0.256 \times density + 39.778) \qquad (4.3)$$

where $V_{gas\ pores}$ is the volume of the gas pores in the packed silage material [L]; $porosity_{silage}$ is the ratio of gas pores in the volume of packed silage [%]; V_{silage} is the volume of the packed silage material in the barrel [L] (28.8 L volume); DM is the dry matter concentration of the silage material [%] (425 $g_{DM}\ kg_{FM}^{-1}$ during silo packing); and density is the packing density [$kg_{DM}\ m^{-3}$] (150.6 $kg_{DM}\ m^{-3}$). At the time of silo closure, the porosity was 74.90% ± 0.02%, $V_{gas\ pores}$ 21.57 ± 0.01 L and V_{gas} 27.57 ± 0.01 L for the twelve barrels.

The cumulative gas emission quantities were calculated for each measurement time point i in Experiment A1 using Equation 4.4:

Equation 4.4 Calculation of the cumulative gas emission quantities.

$$M_{gas,\,i} = V_{gas,\,i} \times c_{gas,\,i} \qquad (4.4)$$

where $M_{gas,\,i}$ is the cumulative gas emission mass [g]; $V_{gas,\,i}$ is the total gas space at this measurement time point i [L] (Equations 4.1 to 4.3); and $c_{gas,\,i}$ is the gas concentration in the gas bag at measuring time point i [$g_{gas}\ L^{-1}$].

Equation 4.4 was also used for calculating the gas emission masses in the various gas bags. After the gas bags of all the zero-pressure systems were changed at ensiling hour 36, the gases in the system (barrel plus second gas bag) were added to the gases in the first bags.

The maximum gas emissions per silo in Experiment A1 were the gas emission masses at that measurement time point when the gas formation quantity – i.e. the sum of the gas volume of the gas bag one and two per silo – reached its maximum.

4.2.6 Data processing and statistics

The following conversion ratios were used: 1 ppm CO_2 = 1.83 (mg CO_2) m^{-3}; 1 ppm CH_4 = 0.67 (mg CH_4) m^{-3}; and 1 ppm N_2O = 1.83 (mg N_2O) m^{-3}. In Experiment A1, the gas quantities formed per silage mass are given for the DM mass at the time of ensiling (day 0), according to Bueno et al. [29]. The following GWP were applied according to the fourth assessment report of the Intergovernmental Panel on Climate Change (IPCC) [58]: CO_2 = 1, CH_4 = 25, and N_2O = 298. The CO_2 equivalent (CO_2eq) emissions derived in this trial considered only climate-relevant gases, i.e. CH_4 and N_2O; GHG emissions also included CO_2. DM losses are indicated by negative values, and DM gains are indicated by positive values.

In Experiment A2, the chemical compositions and microbial counts of the fresh and ensiled materials were compared using one-way analysis of variance (ANOVA). In Experiment A1, the gas formation quantities, the gas concentrations, and the subsequently calculated gas emission masses were compared using mixed ANOVA, with subsequent one-way ANOVAs for each measurement analysis interval. These intervals differ from the measurement time points described above (Sections 4.2.4 and 4.2.5). Multiple measurement time points were combined into one measurement analysis interval to ensure a sufficient sample size for the ANOVA (Table 4.4). For each one-way ANOVA, if homogeneity of variance was given, Tukey's-HSD test was used for post hoc significance comparison; if not, a Welch-ANOVA was followed by a Games-Howell post hoc test. Linear correlations were analysed using Spearman correlation. In all the analyses, $p < 0.05$ was considered to indicate statistical significance.

Microsoft Office Excel 2019 (Microsoft Corporation, Redmond, Washington, DC, USA) was used for descriptive data analysis. IBM SPSS 26.0 (International Business Machines Corporation Armonk, New York, NY, USA) was used for statistical analysis.

4.3 Results

4.3.1 Ambient air and silage temperatures

The maize was harvested at an ambient air temperature of 12.2 ± 0.4°C. At the time of silo closing, the silage temperatures were 13.9 ± 0.1°C for CON, 13.1 ± 0.1°C for BIO, and 13.4 ± 0.1°C for CHE barrels in Experiment A1. After 2 h, the barrels and glass jars were stored indoors (19.0 ± 1.4°C). CON barrels reached the temperature level of ambient air after 1.22 ± 0.02 d, BIO after 1.18 ± 0.01 d, and CHE after 1.23 ± 0.02 d. Afterwards, the temperatures of the BIO barrels were greater than those of the CON (+ 0.3 ± 0.1 K) and CHE (+ 0.3 ± 0.1 K) barrels until ensiling hour 36. The silage temperatures subsequently remained at steady levels in Experiment A1: 18.7 ± 0.9°C for the CON barrels, 18.7 ± 1.0°C for the BIO barrels, and 18.7 ± 1.0°C for the CHE barrels.

4.3.2 Composition of the silage

The DM losses in Experiment A1 and the chemical compositions, the V-Score, and microbial counts of the fresh and ensiled materials of the silage treatments in Experiment A2 are shown in Tables 4.1 and 4.2. In Experiment A1, all the silage barrels exhibited decreasing FM weights throughout the fermentation process. These results were as follows: for d2, CON -0.33% ± 0.01%, BIO -0.36% ± 0.01%, and CHE -0.29% ± 0.01%; for d14, -0.51% ± 0.02%, -0.55% ± 0.02%, and -0.46% ± 0.02%; for d30, -0.56% ± 0.03%, -0.66% ± 0.02%, and -0.54% ± 0.02%; and for d135, -0.74% ± 0.01%, -1.27% ± 0.04%, and -0.71% ± 0.03%, respectively. Considering the DM of the silage material (based on the DM in Experiment A2), CON barrels indicated the most considerable DM losses between ensiling days 0 and 14, followed by BIO and CHE ($p < 0.05$). However, the DM losses were not linear for all the treatments over time (Table 4.1). CON and CHE presented DM mass increases between ensiling days 14 and 30, and subsequent losses occurred between days 30 and 135. BIO showed a constant increase in DM after ensiling day 14. Based on the silo fresh mass decrease, this DM mass increase resulted from rising DM concentrations (Section 4.4.5). In Experiment A2, the glass jars indicated similar FM and DM mass losses throughout the fermentation process.

In Experiment A2, VOC concentrations in the silage material differed between the treatments. At ensiling day 30, the ethanol concentrations did not vary, but BIO had higher EA concentrations than CON and CHE did ($p < 0.05$). On ensiling day 135, BIO had higher ethanol, propanol, 1,2-propanediol, 2-butanol, and EA but lower ethyl lactate concentrations than CON ($p < 0.05$). In the BIO treatment, a decrease in LA and an increase in AA concentrations were consistent with an increase in EA and a decrease in ethyl lactate concentrations.

All silage treatments are characterised by a quick decrease in pH within the first 2 ensiling days in Experiment A2; BIO increases after ensiling day 30. This aligns with a decrease in LA, sugar, and WSC concentrations, and an increase in AA concentrations. All the silages were free of butyric acid (not shown), and only BIO had small amounts of propionic acid at ensiling day 135.

In Experiment A2, all the treatments had higher LAB counts on ensiling day 2 than on day 0. This difference aligns with the findings of LA formation and a decrease in pH during this period. Subsequently, the LAB counts decreased continuously until ensiling day 30 in all the treatments. BIO had significantly greater counts since ensiling day 30; the counts at ensiling day 135 were similar to those at ensiling day 2, while CON and CHE showed noticeable decreases. Yeast counts decreased during anaerobic storage in all the treatments. In detail, the decline is fastest in the CHE treatment and slowest in the CON treatment. The high yeast counts in the CON treatment at ensiling day 135 are due to an outlier (possibly a tiny oxygen leakage in one of the silage glass

jars), proven by the high standard deviation. However, since the other parameters of this sample did not show outliers, this sample was not excluded.

The V-Score showed consistently high values of ≥ 90 indicating favourable silage quality. The lowest values were indicated by the BIO treatment based on the ongoing AA production.

In Experiment A1, the inspection of the silage faces in the silage barrels on the days of opening showed a consistently high silage quality (Tables 4.5 and 4.6). The BIO barrels indicated a slight smell of alcohol on ensiling day 30, even though the BIO ethanol concentrations in the material (Experiment A2) were slightly below the level of CON. At ensiling day 30, BIO and CHE barrels showed a pungent odour of AA, which increased in the BIO treatment until ensiling day 135. In addition, slight mould growth and a recurring yeast formation were evident in individual barrels. At ensiling day 30, 5%–20% of CON's, 15% of BIO's and 15% of CHE's silage faces were covered with yeast spots; at day 135, 15%–30%, < 5%–5%, 5%–15%, respectively (Tables 4.5 and 4.6).

Table 4.1 Chemical composition and energy concentration of fresh maize and ensiled material for the silage treatments in Experiment A2 (unless otherwise stated).

Ensiling time	Treatment[A]	Dry matter[B]	DM losses[C]	pH	Lactic acid	Acetic acid	Propionic acid	Crude ash	Sugar	Starch	Water-soluble carbohydrates	Crude fibre	Crude protein	Utilisable crude protein at the duodenum	Metabolizable energy
[d]		[g kg_{DM}^{-1}]			[g kg_{DM}^{-1}]										[MJ kg_{DM}^{-1}]
0	fresh	**425** ± 6	/	**5.94** ± 0.05	**1.4** ± 0.1	**2.6** ± 0.5	N/D	**30.0** ± 1.7	**56.0** ± 5.3	**353** ± 11	**144** ± 15	**203** ± 4	**65.7** ± 2.1	**128** ± 1	**11.1** ± 0.1
2	CON	**399** ± 2	**-64.2**[a] ± 0.1	**4.61** ± 0.03	**17.0**[a] ± 0.5	**7.4** ± 0.6	N/D	**33.0** ± 1.0	**20.7** ± 1.5	**371** ± 14	**34**[ab] ± 5	**191** ± 5	**70.0** ± 1.0	**131** ± 1	**11.2** ± 0.1
2	BIO	**402** ± 16	**-57.5**[b] ± 0.1	**4.55** ± 0.04	**19.4**[b] ± 0.6	**7.4** ± 0.8	N/D	**33.7** ± 1.5	**18.3** ± 4.6	**372** ± 29	**25**[a] ± 1	**191** ± 12	**71.0** ± 1.0	**132** ± 1	**11.2** ± 0.2
2	CHE	**405** ± 5	**-51.0**[c] ± 0.1	**4.64** ± 0.05	**16.5**[a] ± 0.5	**6.9** ± 0.9	N/D	**32.7** ± 2.1	**21.7** ± 2.5	**393** ± 25	**43**[b] ± 1	**180** ± 11	**70.0** ± 2.6	**133** ± 0	**11.4** ± 0.2
14	CON	**393** ± 5	**-80.3**[a] ± 0.2	**3.94** ± 0.06	**27.0**[a] ± 2.2	**8.1** ± 1.0	N/D	**35.0** ± 1.0	**13.0** ± 1.7	**354** ± 24	**5** ± 1	**195** ± 9	**76.0** ± 1.0	**134** ± 2	**11.2** ± 0.2
14	BIO	**395** ± 10	**-76.4**[b] ± 0.1	**3.98** ± 0.02	**35.9**[b] ± 1.6	**9.1** ± 1.1	N/D	**36.7** ± 0.6	**10.7** ± 4.0	**337** ± 18	**5** ± 1	**206** ± 9	**78.0** ± 0.0	**133** ± 2	**11.0** ± 0.2
14	CHE	**395** ± 4	**-75.5**[c] ± 0.2	**3.97** ± 0.01	**39.9**[b] ± 1.6	**7.7** ± 2.1	N/D	**35.7** ± 0.6	**10.3** ± 2.1	**351** ± 19	**6** ± 1	**197** ± 7	**77.7** ± 0.6	**134** ± 1	**11.1** ± 0.1
30	CON	**397** ± 5	**-71.5**[a] ± 0.3	**3.92** ± 0.00	**41.1**[a] ± 3.0	**9.2**[ab] ± 0.6	N/D	**35.7** ± 1.2	**12.7** ± 2.1	**356** ± 24	**6**[ab] ± 1	**193** ± 12	**78.7** ± 1.5	**135** ± 2	**11.2** ± 0.2
30	BIO	**400** ± 7	**-66.5**[b] ± 0.2	**3.95** ± 0.02	**36.3**[a] ± 1.1	**11.4**[b] ± 0.4	**0.16** ± 0.02	**35.7** ± 0.6	**13.0** ± 1.7	**358** ± 17	**5**[a] ± 0	**193** ± 6	**77.7** ± 0.6	**135** ± 1	**11.2** ± 0.1
30	CHE	**402** ± 7	**-59.1**[c] ± 0.2	**3.91** ± 0.00	**49.2**[b] ± 1.7	**8.7**[a] ± 1.5	N/D	**35.7** ± 0.6	**12.3** ± 2.3	**366** ± 8	**6**[b] ± 0	**189** ± 4	**77.7** ± 0.6	**135** ± 1	**11.2** ± 0.1
135	CON	**393** ± 14	**-83.3**[a] ± 0.1	**3.91**[a] ± 0.07	**52.2**[c] ± 2.2	**11.6**[a] ± 0.6	**0.09** ± 0.15	**37.7** ± 1.5	**16.0**[b] ± 3.0	**329** ± 33	**11**[b] ± 1	**200** ± 14	**81.3** ± 1.2	**135** ± 2	**11.1** ± 0.2
135	BIO	**407** ± 2	**-55.4**[b] ± 0.4	**4.29**[b] ± 0.02	**15.9**[a] ± 1.3	**30.3**[b] ± 2.4	**1.85** ± 0.23	**36.7** ± 0.6	**4.0**[a] ± 1.7	**374** ± 5	**3**[a] ± 0	**187** ± 2	**82.0** ± 1.0	**138** ± 1	**11.3** ± 0.0
135	CHE	**392** ± 8	**-83.4**[a] ± 0.3	**3.92**[a] ± 0.06	**45.1**[b] ± 0.8	**11.7**[a] ± 0.9	**0.** N/D	**37.0** ± 1.0	**16.0**[b] ± 3.0	**346** ± 20	**13**[b] ± 1	**191** ± 10	**81.0** ± 1.0	**136** ± 2	**11.2** ± 0.2

DM = Dry matter (concentration), N/D = Not detectable.

Treatments with different superscript lowercase letters within a parameter and ensiling day differ significantly (Tukey's-HSD or Games-Howell tests, $p < 0.05$).

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE)

[B] Corrected dry matter based on Weißbach and Strubelt [50].

[C] Based on the dry matter weight and losses of the silage barrels (Experiment A1, n = 4 for ensiling days 2–30, n = 2 for ensiling day 135). Losses concerning the silage mass filled into the silos on the harvest day (day 0).

Table 4.2 Chemical composition, the V-Score, and microbial counts of fresh maize and ensiled material for the silage treatments in Experiment A2.

Ensiling time	Treatment [A]	Methanol	Ethanol	Propanol	1,2-Propanediol	2-Butanol	2,3-Butanediol	Ethyl acetate	Ethyl lactate	V-Score	Total bacterial counts	Lactic acid bacteria	Yeasts	Moulds
[d]		[g kg_{DM}^{-1}]									[log_{10} CFU g_{FM}^{-1}]			
0	fresh	**0.04** ± 0.01	**0.00** ± 0.00	N/D	**0.09** ± 0.00	N/D	**0.09** ± 0.00	N/D	N/D	**100** ± 0	**8.20** ± 7.93	**6.64** ± 5.56	**7.48** ± 7.43	**6.63** ± 6.18
2	CON	**0.07** ± 0.01	**5.35** ± 0.80	N/D	**0.10** ± 0.00	N/D	**0.22** ± 0.03	**0.12**[a] ± 0.02	**0.01** ± 0.01	**99** ± 0	**9.43** ± 8.06	**9.29** ± 8.18	**6.56** ± 6.65	**6.04** ± 6.28
2	BIO	**0.07** ± 0.01	**6.62** ± 1.36	N/D	**0.10** ± 0.00	N/D	**0.24** ± 0.02	**0.16**[b] ± 0.02	**0.02** ± 0.00	**99** ± 0	**9.34** ± 8.95	**9.43** ± 8.82	**6.10** ± 5.06	**3.17** ± 3.13
2	CHE	**0.07** ± 0.01	**4.28** ± 0.37	N/D	**0.10** ± 0.00	N/D	**0.12** ± 0.10	**0.08**[a] ± 0.01	**0.01** ± 0.01	**99** ± 0	**9.36** ± 8.86	**9.36** ± 8.80	**6.52** ± 6.64	**3.03** ± 2.55
14	CON	**0.08** ± 0.00	**7.14** ± 1.14	N/D	**0.10**[a] ± 0.00	**0.05**[b] ± 0.00	**0.24** ± 0.04	**0.14** ± 0.03	**0.07** ± 0.00	**99** ± 0	**8.83** ± 7.30	**8.71** ± 8.36	**5.29**[b] ± 4.67	**1.52** ± 1.76
14	BIO	**0.10** ± 0.02	**7.12** ± 0.87	**0.3** ± 0.0	**0.42**[b] ± 0.01	**0.03**[a] ± 0.00	**0.23** ± 0.04	**0.11** ± 0.02	**0.07** ± 0.00	**99** ± 0	**9.15** ± 8.42	**8.76** ± 8.46	**5.09**[b] ± 4.18	**1.52** ± 1.76
14	CHE	**0.08** ± 0.01	**5.18** ± 1.09	N/D	**0.10**[a] ± 0.00	**0.02**[a] ± 0.00	**0.11** ± 0.09	**0.08** ± 0.02	**0.06** ± 0.02	**99** ± 1	**8.88** ± 7.98	**8.63** ± 8.14	**4.33**[a] ± 3.18	N/D
30	CON	**0.12** ± 0.01	**8.25** ± 0.88	**0.1** ± 0.1	**0.10** ± 0.00	**0.11**[b] ± 0.02	**0.24**[b] ± 0.06	**0.17**[a] ± 0.01	**0.12** ± 0.01	**99**[ab] ± 0	**8.00** ± 6.93	**7.97**[a] ± 7.21	**4.49**[b] ± 3.49	N/D
30	BIO	**0.15** ± 0.02	**7.49** ± 1.29	**1.8** ± 0.1	**1.63** ± 0.11	**0.06**[b] ± 0.01	**0.15**[a] ± 0.01	**0.26**[b] ± 0.01	**0.10** ± 0.01	**98**[a] ± 0	**9.29** ± 8.89	**8.62**[b] ± 8.08	**3.16**[a] ± 3.23	N/D
30	CHE	**0.10** ± 0.03	**5.59** ± 1.36	N/D	N/D	**0.03**[a] ± 0.00	**0.15**[a] ± 0.01	**0.15**[a] ± 0.01	**0.10** ± 0.01	**99**[b] ± 1	**8.16** ± 8.23	**7.56**[a] ± 7.26	**2.99**[a] ± 3.12	N/D
135	CON	**0.25** ± 0.03	**7.13**[a] ± 0.25	**1.4**[a] ± 0.3	**0.17**[a] ± 0.06	**0.22**[b] ± 0.06	**0.30**[ac] ± 0.02	**0.11**[a] ± 0.02	**0.17**[b] ± 0.02	**96**[b] ± 0	**7.83**[a] ± 7.95	**7.82**[a] ± 7.96	**6.70** ± 6.94	N/D
135	BIO	**0.20** ± 0.02	**9.98**[b] ± 0.22	**16.0**[b] ± 0.5	**0.34**[b] ± 0.02	**0.58**[c] ± 0.03	**0.13**[ab] ± 0.00	**0.24**[b] ± 0.03	**0.09**[a] ± 0.00	**90**[a] ± 1	**9.05**[b] ± 8.52	**9.05**[b] ± 8.06	N/D	N/D
135	CHE	**0.21** ± 0.02	**6.31**[a] ± 0.66	**1.1**[a] ± 0.1	**0.19**[a] ± 0.04	**0.07**[a] ± 0.02	**0.05**[a] ± 0.09	**0.08**[a] ± 0.02	**0.14**[b] ± 0.01	**97**[b] ± 1	**7.16**[ab] ± 6.36	**7.08**[a] ± 6.00	N/D	N/D

CFU = Colony-forming units, DM = Dry matter, FM = Fresh matter, N/D = Not detectable.

Treatments with different superscript lowercase letters within a parameter and ensiling day differ significantly (Tukey's-HSD or Games-Howell tests, $p < 0.05$).

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

4.3.3 Formation of greenhouse gases

In Experiment A1, the courses of the gas volumes and the corresponding cumulative gas quantities within the barrels' head space and gas bags, respectively, are visualised in Fig. 4.4. The central part of the gas formation occurred within the first ensiling days. The gas bags' volume increased after ensiling hour 8. Until this time, barrels formed 11% ± 1% of the total CO_2 gas quantities. After 2 ensiling days, 78% ± 4% of the CO_2 was generated, and after 5 days, 93% ± 4% was generated. CON indicated increasing cumulative gas quantities until ensiling day 19.5 ± 6.4, BIO until ensiling day 60.0 ± 49.5, and CHE until ensiling day 14.0 ± 0.0 (Figs. 4.4, 4.7 and Table 4.3). CHE had a slower increase in gas volume (for ensiling days 0–14; see Fig. 4.7), with significantly lower volumes since ensiling day 0.5 ($p < 0.05$; see Table 4.7 for selected data). CON and BIO differ significantly ($p < 0.05$) for ensiling days 30–70. However, the large standard deviation in the BIO treatment since ensiling day 70 affects the statistical analysis. The central part of the gas formed is CO_2, which shows a strong positive correlation between the gas and CO_2 quantities (Fig. 4.8). Most of the CO_2 generated during the main fermentation phase (ensiling days 0.3–4.0). During this phase, CO_2 concentrations reach 1.20×10^6 mg m^{-3} ($\approx 6.6 \times 10^5$ ppm), with a subsequent regressive decrease. After a certain point, i.e. between ensiling days 50 and 80, depending on the treatment, the CO_2 quantities within the total gas space remain at a (nearly) constant level.

The CH_4 concentrations in the headspace reached 4.69–5.13 mg m^{-3} ($\cong$ 7.04–7.69 ppm) between ensiling hours 132 and 144. The N_2O concentrations peaked at 49.9–66.2 mg m^{-3} ($\cong$ 27.4–36.4 ppm) at ensiling hour 44. CHE had lower CH_4 quantities than CON did between ensiling days 20 and 135 ($p < 0.05$). The combination of lower gas formation and CH_4 concentrations leads to less CH_4 emissions in the CHE treatment. A noticeable standard deviation of the BIO values reduces the statistical accuracy. For N_2O, CHE had significantly greater quantities than CON and BIO since ensiling day 1.5 ($p < 0.05$).

Several barrels show a constant decrease in gas quantities in the gas bags after 14–25 ensiling days (Figs. 4.4, 4.7 and Table 4.3; exceptions barrels BIO3 and BIO4). However, focusing on the individual gases, CO_2 quantities peaked between ensiling days 4.0 and 7.5, and an average reduction of -25.3% occurred until ensiling day 30 (-44.3% until ensiling day 135). CH_4 quantities peaked between ensiling days 5 and 12 and decreased by -31.0% and -49.0%, respectively; N_2O quantities peaked between ensiling days 1.5 and 2.7 and decreased by -51.2% and -76.3%, respectively.

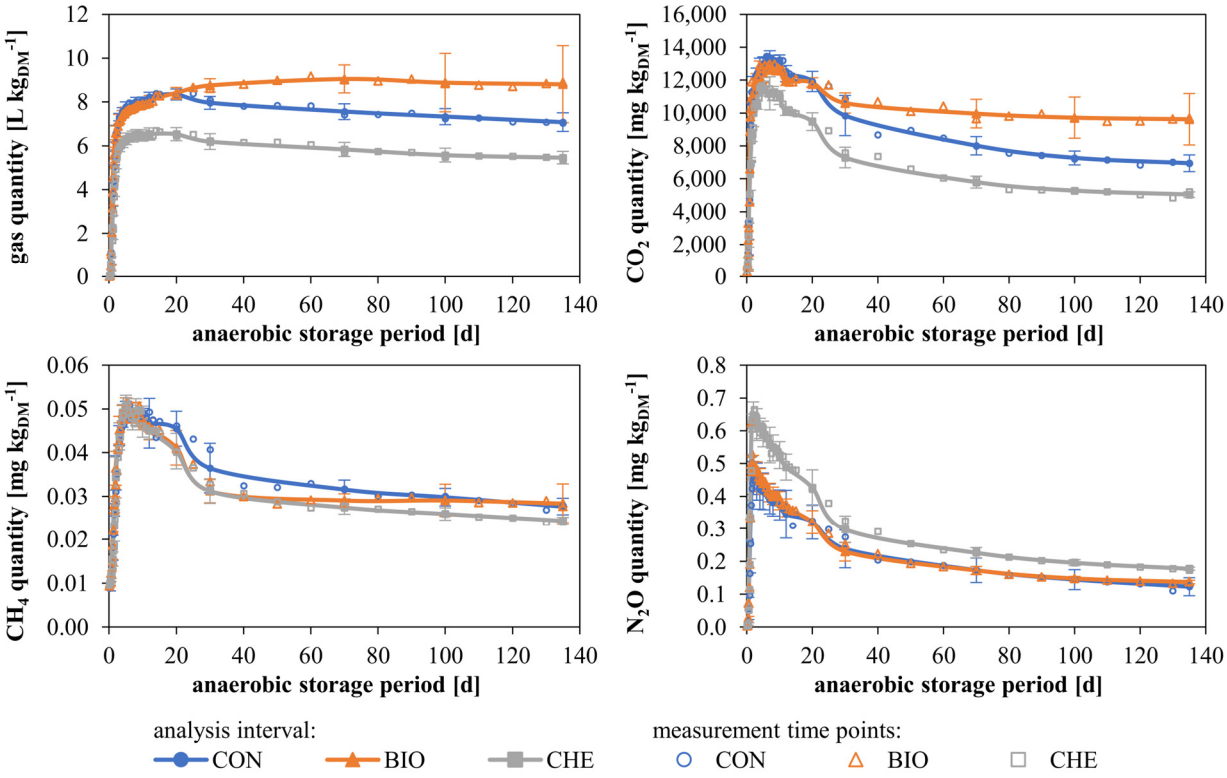

Fig. 4.4 Cumulative gas, CO_2, CH_4 and N_2O quantities within the zero-pressure systems during the ensiling process.
Error bars indicate the standard deviation of all treatments' measurement time point values within each analysis interval.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

All the barrels exhibited DM loss and gas formation until ensiling day 14 (Fig. 4.9). These parameters show strong correlations. Subsequently, the gas quantities decreased, and the DM masses of the silos increased (+ 0.88% for CON, + 0.99% for BIO, and + 1.64% for CHE; Table 4.1) between ensiling days 14–30 compared to ensiling day 14 (Fig. 4.10). Statistical correlations were partly given. However, the net losses were still negative. Between days 30 and 135, CON and CHE exhibited DM losses and a decrease in gas quantities, while BIO exhibited nearly constant gas quantities and an increase in DM mass.

When gas bags were exchanged at ensiling hour 36, GHG concentrations were greater in the headspace than in the first gas bags; on ensiling days 30 and 135, the opposite was true for the second gas bags.

4.3.4 Formation of ethanol and ethyl acetate

The cumulative ethanol and EA quantities during the anaerobic storage period are displayed in Figs. 4.5 and 4.11. Ethanol quantities rapidly increased within the first 5 ensiling days, and the gas quantity peaked at ensiling day 4.7 ± 0.4. After 2 ensiling days, 55% ± 8% ethanol was formed, and after 5 days, 94% ± 6% ethanol was produced. Subsequently, constant quantities were

observed for CON and CHE silos after a certain point during the anaerobic storage period. Ethanol gas quantities decreased by -38% to -47% of the maximum quantities until ensiling day 30 and -30% to -62% until ensiling day 135. BIO showed a constant increase after ensiling day 60 due to the increasing quantities in the BIO4 barrel (Table 4.3). CHE showed the lowest cumulative ethanol formation quantities throughout the anaerobic storage period (ensiling days 0.5 to 135, $p < 0.05$) compared to CON and BIO; between the latter two, CON values were significantly lower than BIO values after ensiling day 70 ($p < 0.05$).

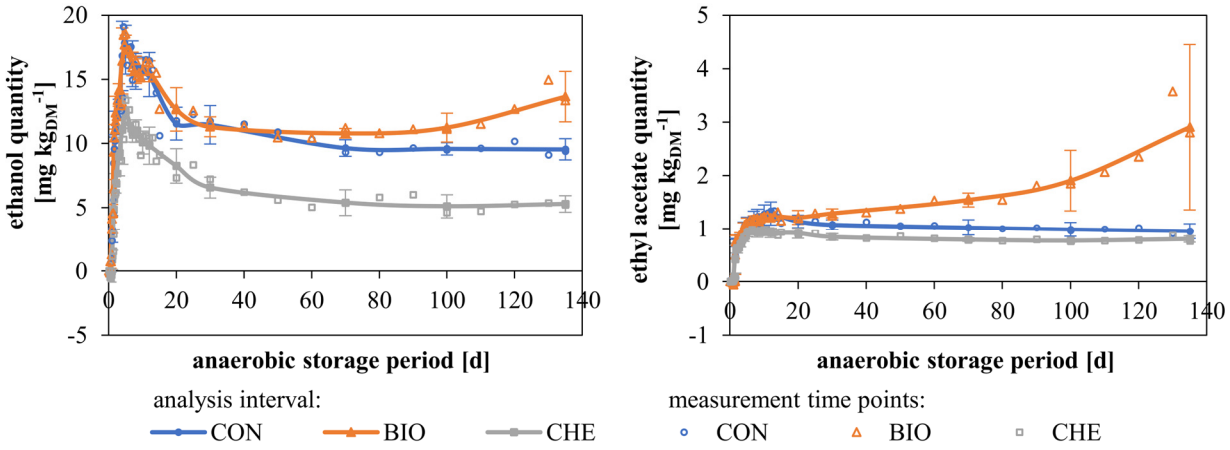

Fig. 4.5 Cumulative ethanol and ethyl acetate quantities within the zero-pressure systems during the ensiling process.
Error bars indicate the standard deviation of all treatments' measurement time point values within each analysis interval.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

For EA, after the gas bags were exchanged at ensiling hour 36, all the treatments exhibited a spontaneous increase in the calculated quantities (Fig. 4.11). Subsequently, CHE increased until ensiling days 5 to 6; CON and BIO increased until ensiling days 12 and 30. After this, the EA quantities remained steady or showed small decreases (Fig. 4.5). At ensiling day 30, CON and CHE exhibited losses of -17% to -23% compared to the peak quantities. The decline changed to -30% to -34% at ensiling day 135. However, the BIO3 and BIO4 barrels exhibited a substantial increase in EA formation during the ongoing storage period (Table 4.3). These barrels formed only 23%–61% of the final quantity of EA gas before ensiling day 30. CHE indicates significantly lower EA gas quantities than CON and BIO at ensiling day 2 and between ensiling days 4 and 100 ($p < 0.05$). BIO had greater quantities than CON did between ensiling days 3 and 100 ($p < 0.05$).

The cumulative ethanol and EA gas quantities exhibited a strong linear correlation for all the treatments (Fig. 4.12).

The relationships between the ethanol and EA concentrations in the silage material and the cumulative gas quantities are shown in Fig. 4.6. For ethanol, the CON material exhibited a concentration increase until ensiling day 30 and a subsequent decrease (Table 4.2). BIO and CHE levels continuously increased. However, the gas quantity peak during the first days cannot be explained by a material concentration peak but aligns with the ethanol formation in the silage material. For EA, concentrations within the silage material increase until ensiling day 30, including local minima on day 14 for BIO and CHE; after this, all treatments indicate a decrease, which is in line with decreasing gas quantities for CON and CHE. Higher concentrations of EA in BIO material beginning on ensiling day 30 led to increased quantities of EA gas. However, higher material concentrations provide a limited explanation for the differences between BIO3 and BIO4.

Ethanol concentrations within the material correlated positively with EA concentrations in CON ($r_S = 0.655$, $p < 0.05$), and tended to in BIO ($r_S = 0.545$, $p = 0.08$). For BIO, the ethanol concentrations in the material ($r_S = 0.609$, $p < 0.05$) and the gas quantities ($r_S = 0.691$, $p < 0.05$) correlate with the quantity of EA gas. The treatment CHE showed no significant correlations.

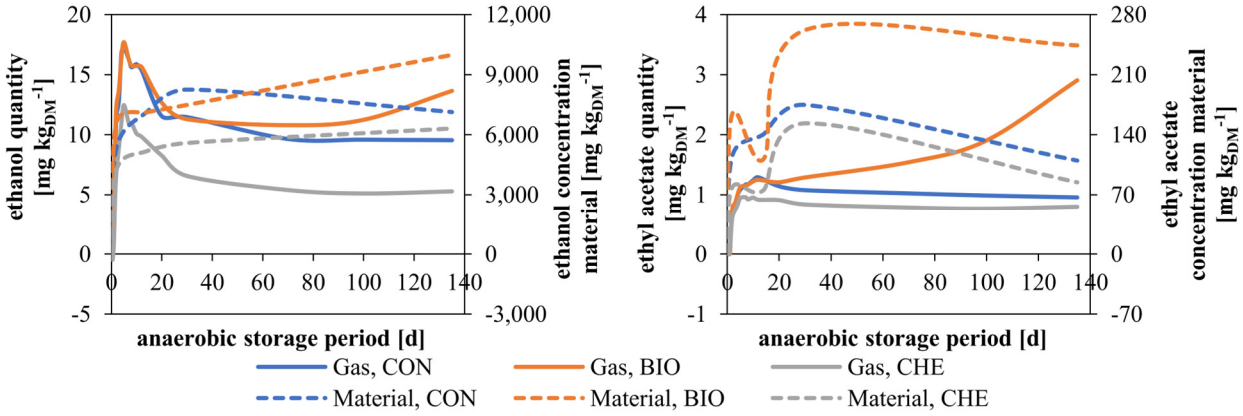

Fig. 4.6 Ethanol and ethyl acetate concentrations in the silage material and cumulative gas quantities in the zero-pressure systems.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

4.3.5 Emissions of climate- and environment-relevant gases

Table 4.3 shows the maximum emission quantities of climate- and environment-relevant gases. CHE indicates significantly lower gas formation quantities than CON and BIO but the highest N_2O and CO_2eq emission quantities ($p < 0.05$). Furthermore, CHE had lower CO_2 and ethanol emission quantities than CON did ($p < 0.05$). EA emission quantities show no differences. BIO indicates lower CH_4, N_2O, and CO_2eq emission quantities than CON ($p < 0.05$).

Table 4.3 Maximum cumulative gas quantity collected within the gas bags and cumulative GHG and VOC quantities at these time points.

Barrel/ treatment [A]	Storage period [B]	Gas quantity	Cumulative gas emission quantities					
			CO_2	CH_4	N_2O	CO_2eq [C]	Ethanol	Ethyl acetate
	[d]	[L kg_{DM}^{-1}]	[mg kg_{DM}^{-1}]					
CON1	25.0	8.4	12,889	0.05	0.37	110	12.2	1.09
CON2	14.0	8.8	12,237	0.05	0.31	94	10.9	1.20
CON3	14.0	8.2	11,419	0.05	0.27	82	14.3	1.05
CON4	25.0	8.4	11,391	0.04	0.33	100	11.3	1.28
BIO1	25.0	8.7	11,973	0.04	0.28	83	13.2	1.36
BIO2	25.0	8.4	11,493	0.04	0.27	80	10.3	1.15
BIO3	60.0	8.9	9,895	0.03	0.18	54	10.2	1.49
BIO4	130.0	10.5	11,091	0.03	0.13	41	14.4	4.00
CHE1	14.0	6.8	10,293	0.05	0.49	147	10.3	1.01
CHE2	14.0	6.5	10,310	0.05	0.50	151	9.5	0.77
CHE3	14.0	6.4	9,418	0.04	0.50	150	6.8	0.84
CHE4	14.0	7.1	10,127	0.04	0.43	130	7.9	0.87
CON	**19.5** ± 6.4	**8.5**^{b} ± 0.2	**11,984**^{b} ± 720	**0.05**^{b} ± 0.00	**0.32**^{b} ± 0.04	**97**^{b} ± 12	**12.2**^{b} ± 1.5	**1.15** ± 0.10
BIO	**60.0** ± 49.5	**9.1**^{b} ± 0.9	**11,113**^{ab} ± 889	**0.03**^{a} ± 0.01	**0.21**^{a} ± 0.07	**65**^{a} ± 21	**12.0**^{ab} ± 2.1	**2.00** ± 1.34
CHE	**14.0** ± 0.0	**6.7**^{a} ± 0.3	**10,037**^{a} ± 421	**0.04**^{ab} ± 0.00	**0.48**^{c} ± 0.03	**144**^{c} ± 9	**8.6**^{a} ± 1.5	**0.87** ± 0.10

Significant differences ($p < 0.05$, analysis based on single measurement time points) among the three treatments are indicated by different lowercase letters.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] Barrels opened on ensiling day 30: CON1, CON2, BIO1, BIO2, CHE2, and CHE3. Barrels opened on ensiling day 135: CON3, CON4, BIO3, BIO4, CHE1, and CHE4.

[B] Measurement time point within the anaerobic storage period when the maximum amount of gas was formed, i.e. the maximum gas quantity in the first and second gas bags. At this point, most of the gases are expelled from the silo.

[C] CO_2eq emissions derived in this trial consider only the climate-relevant gases, i.e. CH_4 and N_2O. Global warming potentials: CH_4 = 25, N_2O = 298.

4.4 Discussion

4.4.1 Composition of the silage

Overall, the DM of the fresh material was greater than the target value of 30%–40% [59, 60]. However, the silage quality meets the requirements for modern maize silage [59] and the V-scores indicate excellent qualities. DM affects microbial activity and subsequent gas formation. With high DM, microbial metabolism seems to be less active: fewer acid quantities are generated [61], gas formation is delayed [2], and less gas is formed [38]. The pH values determined via laboratory analysis are in the recommended range of 3.7–4.0, the latest since ensiling day 14 [60]. A continuous increase in LA is an indicator of homofermentative LAB activity, and an increase in AA is an indicator for heterofermentative LAB activity [6, 18, 62]. The LA concentrations for BIO on ensiling day 135 are below the target range (30–60 g kg_{DM}^{-1}); the opposite applies for AA concentrations (10–30 g kg_{DM}^{-1}) [60]. However, this pattern results from the use of biological inoculants: *Llb. buchneri* leads to the degradation of lactic acid to acetic acid, 1,2-propanediol, ethanol, and CO_2 [18, 62, 63]. The presence of small amounts of propionic acid at ensiling day 135 in BIO aligns with the activity of *Llb. buchneri* [60, 64].

The DM losses showed initial losses between ensiling days 0 and 14 and subsequent increases in DM mass up to day 30 for CON and CHE or day 135 for BIO (Section 4.4.5). The DM losses in the silage barrels are on the levels reported for commercial maize silos [10, 65]. However, as stated by Ostertag et al. [66], farm-scale silos exhibited greater DM losses than laboratory silos. Higher farm-scale losses seem reasonable due to prolonged oxygen supply [34, 41], lower gas tightness of the silos, and possible air leakages within silo sealing [14]. Furthermore, 67%–80% of the maximum DM losses (ensiling day 14) were detected within the first 2 ensiling days. The high DM and low packing density of silage lead to high porosity and high oxygen quantities in the silos at the moment of silo closure. This can promote the activity of plant material and aerobic microbes, e.g. enterobacteria or yeasts [1, 7]. Sun et al. [67] reported a negative correlation between packing density and *Enterobacter* abundance. Moreover, some articles have reported correlations between DM concentration and packing density and subsequent DM losses [7, 9, 68, 69]. For instance, Borreani et al. [7] report the findings of Holmes [70]: DM loss [%] = 29.1 – 0.058 × DM density [kg_{DM} m^{-3}]. The highest losses occurred with increasing DM concentration and low packing density [71]. These findings align with the noticeable GHG and VOC formation observed during the first days of ensiling (Sections 4.4.2 and 4.4.3). In principle, higher DM leads to lower gas formation in the silage if the porosity in laboratory silos is consistently low [38]. Compared to silage with low DM, the fermentation intensity is reduced with dry silage, as the acid tolerance of the microbes is lower. In practical silos, however,

compacting the dry particles is usually associated with greater effort; higher porosities and increased residual oxygen supply are the result. This was modelled in the laboratory silos here with high porosity. Therefore, the regression equations presented should be able to estimate the quantities of gas and CO_2 emitted by farm-scale silos during the main fermentation phase of maize silage with similar DM.

4.4.2 Formation and emissions of greenhouse gases

All three treatments indicate a rapid increase in GHG quantities formed and emitted into gas bags, which aligns with former reports stating a regressive course of accumulative CO_2 emission quantities [4, 5, 13, 38, 72, 73]. Emitted gas quantities vary between plant species [4] and are affected by SA use [5, 72]. In detail, SA did not affect the quantity of gas formed by maize silage during the first 10 ensiling days [5]. However, during the ongoing activity of heterofermentative LAB, additional gas was generated. The initial course of gas formation correlates with DM losses. The initial gas formation in the first phase of the ensiling process [1] is based on the aerobic activity of the plant material and microorganisms [2, 74]. The barrel silos indicated noticeable yeast counts on the silage face at ensiling day 30. This could result from high initial oxygen availability and low yeast inhibition due to lower CO_2 concentrations in zero-pressure systems [75].

Several studies have measured gas concentrations within silos [2, 34, 76, 77]. The CO_2 concentrations presented here (approximately 66%) support the data from Peterson et al. [76] and Wang and Burris [34], who reported CO_2 concentrations of 75%–85% after 48 h of ensiling in commercial farm-scale maize silos. The values seem comparable, considering the gas tightness of the zero-pressure set-up and the quantities of N_2 remaining in the system.

In addition to plant and aerobic microorganism activity, LAB are considered to be tolerant to aerobic conditions and are active in the first phase of ensiling [7, 78, 79, 80]. For instance, *Lpb. plantarum* can metabolise glucose first to lactate and subsequently to acetate under aerobic circumstances resulting in CO_2 formation [78, 81, 82]. However, based on the abundance in the epiphytic microbial community [1, 83], proteobacteria and particularly enterobacteria seem to be the most important microorganisms for oxygen depletion, CO_2 formation, and pH decrease in this phase [80, 84]. Other microorganisms such as yeasts contribute to CO_2 formation during aerobic respiration.

Unfortunately, gas sampling and analysis could not measure the oxygen concentrations within the silos' headspaces. Therefore, it is unclear when absolute anaerobic conditions were present. Some of the O_2 within the barrel may already be respired between filling and sealing the barrel. The availability of oxygen differs for the various layers and positions within silage due to metabolic respiration and limited oxygen diffusion [2, 20, 85, 86]. In former trials, the aerobic

phase in maize silos lasted only for several hours, e.g. 1.4–3.0 h in laboratory silos and 6.0 h in farm-scale silos [34, 41, 80]. The rapid decrease of pH until ensiling day 2 confirmed anaerobic conditions before this point. It is assumed that anaerobic conditions apply the latest at ensiling hour 8, based on the literature review, and that the gas bag volume starts to increase. The respiration of O_2 to CO_2 considers the constant gas volume of both gases.

After this point, enterobacteria, yeasts, LAB, and propionic acid bacteria can form CO_2 during anaerobic fermentation [8, 87]. Approximately 87%–93% of the total CO_2 is generated after ensiling hour 8, i.e. in the anaerobic phase. The formation of LA – in combination with decreased water activity, microbial substrate concurrence, and additional factors – inhibits microbial activity when the pH tolerance of the bacteria is undercut. Currently, it is unclear which ratios of total CO_2 production can be assigned to which phyla or genera of microorganisms. Sun et al. [80] described a high abundance of enterobacteria in the first hours after silo closure. The increase in 2,3-butanediol quantities formed within the first 2 ensiling days indicates the metabolism of enterobacteria during neutral fermentation [8, 79, 87]. In this phase, anaerobic fermentation by enterobacteria leads to the synthesis of AA or CO_2, among other products, and DM losses of -17% occur [79]. For this purpose, LA is a potential substrate [88] that affects the slope of the pH decrease. Vigne [84] reported that enterobacterial metabolism is the major gas producer in this phase. Upon further metabolism under acidic conditions (down to $pH \geq 4.5$) [1], glucose is metabolised to lactate, acetate, ethanol and CO_2 [79]. However, the abundance of enterobacteria decreased with decreasing pH to > 5.5 [80]. Yeasts ferment glucose to ethanol and CO_2, leading to DM losses of -49% [7, 79]. Therefore, the formation of 1 mol of ethanol by yeasts is associated with greater DM losses (-24.5%) than is the formation of ethanol by enterobacteria (-17%) or heterofermentative LAB (-17%). The ratio of ethanol production during the first 2 ensiling days of maximum ethanol quantities (65%–68%) was less than the initial CO_2 and gas quantity formation (71%–79%). Schmidt et al. [13] measured unrestrained CO_2 production in the first 2 ensiling days after treatment with natamycin. This food and feed additive should inhibit yeast spoilage [29] but not enterobacterial activity. Thus, in this study, a significant portion of the initial gas formation seemed to be metabolised by microorganisms such as enterobacteria or LAB rather than by yeasts. Enterobacteria counts were not quantified, but the literature reports significant counts and activity in the initial days [8]. A more detailed analysis of the microbiological community and fermentation products, such as formic acid, 1-butanol, and hydrogen gas, could provide additional information concerning CO_2 formation.

After the final pH values were reached, most of the CO_2 formed could be assigned to obligate heterofermentative LAB, such as *Llb. buchneri*. These bacteria convert LA to 1,2-propanediol,

ethanol, AA, and CO_2 [6, 18]. This anaerobic fermentation, which also occurred in the late phase of the storage period, explains the increase in gas quantities (up to ensiling day 60 or 135) measured in barrels BIO3 and BIO4 in combination with the increase in 1,2-propanediol, ethanol, and AA concentrations. Thus, the application of heterofermentative LAB using biological SA can lead to increased CO_2 formation. However, the cumulative CO_2 quantities did not differ between CON and BIO despite increasing gas bag volumes in BIO3 and BIO4. This aspect may be attributed to the short fermentation period of 30 days for some barrels and to CO_2 degradation or fixation pathways (Section 4.4.5). To the best of the author's knowledge, scientific research on gas formation during the fermentation process of maize silage treated with chemical SA is lacking.

After 80 ensiling days, CON (425 g_{DM} kg_{FM}^{-1}) formed ≈ 7.4 (L gas) kg_{DM}^{-1}. A preliminary trial by our working group (unpublished data) determined cumulative gas quantities of 10.0 L kg_{DM}^{-1} for untreated maize silage (400 g_{DM} kg_{FM}^{-1}) after 83 days of storage. Daniel et al. [5] reported gas quantities of ≈ 13.0 L kg_{DM}^{-1} for untreated maize silage after 83 days (380 g_{DM} kg_{FM}^{-1}). Therefore, gas quantity formation seems to increase with decreasing DM. These findings align with the observations of Gomes et al. [38], who reported decreased gas formation with increased wilting intensity in oat silage. Nevertheless, while high DM seem to decrease CO_2 emissions during anaerobic storage, higher emissions are possible during the aerobic phases after silo closure or in the feed-out phase (Part B) [7]. Therefore, managing the DM seems to be a compromise but is important for minimising DM losses and gas formation.

Although other gases remain in the zero-pressure system, CH_4 concentrations (maximum of 6.8–8.0 ppm) exceed the values in grass and lucerne silage (maximum of 4.6–5.8 ppm) [2]. CH_4 concentrations are on the level of Schmidt et al. [13], who measured 7 ppm methane in maize silo emissions on ensiling days 5 and 15. After oxygen depletion in the whole gas volume or in some areas of silage [2, 20, 85, 86], enterobacteria can provide free hydrogen (H_2) during formate degradation in the first hours of the (partially) anaerobic phase [1, 2]. This H_2 can be used partly by archaea for methanogenesis. This process can stop if the pH is ≤ 6.8, a typical threshold for common archaea in silage-based methanogenesis in biogas plants [89]. However, the highest CH_4 concentrations were measured at ensiling days 5.5–6.0, and laboratory analysis indicated that the pH was ≤ 5.9 beginning on day 0. A literature review indicated that methanogenesis occurs in acidic environments by acetoclastic methanogens, using acetate as a substrate, or via hydrogenotrophic methanogenesis by specific archaea, using H_2 as an electron donor [90]. A decrease in the pH to 3.8 promotes the latter pathway based on the pH tolerance of various microorganisms, e.g. *Methanobacterium* and *Methanothermobacter* [90, 91, 92, 93]. However, to the author's knowledge, little is known about the presence of these microorganisms in maize

silage. In particular, the sensitivity of various microorganisms to changes in pH [90, 94] requires additional research. Other biochemical pathways may also be involved [2]. Modified gas analysis, which involves measuring H_2 concentrations within the silos, could supply additional information regarding methanogenesis and enterobacterial activity.

The N_2O concentrations (maximum of 24–38 ppm) are less than the maximum values mentioned by Schmithausen et al. [2] (686–1,118 ppm), Zhao et al. [33] (1,806–1,836 ppm), and Wang and Burris [34] (10,000–43,500 ppm). On the other hand, Schmidt et al. [13] reported concentrations of 1 ppb in the cumulative emissions of maize silage. The variety of values can be partly explained by the varying experimental set-ups, sampling points, or technical limitations [33]. N_2O formation seems to be part of denitrification [2, 95, 96]. Franco [97] reported that silage treated with potassium sorbate emitted greater amounts of nitrogen oxides (NO_x) during the initial fermentation period. The same applies to N_2O in the CHE treatment. Franco [97] assumed that potassium sorbate has an inhibitory effect on denitrificating microorganisms and limits the degradation of nitrate and nitrite to N_2. However, all three treatments showed no signs of extended proteolytic breakdown, which could lead to additional N_2O formation.

The LAB inoculation in BIO was able to reduce organic acids in CHE increased CO_2eq emission quantities. Thus, the use of SA affects the formation of climate-relevant emissions, but the relevance of these differences has to be contextualised (Part B). All treatments show higher N_2O emission quantities than CH_4. This, in combination with the different GWPs, shows that the climate relevance of N_2O emissions is more significant than that of CH_4. Any mitigation measures should therefore start with a reduction in N_2O emissions. To minimise DM losses and CO_2 formation, the aim should be to achieve rapid oxygen exclusion through low porosity and rapid covering of the silo as well as a rapid pH reduction to minimise the activity of yeasts and enterobacteria.

4.4.3 Formation and emissions of ethanol and ethyl acetate

The course of ethanol concentrations within the material aligns with values stated by Weiß et al. [20], who reported a rapid increase within the first two days of the ensiling period and a subsequent slow increase until ensiling day 30. Weiß et al. [98] support these data, stating that high proportions of maximum ethanol concentrations are formed in the early fermentation phase. The formation of ethanol starts in silos with temporal-spatial variation considering oxygen depletion [20] and pH [32]. Weiß et al. [20] reported a very similar pattern in yeast counts and ethanol concentrations in promptly or delayed sealed maize silage and a strong linear correlation between DM losses and ethanol concentrations. However, the role of enterobacteria in ethanol fermentation should also be considered. The initial DM losses and gas formation,

possibly unrestrained by antifungal SA (Section 4.4.2), led to the assumption that the production of ethanol was also based on the metabolism of enterobacteria. However, further research is necessary to clarify this phenomenon in various silages.

Concerning the continuous increase in BIO, Weiß et al. [47] measured heterogeneous ethanol concentrations in farm-scale silos, but the concentrations decreased with the addition of heterofermentative LAB. These findings align with those of Arriola et al. [6]. The laboratory-scale trial of Hafner et al. [19] measured higher ethanol and EA concentrations in heterofermentative LAB-treated maize silage than in CON silage ($p < 0.01$). The following assumptions for ethanol formation apply: (a) Ethanol can be produced in a pathway parallel to the degradation of LA to 1,2-propanediol or AA by *Llb. buchneri* [18]. Therefore, increasing ethanol quantities are an indicator of the activity of heterofermentative LAB [18, 19, 32]. In addition to the formation of AA, heterofermentative LAB can produce ethanol during the anaerobic fermentation of glucose [79]. As shown, the counts of LAB and AA and ethanol concentrations are greater in BIO; (b) yeasts are a typical producer of ethanol in silage production [16, 20]. The increasing AA concentrations and decreasing yeast counts in the glass jars exclude this formation pathway in the BIO treatment. Furthermore, the excellent silage face scoring of the BIO barrels excludes a difference between the glass jars and the silo barrels. The formation of ethanol by yeasts may be from higher priority in practical silos than in laboratory silos which are characterised by rapid compaction and sealing. Based on the literature review, assumption a) seems the most reasonable in this trial. Thus, heterofermentative LAB may be beneficial for ethanol reduction in practical silos by suppressing yeast metabolism but contradictory in laboratory silos.

The only article considering the measurements of ethanol gas formation during silage-related, broth-based anaerobic fermentation was by Shan et al. [32]. Their results indicate rapid ethanol production within the first 20–30 h with reduced gas formation quantities for the treatment supplemented with *Llb. buchneri* and *Lpb. plantarum* compared to that of *Llb. buchneri* alone. However, the trial presented here indicated no difference between CON and BIO during the first days of the ensiling process. Ethanol gas formation stopped at ensiling day 4.67, at which point no further increase in ethanol gas quantity occurred. The ethanol concentrations in the silage material further increased. At present, why gas formation stopped is unclear, but pH may be a factor. In the trials of Shan et al. [32], ethanol gas formation stopped rapidly at pH 3.7–4.0.

Furthermore, previous research has shown that the gas quantities in silos are based on the VOC concentrations of the material considering Henry's law [16, 31, 32]. Moreover, ethanol gas formation may depend on ambient and silage temperatures during anaerobic fermentation, aligning with emission patterns in the feed-out phase [21, 36]. This may be true, but correlations between

ethanol and EA concentrations and between ethanol and gas quantities are small or nonexistent. Therefore, measurements of ethanol or ethyl acetate concentrations within the gas phase of farm-scale silos may provide only limited information for assessing material concentrations. Consequently, this procedure would not be an improvement over a standard laboratory analysis.

Hafner et al. [31] established a calculation model for VOC emissions during the fermentation process and calculated losses of 0.2%–1.0% of the present ethanol. For this purpose, they assumed uniform ethanol concentrations for the ensiling process, which should be constant at the final level. Based on the results presented here, the ratios of treatments CON, BIO, and CHE were 0.2%, 0.1%, and 0.1%, respectively. The percentages for EA are 1.1% for CON, 0.8% for BIO, and 1.1% for CHE. The experimental data are lower than the calculated data, which aligns with the difference between assumed continuous ethanol concentrations and the actual pattern measured in the material [16].

The course of EA concentrations is, in principle, in line with the previous literature showing an increase in the first 16 [98] to 30 ensiling days [20], followed by a subsequent decrease. However, local minima at ensiling day 14 have not yet been reported.

The formation of EA by yeasts was detected under various metabolic and processing conditions [99, 100]. In silage, EA can be produced through several biochemical pathways as previously described by Weiß et al. [20]. EA formation within the first days after silo closure can be mainly attributed to the enzymatic yeast activity of esterase, hemiacetal dehydrogenase, and alcohol acetyltransferase. Thus, EA may be formed by dehydration of acetate and ethanol, from acetyl-CoA and ethanol or from reduced hemiacetals like ethanol and aldehyde, respectively [20, 101]. In detail, the metabolism of some yeast genera shows a substantial increase in ethanol and especially EA formation shortly after oxygen depletion [102]. This could explain the rapid increase in material concentrations and gaseous emissions of EA during the initial phase of the ensiling process. Unfortunately, changing the gas bags at ensiling hour 36 could have affected the gas concentration measurements. In addition, the enzymatic activity of esterase in *Acetobacter* sp. [103] could also be of minor relevance in this first period.

Due to microbial analysis and silage face scoring, the activity of yeasts can be neglected during the late increase in EA in the BIO treatment. At this point, other possible explanations could be the metabolism of LAB. Previous research has focused on the activity of the ferulate-esterase formed by *Llb. buchneri* to affect silage digestibility by ruminants [104]. Furthermore, naturally occurring LAB on plants cause EA formation [105]. Therefore, LAB can synthesise esters and probably also EA in silage. Thus, the increase in the EA concentration in BIO may be attributed to the activity of (heterofermentative) LAB. This aligns with the increased EA concentrations

in *Llb. buchneri*-treated silage treatments observed by Gomes et al. [38]. Moreover, combining lactic acid and acetic acid bacteria can enhance EA formation [106]. However, during the ongoing fermentation phase, enzyme activity seems to decrease with increasing concentrations of acids and decreasing pH [20, 107]. Thus, further research to determine a more precise bacterial species and strain specification is required concerning EA synthesis.

According to the authors' information, the formation of gaseous EA emissions during the anaerobic fermentation of silage has not yet been reported. Therefore, this study presents novel information concerning potential VOC emissions from silage production.

Overall, the VOC concentrations in all silage treatment materials were lower than those in the former literature [20, 39, 47, 98, 108, 109]. Reviewing these studies leads to the assumption that ethanol (2.6–33.6 g kg_{DM}^{-1}) and EA (0.02–1.60 g kg_{DM}^{-1}) concentrations vary widely and EA concentrations decrease with increasing DM in maize silages. However, the literature data show high variation in trial set-up, plant variety, and storage length and conditions. Weiß et al. [110, 111] reported that VOC concentrations increase with strict anaerobic conditions, lower ambient air storage temperatures, and high packing density. For legume silage, VOC concentrations decrease with increasing DM [111]. Therefore, low CO_2 concentrations within zero-pressure barrels, constant indoor storage temperatures, low packing density, and high DM could lead to reduced ethanol and subsequent EA formation compared to farm-scale silos. Whether practice silos with higher DM also form less EA cannot be generalised. The multifactorial influences, such as the achieved compaction, O_2 supply, and resulting microbial activity, can have an impact.

4.4.4 Examination of the methodological procedure

The packing density of 150.60 ± 0.08 kg_{DM} m^{-3} was significantly below the theoretical recommendations for farm-scale silos of approximately 346 kg_{DM} m^{-3} for fresh material with 42.5% DM [112, 113]. However, these theoretical target values cannot be achieved in practice silos, where a density of around 260–300 kg_{DM} m^{-3} should be aimed for. The calculated porosity of the material was, therefore, very high. The trial set-up involved the combination of high DM and low packing density to provoke heating of the silage during the aerobic storage period (Article Part B) according to the German procedure for assessing the ASTA of silages [114]. With this, DM losses seem to be high compared to former laboratory-scale studies but at the level of well-managed commercial silos [7, 10, 65, 115, 116, 117]. Therefore, the correlation between DM losses and CO_2 emissions could also apply to farm-scale silos, since the basics of metabolic processes differ only slightly between systems [14]. Nevertheless, diverse microbiota [75] should be considered, and farm-scale silos with similar properties (high DM and low packing density)

are likely to suffer greater DM losses. This aspect is especially relevant for increased oxygen exposure due to small leakages or during the aerobic feed-out phase (Part B).

The set-up of barrels and gas bags is a variation of previous trials in silage [2, 30] and differs from the approaches of Brazilian researchers [4, 26, 38]. The importance of short measurement intervals has been discussed previously [2]. Barrel silos are established for laboratory-scale investigations and, at the same time, have larger masses than glass jars. This fact was utilised to achieve larger emission quantities. As a result, these corresponded to the measuring ranges of gas measurement technology, especially during the aerobic emission measurement periods (Part B). The addition of gas bags enables the quantification of GHG and VOC emissions. If no bags had been used, the gas quantities would have been released through the lid seal, and it would only have been possible to determine gas concentrations in the headspace and not emission quantities [2]. However, the storage of all gases, especially residual N_2 and formed CO_2, could affect the emission behaviour or gas fixation of silage due to varying volatilisation and diffusion rates based on Fick's and Henry's laws [15, 41]. Furthermore, little is known about the dispersion of gases in silos. The first gas bags had lower gas concentrations than did the headspace; the second gas bags had lower gas concentrations. A significant portion of the residual N_2 was transferred into the first gas bag due to the formation of CO_2 in the silage material. Thus, further trials should use larger gas bags (≥ 15 L kg_{DM}^{-1}) to avoid methodological implications. Nevertheless, whether gases are subject to stratification in barrels and gas bags is unclear. Therefore, some variation between the calculated and actual gas quantities can apply. Furthermore, the storage of formed gases within a zero-pressure silo system differs from that of commercial silos with outwards-directed mass flow. Thus, gas dynamics could vary between this trial and farm-scale silos.

The silage treatments showed an increase in DM mass at certain stages of the fermentation process. The weights of the barrel and glass jar silos were measured using scales with adequate measuring ranges. The method of DM analysis and correction was frequently used [2, 20, 39, 47, 50, 98] and is considered the best practice. Nevertheless, the DM at ensiling day 14 showed a local minimum. Therefore, the increase in DM could be based on methodological errors, but a literature review suggested that gas fixation may be possible (Section 4.4.5). However, further studies are needed to verify these hypotheses in this new field of research [42].

In this trial, barrel silos were employed in Experiment A1 and glass jar silos in A2. The variation in silo geometry and storage conditions may influence the fermentation process and silage characteristics. For instance, glass jar silos were packed to provide the same silage porosity as the barrel silos, but lacked a head or floor space. Consequently, the residual oxygen supply within each glass jar was likely to be smaller affecting the microbial activity in this phase.

However, this compromise was unavoidable due to the overarching trial set-up. The silage material in the barrels (Experiment A1) was to be stored untouched. This also meant that the quality of the silage in experiment A1 could only be qualitatively assessed once. A laboratory analysis of the material would have been desirable, but was not possible. However, the specific silo characteristics were needed to conduct emission measurements during fermentation and feed-out (Part B). Furthermore, the trial was conducted in a way to minimise differences in external effects such as ambient air temperature.

For statistical analysis, single measurement time points were merged into measurement intervals. This approach can lead to minor deviations from actual and stated time points for gas formation. However, the differences in gas formation patterns among the various silages were obvious. Furthermore, minor differences in the methodological procedure used by the two laboratories for microbial analysis may have affected the values. However, the course of microbial counts seems reasonable based on changes in chemical composition and gas formation.

The use of these measurement technologies limits the number of silos used during subsequent aerobic emission measurement periods (Article Part B). More precisely, the Multipoint Sampler and Doser (INNOVA 1303, LumaSense Technologies SA, Ballerup, Denmark) used in the aerobic emissions measurement periods for the PAS technology has 6 measuring points, which limited the number of barrels ($n = 2$; Article Part B). Due to the extensive manual sampling and dilution, the experimental design was limited to a maximum of 2 aerobic measurement periods – i.e. a total of 12 barrels. This affects the fermentation trial and restricts the statistical significance of the results shown. In particular, the significant deviation of the BIO3 and BIO4 barrels highlights the weakness of the small sample size. Nevertheless, new essential findings in silage research can be presented due to the extensive and detailed execution of the experiments and subsequent literature review. This case study should be considered an additional step in silage emission research and should be supplemented by further studies.

4.4.5 Gas quantities decrease and DM mass increase

CON and CHE showed a substantial increase in DM and a decrease in gas quantities between ensiling days 14 and 30, and BIO between days 14 and 135. On this basis, the following hypotheses were derived to explain the measured phenomena.

When setting up the experiment, care was taken to use barrel, hose, and gas bag materials impermeable to relevant gases. In addition, all the hose connectors were checked regularly. Knicky et al. [30] and Schmithausen et al. [2] used similar approaches in silage research. Other trial set-ups [26, 29] used silicone hoses known to be CO_2 permeable, leading to losses.

Long-term gas losses cannot be excluded in this trial. The decreasing quantities of gases in the zero-pressure system could result from tiny leakages, e.g. inside the seal of hose connectors. Similar loss rates for all silos and treatments are assumed if these losses apply, but it is unclear if they vary for the individual gases. For example, a greater decrease in N_2O could result from a greater permeability of this gas through the materials of the set-up. Furthermore, gas loss via permeability through the material could also be applied to other laboratory or commercial silos [7]. However, the constant gas and CO_2 quantities in the late phase of the storage period make leakage unlikely. Rather, the regressive course of CO_2 quantities strengthens the suspicion of controlled – and, from a particular point, time-saturated – gas fixation in contrast to uncontrolled, linear gas escape due to leakage [42].

Although an increase in DM during extended anaerobic storage periods is not mandatory, this phenomenon has been reported previously [6, 14, 118]. Savoie et al. [118] assumed errors in methodology and analysis (Section 4.4.4) or in the formation of effluent. The effluent was not detectable in this trial due to the set-up but seemed unlikely due to high DM [119].

These decreasing gas volumes contradict the findings of Daniel et al. [4, 5], who described degressive courses. The Brazilian silos indicate positive pressure, and gases were released after pressure measurements. The set-up used here is based on the zero-pressure principle. These differences could depend on the various solubilities of CO_2 in the liquid phase, considering the varying DM. Furthermore, the concentrations in the gas space of the silos affect the solubility and volatilisation of substances, but this methodology was necessary to quantify the decrease in gas quantities. Furthermore, BIO had a pH value of 4.3, whereas it was 3.8 for the treatment 'ComboHigh' (*Llb. buchneri* and *Lpb. plantarum*) in Daniel et al. [5]. This could increase the solubility of CO_2 in the form of carbonic acid in the liquid phase [120].

Other studies have reported negative pressure in silos after the completion of the main fermentation phase [2, 26, 44]. Krueger et al. [14] discussed the variance between the expected and calculated CO_2 emissions of maize silage. Furthermore, CO_2 absorption by maize silage exceeded the CO_2 solution potential in the liquid phase [26, 42, 43]. In those studies, this phenomenon can be attributed to the CO_2 permeability of the silicone hoses used in the trial. However, the decrease in the CO_2 quantity also exceeded the solution potential in the trial presented here.

Gas fixation in silage was described in recent reviews [42, 84], but these findings may be affected by erroneous methodology. Currently, two pathways of CO_2 fixation in silage are a matter of conjecture. First, Brazilian researchers assumed microbiological CO_2 reduction to acetate by acetogenic bacteria (Wood-Ljungdahl pathway) [26]. In this pathway, CO_2, in combination with

'free' protons and electrons, is the substrate for the formation of acetate and water [121]. Several species are known for this reductive acetyl-coenzyme A pathway [121]. However, to the authors' knowledge, only one species (*Sporomusa ovata*) has been detected in silage [122, 123]. Additional species could be present in the epiphytical microbial community. The application of this pathway could explain the differences in AA concentrations between laboratory-scale and farm-scale silages [124, 125]. The improved gastightness of laboratory-scale silos could lead to higher quantities of CO_2 remaining inside for additional acetate provision. These findings align with those of Vigne et al. [45] reported in Schmidt and Vigne [42]. In particular, this could explain the increase in AA concentrations during the late phase of the ensiling process, while LAB counts remain at similar levels [125]. For commercial silos, the improved gastightness of tower silos could affect this pathway compared to bunker silos.

In the second pathway, CO_2 may be chemically fixed in the liquid phase during the ensiling process [41], e.g. in the form of solved CO_2, bicarbonate (HCO_3^-), or carbonic acid (H_2CO_3). At pH 4, no bicarbonate should actually occur, and the most common form should be protonated carbonic acid ($H_3CO_3^+$), which is in equilibrium with CO_2. Furthermore, former titration trials indicated no presence of carbonates or bicarbonates in the press juice of silage samples [126, 127]. Nevertheless, microbial activity could affect the equilibrium. This includes transmembrane CO_2 transport by LAB using aquaglyceroporin [128]. The transport of CO_2 into the cytosol and its hydration to H_2CO_3 and bicarbonates are assumed to regulate the pH of the cell [14]. Furthermore, LAB can convert CO_2 to H_2CO_3 and HCO_3^- through carbonic anhydrase activity [129]. Subsequently, bicarbonate can be used for the formation of pyrimidines and arginines [129, 130]. This pathway can be auxotrophic at low CO_2 concentrations in one-third of *Lpb. plantarum*-related strains [131]. Other LAB exhibit phosphoenolpyruvate carboxylase activity and upregulated bioenergetic metabolism in a CO_2-enriched atmosphere [132]. The relevance of this pathway has yet to be proven for silage, and the possible impacts of chemical or physical pathways, e.g. buffer systems or pH changes, have yet to be assessed.

CH_4 quantities substantially decreased between ensiling days 5 and 30. Little is known about the underlying reasons for this pattern, but two assumptions apply: (a) methane leakage or relocation within the zero-pressure system, or (b) special acid-tolerant, anaerobic, methane-oxidising archaea metabolise methane to CO_2 in 'reverse methanogenesis' [133, 134]. Concerning a), low-density CH_4 could have been shifted earlier and in larger parts into the gas bags. After formation, the low density leads to an accumulation in the headspace of the barrel from where it is subsequently driven into the bag. This would influence the calculation of the gas quantities in the zero-pressure system, as this is based on the gas concentration in the headspace

(Section 4.4.4). At this point, it is unknown whether the pathways of assumption b) are relevant for anaerobic fermentation of silage.

After the initial peaks, N_2O exhibited a greater decrease in quantity than did CO_2 and CH_4 for all the treatments. This pattern could be based on (a) N_2O gas relocation or leakage at higher rates than CO_2 and CH_4, (b) denitrification of N_2O to N_2 in the silos, or (c) solubility of N_2O in the liquid phase of silage material [95, 135]. Pathway a) was already discussed. Pathway b) is also possible, since N_2O gas concentrations peak at ensiling hour 44 at pH values approximately or slightly above 4.6–4.7, and denitrification was shown to possibly occur under these acidic conditions [136]. Theoretically, denitrification leads to N_2 formation. However, in a former trial by Wang and Burris [34], a decrease in N_2O concentrations did not lead to detectable increases in N_2 concentration. However, denitrification of the small quantities of N_2O in the present study did not impact N_2 or other gas concentrations. Concerning pathway c), N_2O dissolves in the liquid phase spontaneously and in parallel with the increase in gas concentration. The increase in N_2O emissions during aerobic emission measurements (Article Part B) underlines this fact. Thus, a mix of pathways b) and c) seems most reasonable.

Little is known about ethanol degradation during the ongoing ensiling process. Some LAB and acetic acid bacteria are reported to metabolise ethanol to organic acids, e.g. AA [137, 138]. To the authors' knowledge, no such metabolic pathways have been detected in silage. The same applies to EA degradation, considering the lower rates of decrease.

Krueger et al. [14] mentioned that silage could serve as a sink and thus absorb CO_2. Schmidt and Vigne [42] expressed similar hopes, stating the open question: '*Can silage absorb more carbon than what it emits during fermentation?*'. The results presented here clearly show that although silage can absorb gases during anaerobic storage, the net balance of the fermentation process is still negative. Consequently, reducing avoidable losses in the early phases of ensiling seems more of a matter.

4.4.6 Implications for ensiling management and ensiling research

Ongoing climate change can make optimal harvesting and silage management difficult for farmers worldwide. Sudden rainfall can lead to short wilting periods [2]. On the other hand, draughts increase temperatures and solar radiation, leading to high forage DM. Improving ensiling management – i.e. by shortening chopping lengths, increasing packing density, and rapidly closing the silo – or applying SA can minimise losses during anaerobic storage. Nevertheless, other types of forage conservation, e.g. hay making, are also related to CO_2 emissions and should be compared to the silage process chain.

Further research should include O_2 analysis at narrow intervals during the first days of the ensiling process to determine the start of anaerobic conditions. For this purpose, gas sampling at different positions within the silos and more detailed specifications for microbiological analysis are recommended in future studies. With this, further conclusions can be drawn regarding the temporal-spatial variation in microbial activity. Calculating the molar masses in the zero-pressure system could also help.

To the authors' knowledge, this research article is the first to quantify the formation and emission of gaseous ethanol and EA during the ensiling process of (maize) silage. Currently, at least 46 different VOCs have been identified in silage [16]. However, ethanol can be used as an indicator substance for the VOC formation pattern since approximately 80% of silage VOC emissions are alcohol [15, 16, 139], with ethanol as the primary contributor (> 70% of alcohol emissions). Furthermore, ethanol is essential for forming esters, e.g. ethyl lactate or EA [20]. EA is reported to have antibacterial and antifungal properties. This phenomenon is primarily important for industrial processes and products, indicating the inhibitory effects of EA on yeast growth or specific bacteria [22, 23]. Thus, higher concentrations within BIO barrels could be a factor in suppressing adverse microorganisms and improving the ASTA of the BIO treatment.

VOC can lead to ground-level ozone formation and affect air pollution and climate change [15, 17]. However, the impacts are difficult to quantify, but emissions are relevant for ecological systems and can lead to substantial DM losses. This process is especially important for transforming photosynthetically bonded CO_2 to carbohydrates and subsequent emissions of methane, considering the higher GWP or high-energy and environment-relevant VOC.

Measurements of GHG and VOC gas concentrations within silos can provide important information for assessing microbial activity and ensiling quality. For instance, a previous study shows that increasing CH_4 concentrations in the silo can be used as an indicator for clostridial activity and quality decrease [2]. Furthermore, the formation of CO_2 or VOC gases may be used to determine the efficiency of microbial metabolism in silage fermentation [32]. Therefore, this approach can complement the preliminary methodology used in silage research. If applied in reverse, easily assessed parameters such as DM losses could be used to derive accurate models to calculate silage emissions and effects on the CF of silage production (Part B). VOC concentrations in the material have been part of the silage evaluation criteria for a long time, e.g. in the methods used in this trial. However, it is still unclear whether VOC gases in silos can be used to assess fermentation quality. Data show that correlations between concentrations in the material and the quantities in the gas phase are limited.

Nevertheless, even if the basic principles of anaerobic fermentation in laboratory and practical silos are similar, the transferability of the test results to the field requires special attention. The larger dimensions, extended oxygen provision during silo filling and compaction, the greater risk of air infiltration through small leakages, and the fundamental difference in gas exhaustion compared to gas storage in zero-pressure gas bags can influence gas dynamics of practical silos. Thus, future tests should also be carried out on commercial silos. The integration of experiments conducted at both scales could potentially yield a synergistic enhancement in the quality of the findings. Furthermore, parameters of ensiling management, e.g. cutting length, packing density, time period until silo sealing, or external factors, e.g. ambient temperature, may be relevant factors.

4.5 Conclusions

This study effectively measured the gaseous emissions of GHG, ethanol, and ethyl acetate during the fermentation process of maize silage. These findings aid in the evaluation of the distinct gas formation patterns of laboratory-scale silos during the various stages of the ensiling process. Notably, CO_2 formation is linked to DM loss under aerobic conditions and before the pH drops to 3.9–4.3. The BIO treatment, which was supplemented with *Llb. buchneri* and *Lpb. plantarum*, exhibited prolonged CO_2 and ethyl acetate formation. For the first time, records of ethanol and ethyl acetate gas formation during the fermentation process were made, validating earlier emission models. However, a few relationships were found between the material's VOC levels and gaseous emissions. BIO emitted less methane and nitrous oxide than the untreated control (CON). The CHE treatment, supplemented with sodium benzoate and potassium sorbate, emitted more nitrous oxide than CON ($p < 0.05$). CHE had the highest climate-relevant CO_2eq emission quantities, BIO the lowest ($p < 0.05$). After a certain point, the data indicate decreasing gas quantities, particularly CO_2. This suggests a shift from formation to fixation, which coincides with an increase in DM. The results of these experiments are worthy of further research. Nevertheless, gas formation clearly exceeds gas fixation during anaerobic storage of silage. Further research should assess parameters that may influence silage gas dynamics, e.g. plant species, forage DM and maturity, epiphytic microbial counts, silo type, including laboratory- and farm-scale silos, management decisions such as chopping length or compaction effort, and malfermentation or external factors such as ambient temperature.

4.6 Supplementary material

Table 4.4 Assignment of actual measurement time points to measurement analysis intervals for ANOVA analysis.

Measurement time point [d]	Analysis interval [d]	Measurement time point [d]	Analysis interval [d]
0.08	0.25	8.00	8.00
0.17	0.25	8.50	8.00
0.33	0.25	9.00	8.00
0.50	0.50	9.50	10.00
0.67	0.50	10.00	10.00
0.83	0.50	11.00	10.00
1.00	1.00	12.00	12.00
1.17	1.00	13.00	12.00
1.33	1.00	14.00	12.00
1.50	1.50	15.00	20.00
1.67	1.50	20.00	20.00
1.83	1.50	25.00	20.00
2.00	2.00	30.00	30.00
2.33	2.00	40.00	30.00
2.67	2.00	50.00	30.00
3.00	3.00	60.00	70.00
3.33	3.00	70.00	70.00
3.67	3.00	80.00	70.00
4.00	4.00	90.00	100.00
4.33	4.00	100.00	100.00
4.67	4.00	110.00	100.00
5.00	5.00	120.00	135.00
5.50	5.00	130.00	135.00
6.00	5.00	135.00	135.00
6.50	7.00	/	/
7.00	7.00	/	/
7.50	7.00	/	/

Table 4.5 Silo face characteristics for the barrel silos and silage treatments in Experiment A1 at silo opening at ensiling day 30 (right before the first aerobic emission measurement period started).

	Parameter		Quality point deduction	Treatment					
				CON		BIO		CHE	
				CON1	CON2	BIO1	BIO2	CHE2	CHE3
Sensory test [A]	Smell	Pleasantly acidic, aromatic, bread-like	0	X	X			X	X
		Slightly alcoholic or slight acetic acid odour	1			X	X		
		Strong alcoholic or roasted smell	3						
		Musty or slight smell of butyric acid	5						
		Disgusting, rotten smell, yeasty	7						
	Structure	Unchanged (like the original material)	0	X	X	X	X	X	X
		Easily attacked, plant parts friable	1						
		Vigorously attacked, greasy, slimy	2						
		Rotten	4						
	Colour	Colour similar to the original material	0	X	X	X	X	X	X
		Colour little changed	1						
		Colour strongly changed	2						
	Moulds	Visible mould infestation: Do not feed silage!	7	N/D	N/D	N/D	N/D	N/D	N/D
	Total	Quality (Mean ± SD)		Very good (**0.00** ± 0.00)		Very good (**1.00** ± 0.00)		Very good (**0.00** ± 0.00)	
Qualitative observation [B]	Moulds	Scale		N/D	N/D	N/D	0.50	N/D	0.50
	Yeasts	Scale		1.00	2.50	2.00	2.00	2.00	2.00

N/D = Not detectable, SD = Standard deviation.

Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] Based on the official procedure for silage scoring from the German Agricultural Society [55, 56].

[B] Qualitative, subjective observation via a scale from 0 (very good) to 5 (very bad): N/D = no mould/yeast spots; 0.50 = occasional mould/yeast spots, approx. < 5% of the surface; 1.00 = occasional mould/yeast spots, approx. 5% of the surface; 2.00 = small yeast nest, approx. 15% of the silo face, 2.50 = small yeast nest, approx. 20% of the silo face; 3.50 = multiplied yeast nests, approx. 30% of the silo face.

Table 4.6 Silo face characteristics for the barrel silos and silage treatments in Experiment A1 at silo opening at ensiling day 135 (right before the second aerobic emission measurement period started).

		Parameter	Quality point deduction	Treatment					
				CON		BIO		CHE	
				CON3	CON4	BIO3	BIO4	CHE1	CHE4
Sensory test [A]	Smell	Pleasantly acidic, aromatic, bread-like	0	X	X				
		Slightly alcoholic or slight acetic acid odour	1			X	X	X	X
		Strong alcoholic or roasted smell	3						
		Musty or slight smell of butyric acid	5						
		Disgusting, rotten smell, yeasty	7						
	Structure	Unchanged (like the original material)	0	X	X	X	X	X	X
		Easily attacked, plant parts friable	1						
		Vigorously attacked, greasy, slimy	2						
		Rotten	4						
	Colour	Colour similar to the original material	0	X	X	X	X	X	X
		Colour little changed	1						
		Colour strongly changed	2						
	Moulds	Visible mould infestation: Do not feed silage!	7	N/D	N/D	N/D	N/D	N/D	N/D
	Total	Quality (Mean ± SD)		Very good (**0.00** ± 0.00)		Very good (**1.00** ± 0.00)		Very good (**1.00** ± 0.00)	
Qualitative observation [B]	Moulds	Scale		N/D	1.00	N/D	N/D	N/D	N/D
	Yeasts	Scale		3.50	2.00	1.00	0.50	1.00	2.00

N/D = Not detectable, SD = Standard deviation.

Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] Based on the official procedure for silage scoring from the German Agricultural Society [55, 56].

[B] Qualitative, subjective observation via a scale from 0 (very good) to 5 (very bad): N/D= no mould/yeast spots; 0.50 = occasional mould/yeast spots, approx. < 5% of the surface; 1.00 = occasional mould/yeast spots, approx. 5% of the surface; 2.00 = small yeast nest, approx. 15% of the silo face, 2.50 = small yeast nest, approx. 20% of the silo face; 3.50 = multiplied yeast nests, approx. 30% of the silo face.

Table 4.7 Cumulative gas formation, and CO_2, CH_4, N_2O, CO_2eq, ethanol and ethyl acetate emissions during the fermentation in Experiment A1.

Treatment [A]	Ensiling time	Gas quantity	Cumulative gas emissions					
			CO_2	CH_4	N_2O	CO_2eq [B]	Ethanol	Ethyl acetate
	[d]	[L kg_{DM}^{-1}]	[mg kg_{DM}^{-1}]					
CON	0.5	**1.2**^{b} ± 0.6	**3,393** ± 1,078	**0.01** ± 0.00	**0.06** ± 0.03	**17** ± 10	**0.6**^{b} ± 0.6	**-0.01** ± 0.02
BIO	0.5	**1.2**^{b} ± 0.7	**3,307** ± 1,040	**0.01** ± 0.00	**0.07** ± 0.04	**22** ± 12	**0.7**^{b} ± 0.5	**-0.01** ± 0.01
CHE	0.5	**0.6**^{a} ± 0.3	**2,573** ± 733	**0.01** ± 0.00	**0.06** ± 0.05	**18** ± 13	**-0.5**^{a} ± 0.4	**0.00** ± 0.00
CON	1.0	**3.8**^{b} ± 0.5	**8,793**^{ab} ± 1,762	**0.02**^{ab} ± 0.00	**0.26** ± 0.10	**79** ± 29	**3.3**^{b} ± 1.1	**0.01**^{a} ± 0.05
BIO	1.0	**3.9**^{b} ± 0.6	**8,887**^{b} ± 1,759	**0.02**^{b} ± 0.00	**0.34** ± 0.13	**102** ± 40	**4.1**^{b} ± 1.1	**0.01**^{ab} ± 0.08
CHE	1.0	**2.3**^{a} ± 0.5	**6,293**^{a} ± 1,000	**0.02**^{a} ± 0.00	**0.33** ± 0.13	**100** ± 38	**0.9**^{a} ± 0.8	**0.06**^{b} ± 0.03
CON	2.0	**6.6**^{b} ± 0.4	**11,380** ± 850	**0.04** ± 0.00	**0.46**^{a} ± 0.05	**137**^{a} ± 15	**11.5**^{b} ± 1.0	**0.72**^{b} ± 0.07
BIO	2.0	**6.6**^{b} ± 0.3	**11,385** ± 455	**0.04** ± 0.00	**0.49**^{a} ± 0.04	**146**^{a} ± 11	**12.4**^{b} ± 1.3	**0.79**^{b} ± 0.08
CHE	2.0	**5.4**^{a} ± 0.4	**10,014** ± 837	**0.03** ± 0.00	**0.65**^{b} ± 0.04	**194**^{b} ± 12	**6.9**^{a} ± 0.8	**0.63**^{a} ± 0.09
CON	3.0	**7.3**^{b} ± 0.2	**12,369**^{b} ± 360	**0.04** ± 0.00	**0.44**^{a} ± 0.06	**134**^{a} ± 17	**12.9**^{b} ± 0.5	**0.81**^{ab} ± 0.08
BIO	3.0	**7.2**^{b} ± 0.2	**12,062**^{b} ± 337	**0.05** ± 0.00	**0.47**^{a} ± 0.03	**141**^{a} ± 10	**13.8**^{b} ± 0.9	**0.87**^{b} ± 0.05
CHE	3.0	**6.0**^{a} ± 0.3	**10,634**^{a} ± 402	**0.04** ± 0.00	**0.62**^{b} ± 0.05	**185**^{b} ± 14	**9.0**^{a} ± 1.5	**0.74**^{a} ± 0.09
CON	4.0	**7.6**^{b} ± 0.2	**12,793**^{b} ± 544	**0.05**^{a} ± 0.00	**0.42**^{a} ± 0.06	**127**^{a} ± 19	**16.8**^{b} ± 2.7	**1.00**^{b} ± 0.13
BIO	4.0	**7.5**^{b} ± 0.2	**12,788**^{b} ± 382	**0.05**^{b} ± 0.00	**0.45**^{a} ± 0.04	**135**^{a} ± 11	**16.4**^{b} ± 2.6	**1.01**^{b} ± 0.10
CHE	4.0	**6.2**^{a} ± 0.2	**11,529**^{a} ± 359	**0.05**^{b} ± 0.00	**0.61**^{b} ± 0.03	**182**^{b} ± 9	**11.1**^{a} ± 2.7	**0.84**^{a} ± 0.08
CON	7.0	**7.9**^{b} ± 0.2	**13,350**^{b} ± 444	**0.05** ± 0.00	**0.39**^{a} ± 0.05	**118**^{a} ± 14	**16.2**^{b} ± 1.8	**1.17**^{b} ± 0.16
BIO	7.0	**7.7**^{b} ± 0.2	**12,846**^{a} ± 367	**0.05** ± 0.00	**0.41**^{a} ± 0.03	**124**^{a} ± 8	**16.2**^{b} ± 0.9	**1.16**^{b} ± 0.09
CHE	7.0	**6.4**^{a} ± 0.3	**11,199**^{a} ± 1,005	**0.05** ± 0.00	**0.56**^{b} ± 0.05	**167**^{b} ± 16	**10.9**^{a} ± 0.8	**0.96**^{a} ± 0.08
CON	10.0	**8.0**^{b} ± 0.2	**13,182**^{b} ± 360	**0.05** ± 0.00	**0.37**^{a} ± 0.05	**112**^{a} ± 14	**15.9**^{b} ± 0.9	**1.24**^{b} ± 0.20
BIO	10.0	**7.9**^{b} ± 0.2	**12,676**^{b} ± 359	**0.05** ± 0.00	**0.39**^{a} ± 0.03	**116**^{a} ± 8	**15.7**^{b} ± 0.9	**1.23**^{b} ± 0.06
CHE	10.0	**6.5**^{a} ± 0.3	**10,946**^{a} ± 416	**0.05** ± 0.00	**0.53**^{b} ± 0.04	**158**^{b} ± 12	**10.1**^{a} ± 0.9	**0.96**^{a} ± 0.09
CON	30.0	**8.0**^{b} ± 0.3	**9,845**^{b} ± 1,225	**0.04**^{b} ± 0.01	**0.24**^{a} ± 0.06	**72**^{a} ± 17	**11.5**^{b} ± 1.5	**1.08**^{b} ± 0.09
BIO	30.0	**8.8**^{c} ± 0.3	**10,610**^{c} ± 619	**0.03**^{a} ± 0.00	**0.23**^{a} ± 0.03	**69**^{a} ± 8	**11.3**^{b} ± 0.8	**1.29**^{c} ± 0.08
CHE	30.0	**6.2**^{a} ± 0.4	**7,295**^{a} ± 619	**0.03**^{a} ± 0.00	**0.30**^{b} ± 0.04	**89**^{b} ± 12	**6.6**^{a} ± 0.8	**0.84**^{a} ± 0.06
CON	70.0	**7.6**^{b} ± 0.4	**8,015**^{b} ± 561	**0.03**^{b} ± 0.00	**0.17**^{a} ± 0.04	**52**^{a} ± 11	**9.6**^{b} ± 0.6	**1.02**^{b} ± 0.15
BIO	70.0	**9.1**^{c} ± 0.6	**9,967**^{c} ± 871	**0.03**^{ab} ± 0.00	**0.17**^{a} ± 0.01	**52**^{a} ± 4	**10.8**^{c} ± 0.4	**1.54**^{c} ± 0.13
CHE	70.0	**5.8**^{a} ± 0.3	**5,798**^{a} ± 357	**0.03**^{a} ± 0.00	**0.23**^{b} ± 0.02	**68**^{b} ± 5	**5.4**^{a} ± 1.0	**0.79**^{a} ± 0.03
CON	135.0	**7.1**^{b} ± 0.4	**6,944**^{b} ± 517	**0.03**^{b} ± 0.00	**0.12**^{a} ± 0.03	**37**^{a} ± 8	**9.5**^{b} ± 0.8	**0.96**^{ab} ± 0.14
BIO	135.0	**8.8**^{b} ± 1.8	**9,625**^{c} ± 1,561	**0.03**^{ab} ± 0.00	**0.14**^{a} ± 0.01	**41**^{a} ± 2	**13.6**^{c} ± 2.0	**2.91**^{b} ± 1.55
CHE	135.0	**5.5**^{a} ± 0.3	**5,043**^{a} ± 165	**0.02**^{a} ± 0.00	**0.18**^{b} ± 0.00	**53**^{b} ± 1	**5.3**^{a} ± 0.6	**0.80**^{a} ± 0.06

Treatments with different superscript lowercase letters within a parameter and ensiling day differ significantly (Tukey's-HSD or Games-Howell tests, $p < 0.05$).

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[B] CO_2eq emissions consider only the climate-relevant CH_4 and N_2O emissions. Global warming potentials: CH_4 = 25, N_2O = 298.

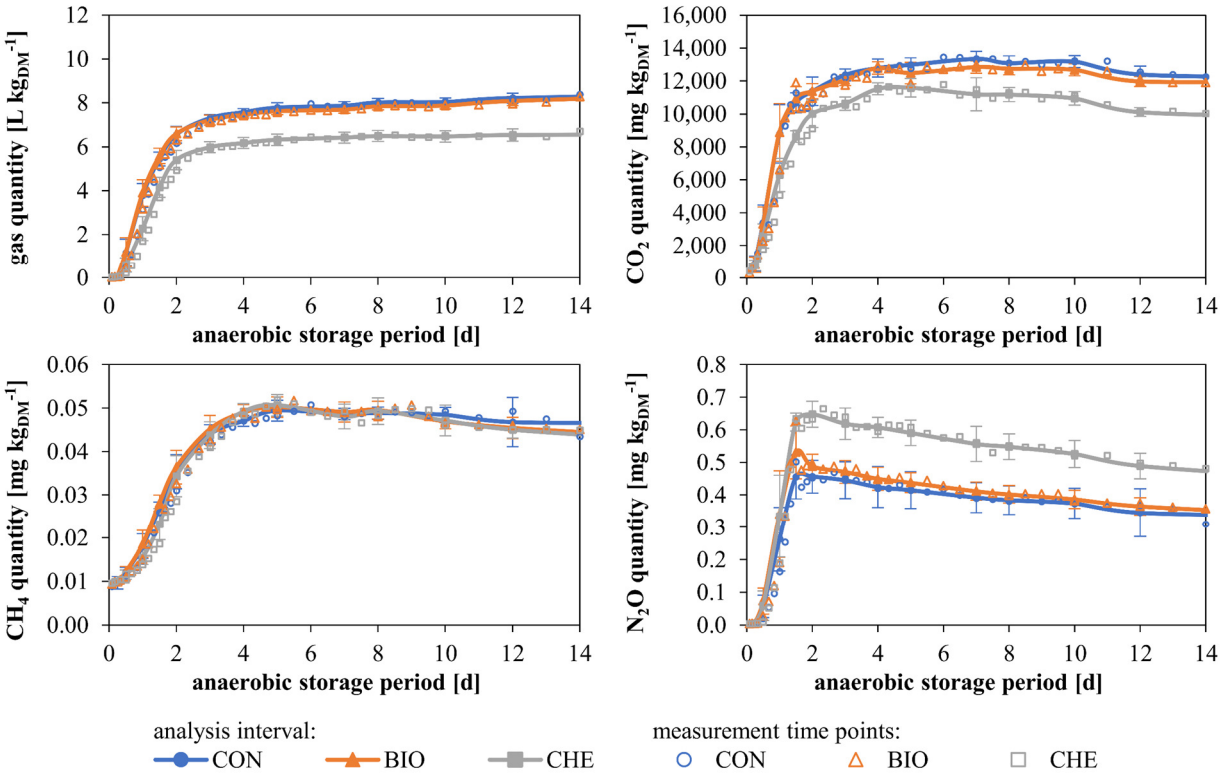

Fig. 4.7 Cumulative gas, CO_2, CH_4 and N_2O quantities within the zero-pressure systems during the main fermentation phase (ensiling days 0–14).
Error bars indicate the standard deviation of all treatments' measurement time point values within each analysis interval.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

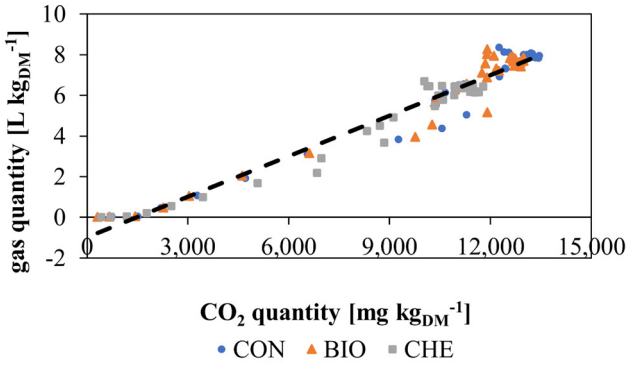

Fig. 4.8 Correlation of cumulative CO_2 and gas quantities within the zero-pressure systems during the main fermentation phase (ensiling days 0–14).
The regression parameters are: $y = -0.0007 \times x - 0.9744$, $r_S = -0.925$, $p < 0.05$.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

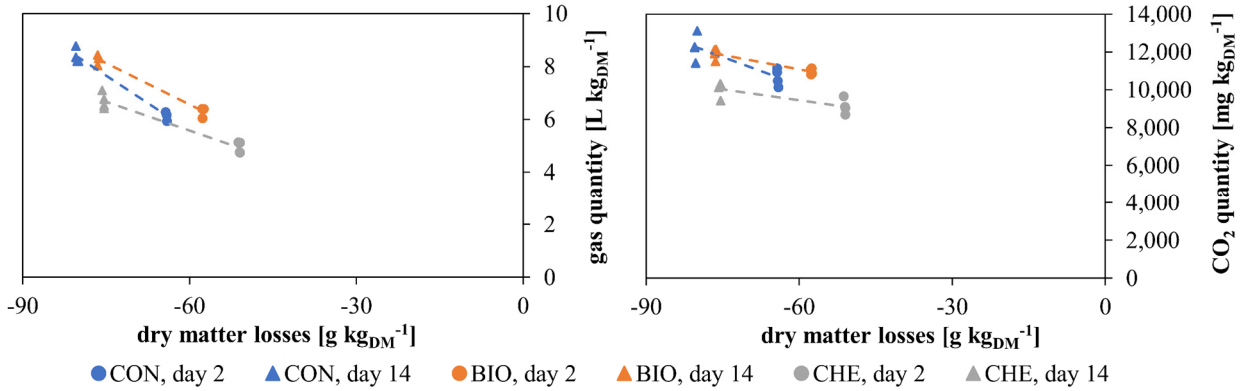

Fig. 4.9 Correlations between dry matter losses and cumulative gas quantity (left) or cumulative CO_2 emission quantity (right) during the main fermentation phase.
For gas quantities, the regression parameters are: for CON, $y = -0.1386 \times x - 2.742$, $r_s = -0.976$, $p < 0.05$; for BIO, $y = -0.1047 \times x + 0.2779$, $r_s = -0.810$, $p < 0.05$; for CHE, $y = -0.0724 \times x + 1.238$, $r_s = -0.934$, $p < 0.05$ For CO_2 quantities, the regression parameters are: for CON, $y = -99.579 \times x + 4266.5$, $r_s = -0.976$, $p < 0.05$; for BIO, $y = -50.549 \times x + 8048.5$, $r_s = -0.810$, $p < 0.05$; for CHE, $y = -37.982 \times x + 7172.8$, $r_s = -0.905$, $p < 0.05$.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

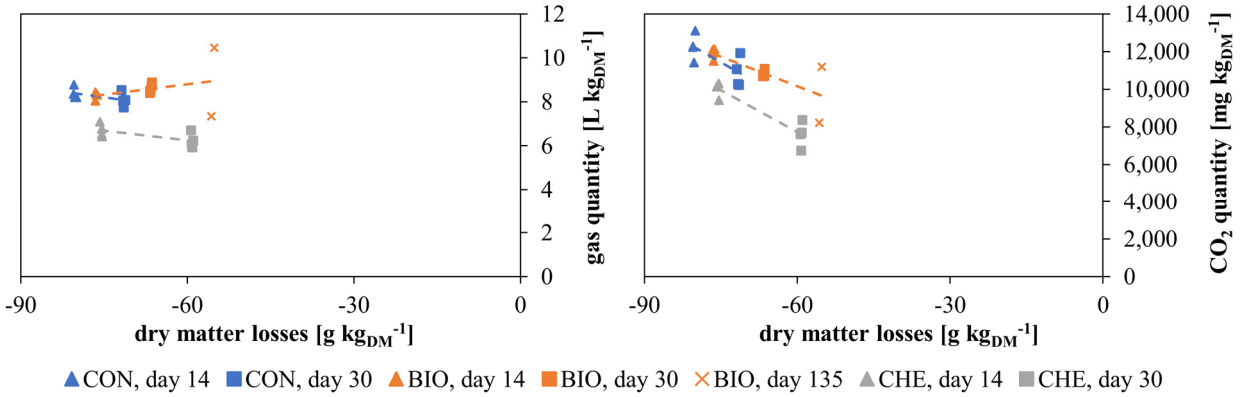

Fig. 4.10 Correlations between dry matter losses or increase and cumulative gas quantity (left) or cumulative CO_2 emission quantity (right) during the time of DM increase (for CON and CHE ensiling days 14–30, for BIO ensiling days 14–135).
For gas quantities, the regression parameters are: for CON $y = -0.0346 \times x + 5.6093$, $r_S = -0.738$, $p < 0.05$; for BIO, $y = 0.0314 \times x + 10.685$, $r_S = 0.442$, $p = 0.200$; for CHE, $y = -0.0294 \times x + 4.4836$, $r_S = -0.810$, $p = 0.200$. For CO_2 quantities, the regression parameters are: for CON $y = -154.67 \times x - 170.44$, $r_S = -0.595$, $p = 0.120$; for BIO, $y = -104.19 \times x + 3920.4$, $r_S = -0.648$, $p < 0.05$; for CHE, $y = -148.73 \times x - 1190.4$, $r_S = -0.714$, $p < 0.05$.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

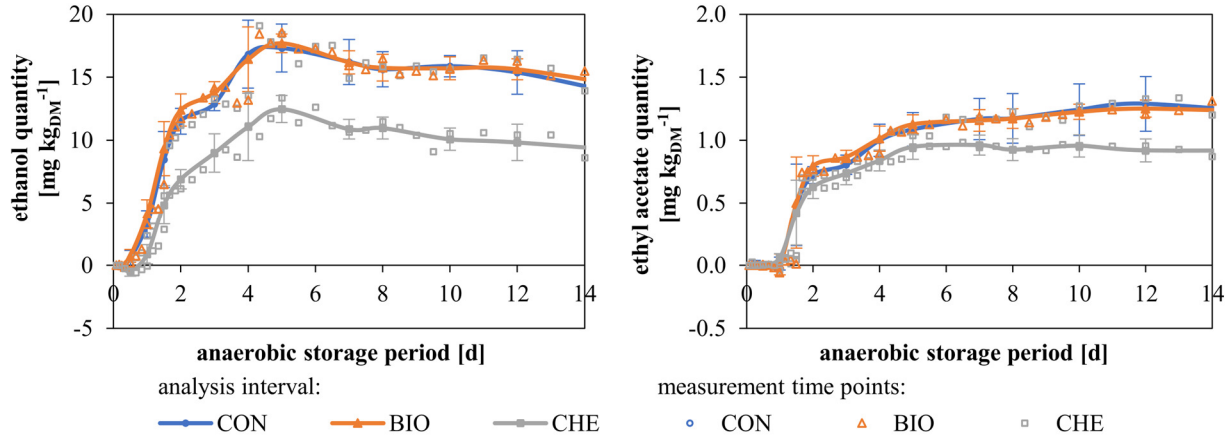

Fig. 4.11 Cumulative ethanol and ethyl acetate quantities within the zero-pressure systems during the main fermentation phase (ensiling days 0–14).
Error bars indicate the standard deviation of all treatments' measurement time point values within each analysisinterval.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

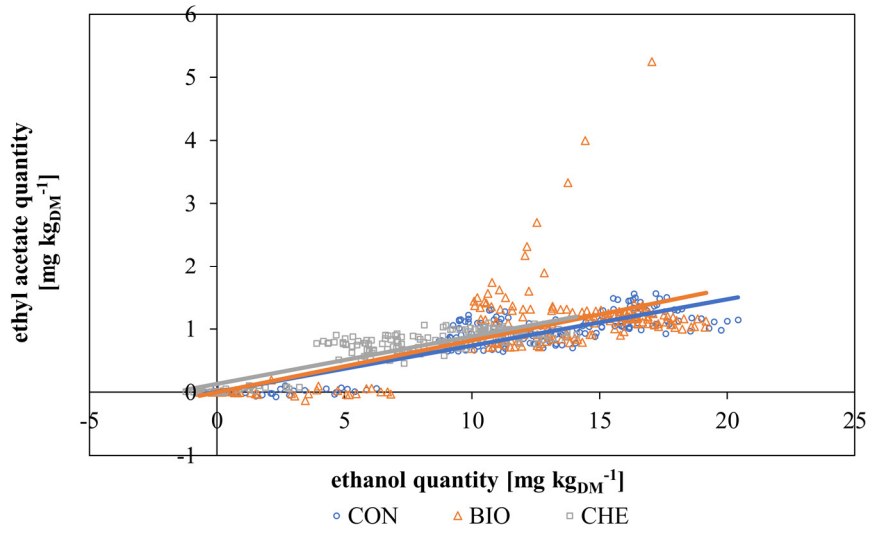

Fig. 4.12 Correlation between ethanol and ethyl acetate gas quantities in the zero-pressure systems.
The regression parameters were as follows: for CON, $y = 0.0732 \times x + 0.0038$, $r_s = 0.763$, $p < 0.05$; for BIO, $y = 0.0819 \times x - 0.0026$, $r_s = 0.529$, $p < 0.05$; and for CHE, $y = 0.0755 \times x + 0.1300$, $r_s = 0.794$, $p < 0.05$.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

4.7 Supplementary information

List of abbreviations

AA	Acetic acid
AEMP	Aerobic emission measurement period(s)
ANOVA	Analysis of variance
ASTA	Aerobic stability
BIO	Treatment containing biological additive
C	Carbon
CF	Carbon Footprint
CFU	Colony-forming units
CH_4	Methane
CHE	Treatment containing chemical additive
CO_2	Carbon dioxide
CO_2eq	CO_2 equivalent
CON	Treatment containing no additive
DM	Dry matter (concentration)
EA	Ethyl acetate
FM	Fresh matter
GC	Gas chromatography
GHG	Greenhouse gas(es)
GWP	Global warming potential(s)
H_2	Hydrogen
H_2CO_3	Carbonic acid
$H_3CO_3^+$	Protonated carbonic acid
HCO_3^-	Bicarbonate
IPCC	Intergovernmental panel on climate change
kg_{DM}	Mass of dry matter material in kg
kg_{FM}	Mass of fresh matter material in kg
LA	Lactic acid
LAB	Lactic acid bacteria
N_2	Nitrogen
N_2O	Nitrous Oxide
NO_x	Nitrogen oxides
PAS	Photoacoustic spectroscopy

r_S	Spearman's correlation coefficient
SA	Silage additive(s)
SD	Standard deviation
VOC	Volatile organic compound(s)
WSC	Water-soluble carbohydrates

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Authors' contributions

HFD: project administration, supervision, resources, conceptualisation, methodology, investigation, data curation, formal analysis, validation, visualisation, writing – original draft, and writing – review & editing. WB: funding acquisition, project administration, supervision, resources, and writing – review & editing. MT: resources, methodology, validation, and writing – review & editing. AJS: resources, investigation, conceptualisation, methodology, and writing – review & editing. KW: resources, investigation, and writing – review & editing. AL: resources, investigation, and writing – review & editing. G-CM: project administration, supervision, resources, conceptualisation, methodology, investigation, validation, and writing – review & editing.

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Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available due to further use of the data for the PhD thesis of Hauke F. Deeken but are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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5 Study 3:

Greenhouse gas and volatile organic compound emissions of additive-treated whole-plant maize silage: part B—aerobic storage period and carbon footprint of silage additive use

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Abstract

Background: Silage emits climate- and environment-relevant gases during anaerobic fermentation and aerobic feed-out periods. This trial should determine the unknown CO_2, methane, nitrous oxide, ethanol and ethyl acetate emissions of constant maize silage over both periods. The results will be published in two consecutive articles (Part A: anaerobic fermentation period; Part B: aerobic storage period).

Methods: Three silage treatments were observed ($n = 4$): The untreated control (CON) was compared to the chemical additive treatment (CHE; 0.5 g sodium benzoate and 0.3 g potassium sorbate per kg fresh matter) and the biological additive treatment (BIO; 1×10^8 colony-forming units *Lentilactobacillus buchneri* and 1×10^7 colony-forming units *Lactiplantibacillus plantarum* per kg fresh matter). During the two aerobic emission measurement periods (AEMP), the silos were ventilated mechanically to supply 2–6 (L air) min^{-1} to the two faces of the material (150.6 kg dry matter m^{-3}). AEMP1 (duration 14 days) began on ensiling day 30, AEMP2 (19 days) on day 135.

Results: In AEMP1, aerobic stability differed among the treatments ($p < 0.05$): 5.17 ± 0.75 days for CON, 6.33 ± 0.15 days for BIO, and 7.33 ± 0.57 days for CHE. In AEMP2, only CON showed a temperature increase of 2 K above ambient temperature after 7.75 ± 0.31 days. BIO and CHE indicated higher ethanol and ethyl acetate emission rates during the first period of the heating process. Furthermore, 20.0%–70.4% of ethanol and 169.0%–953.6% of ethyl acetate quantities present in the material at the silo opening emitted as gases.

Conclusion: Methane and nitrous oxide emissions during anaerobic fermentation exceeded the quantities during aerobic storage in all treatments. However, compared with those of crop production, the total climate-relevant CO_2eq emissions are small. Microbial respiration during heating leads to climate-neutral CO_2 emissions and dry matter losses. Minimising these losses is promising for mitigating climate-relevant emissions directly during silage storage and indirectly during crop production since less forage input is needed. Thus, silage additives can help improve the silage carbon footprint by improving aerobic stability and silage deterioration.

Keywords

Carbon dioxide, Carbon footprint, Corn silage, Ethanol, Ethyl acetate, Lactic acid bacteria, Methane, Nitrous oxide, Silage additives

Graphical abstract

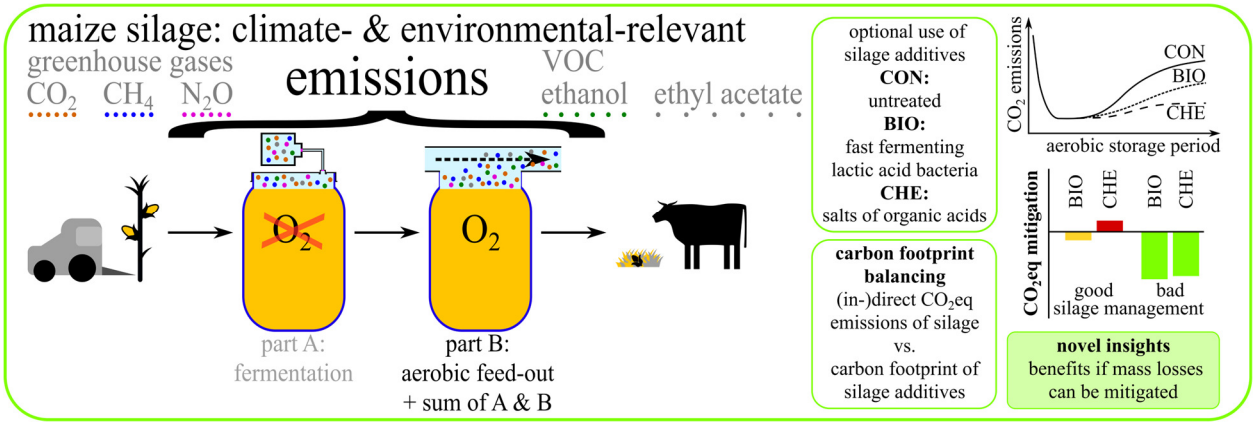

Fig. 5.1 Graphical abstract of Study 3.

5.1 Introduction

Silage is an essential global feedstuff, with the opportunity to conserve one-time crop yields. The supply of high-quality feed is crucial to feed ruminants resource-efficiently throughout the year. The same applies to biogas plants. The ensiling process includes, among others, the anaerobic main fermentation and aerobic feed-out phase [1]. One of the main objectives is to minimise dry matter (DM), energy and quality losses to maintain the resource cycle and the nutritional value of harvested plant material in the best possible manner. DM losses in silage are generally accompanied by gaseous emissions [2, 3, 4, 5] or effluent losses.

Losses are partially unavoidable for high-quality silage fermentation, e.g. heterofermentative metabolism of lactic acid bacteria (LAB) [6, 7], but include avoidable losses, too. The latter consists of exceeding activity of undesirable microbes such as enterobacteria, yeasts or moulds during anaerobic fermentation or aerobic storage. Several authors provided overviews [7, 8, 9] concerning losses and management effects, e.g. silage additive (SA) use, packing density, or aerobic stability (ASTA). Köhler et al. [10] reported losses of -5% to -15% for farm-scale maize silos during anaerobic fermentation. According to Wilkinson [9], the expected total loss of maize silage production was -20.6% from field to trough. SA, such as LAB inoculants and organic acids or their salts, can influence microbial metabolism and losses in various ways. This article focuses on the specific group of SA that achieves a prolongation of ASTA through increased acetic acid (AA) production or antimicrobial properties [1, 6, 7, 11, 12].

Silage production leads to the emission of climate-relevant greenhouse gases (GHG) [2, 4, 5, 13, 14] with various global warming potentials (GWP) and other climate- and environment-relevant gases, e.g. volatile organic compounds (VOC), which are precursors of ground-level ozone formation [15, 16, 17]. The inoculation of ensiling material with heterofermentative LAB can increase DM losses [6] and gas formation during the anaerobic

fermentation period [5] due to AA and carbon dioxide (CO_2) production [18]. Chemical additives can decrease DM losses [7] and the formation of VOC during anaerobic fermentation [19, 20]. Both additives can improve ASTA and, therefore, reduce respiratory emissions during the feed-out phase [6, 7]. Ethanol can be used as an indicator of VOC formation patterns since alcohols contribute to the majority of VOC in silage [15, 16, 21]. Ethyl acetate (EA) is reported to have antibacterial and antifungal properties and may affect microbial metabolism [22, 23]. Furthermore, the high vapour pressure of EA could lead to increased volatilisation into the gaseous phase [20].

According to Schmidt et al. [13], most of the gas produced during anaerobic fermentation is CO_2. The same applies to the aerobic feed-out phase based on respiration pathways. CO_2 can be considered climate-neutral. In the carbon (C) cycle of agricultural resources, photosynthesis converts CO_2 to biomass, which will be converted back to CO_2 in later stages, for instance, anaerobic fermentation, aerobic silage storage, farm animal digestion or manure degradation. While photosynthesis is considered a CO_2 sink, the other stages are CO_2 sources. If biomass is degraded to CO_2 during silage storage, those energy-rich C-molecules are unavailable in the later stages of the cycle. Therefore, DM losses during silage storage affect the C retention efficiency of the resource cycle. As far as the authors are aware, quantification of GHG and VOC emissions from anaerobic fermentation to feed-out of constant silage material is lacking in the scientific literature. Former trials examined either the emissions of ensiling material during anaerobic fermentation or of ensiled material during the feed-out period. Total quantities could be used to compare the emissions during silage storage with those during the other stages of the cycle or with alternative methods of conserving animal feed. Moreover, a comparison between the carbon footprint (CF) of SA and their effect on silage emissions can be made. Therefore, the CO_2 emissions from silage storage are not classified as climate-relevant emissions but rather as emissions of climate-relevant gases.

Schmidt et al. [13] estimated that silage emissions during anaerobic fermentation are lower than those during animal husbandry. However, others demanded more research to assess the relevance of all silage production stages for VOC – and the same applies to GHG – emissions [15]. Henriksson et al. [24] stated: '*In-depth knowledge of GHG emissions associated with silage production is, therefore, crucial in mitigating GHG emissions on farm level*'. This applies in modern times, to assess the CF of various agricultural food products [25]. However, some studies have reported the opposite behaviour, i.e. a gas fixation and DM increase during anaerobic fermentation, based on unclear biological or chemophysical processes [14, 26].

At the silo opening, gases, which are stored inside, are expelled [3, 27, 28, 29]. The anaerobic atmosphere of the silo is flooded with air [27, 30], and aerobic microorganisms are reactive.

The counts of acetic acid bacteria, enterobacteria, yeasts and moulds and concentrations of inhibiting substances, e.g. AA, are critical for ASTA. Acetic acid bacteria or yeasts initiate aerobic deterioration followed by substantial mould activity [1, 7, 31]. This can lead to substantial DM losses and CO_2 emission quantities. The measurement of the temperature of aerobically stored, uncompacted silage is an established methodology for determining the ASTA [32].

The volatilisation rate of VOC is influenced by parameters like temperature and the quantities present in the material [33, 34, 35]. American researchers focused on VOC emissions during short-term aerobic storage [21, 33]. Those researchers used a wind tunnel to monitor the emission pattern of silage. That procedure used small silage masses, e.g. 1.2 kg fresh matter (FM) [34], and may have led to uncontrolled gas discharge before the measurements. Hafner et al. [16] and Montes et al. [21] reported that 10% of ethanol quantities in the material emit during the first 12 h of aerobic storage. Hafner et al. [34] derived an emission model for ethanol. Empirical data are needed to validate this model because of the heterogeneity of silage material. Shan et al. [27] derived the emissions of maize silage bales during a fortnight feed-out phase. Ethanol emissions were unaffected by the silage temperature increase during initial heating. However, changes in silage and boundary layer temperatures could affect the emission rate of silage faces. Several biochemical processes convert ethanol to, among others, esters like EA [20, 36, 37]. The formation processes of various VOC include aerobic processes [38, 39], and some are affected by the use of SA [15, 16, 20, 40]. Thus, the VOC composition pattern may change during aerobic storage periods. To the authors' knowledge, the literature lacks reports of gaseous ethanol and EA emissions from silage during the heating process of additive-treated maize silage.

The objectives of this article are: (1) to combine the methodology of previous research, i.e. to use barrel silos containing uncompacted silage in a wind tunnel to assess ASTA; (2) to derive the GHG and VOC emissions of untreated or treated (biological inoculants or chemical additives) maize silage during aerobic storage periods of 14 days after varying ensiling periods (duration 30 or 135 days); and (3) to assess the temporal changes in gas formation during the ongoing aerobic storage period and heating process.

The GHG and VOC emissions of maize silage, optionally treated with SA, during the feed-out phase, have yet to be compared to the emissions during anaerobic fermentation. Based on the articles Parts A and B, additional objectives were addressed: (4) to quantify the sum of GHG and VOC emissions of constant silage material during anaerobic and aerobic storage periods with minor measurement errors possible; (5) to quantify emission mitigations by SA use; and (6) to assess the balance between the carbon footprint of crop production, silage storage emissions, and SA use (emission mitigation vs. production and application efforts).

5.2 Methods

5.2.1 Principles of the overarching trial and the two consecutive articles

A trial was conducted to determine the emissions of CO_2, nitrous oxide (N_2O), methane (CH_4), ethanol and EA as indicators of VOC emissions from constant maize silage during anaerobic and aerobic storage. Constant material means that the forage was filled into silos, where it remained intact and unchanged for the entire trial duration (both storage periods). Forage material treatments were supplemented with SA to affect microbial metabolism. Heterofermentative LAB may lead to a trade-off between increased CO_2 formation during anaerobic fermentation and decreased respiratory losses during the feed-out phase. The impact of chemical SA on VOC gas formation during anaerobic fermentation has yet to be examined. To the authors' knowledge, this trial is the first to determine the emission quantities of constant silage material during all phases of silage storage. The results are to be presented in two consecutive research articles. The first article (Part A) describes two sub-experiments (see Fig. 5.2). Experiment A1 focuses on gas formation and fixation during the anaerobic fermentation period using barrel silos. Furthermore, Part A includes the analysis of chemical and microbial composition of the treatments ensiled in glass jars used in parallel (Experiment A2). This article (Part B) addresses the emissions from the constant silage ensiled in barrel silos during two aerobic emission measurement periods (AEMP1 and AEMP2) and the sum of emissions during anaerobic fermentation and feed-out. Furthermore, this article provides a first step toward balancing SA's CF and additive effects on emission quantities during silage storage.

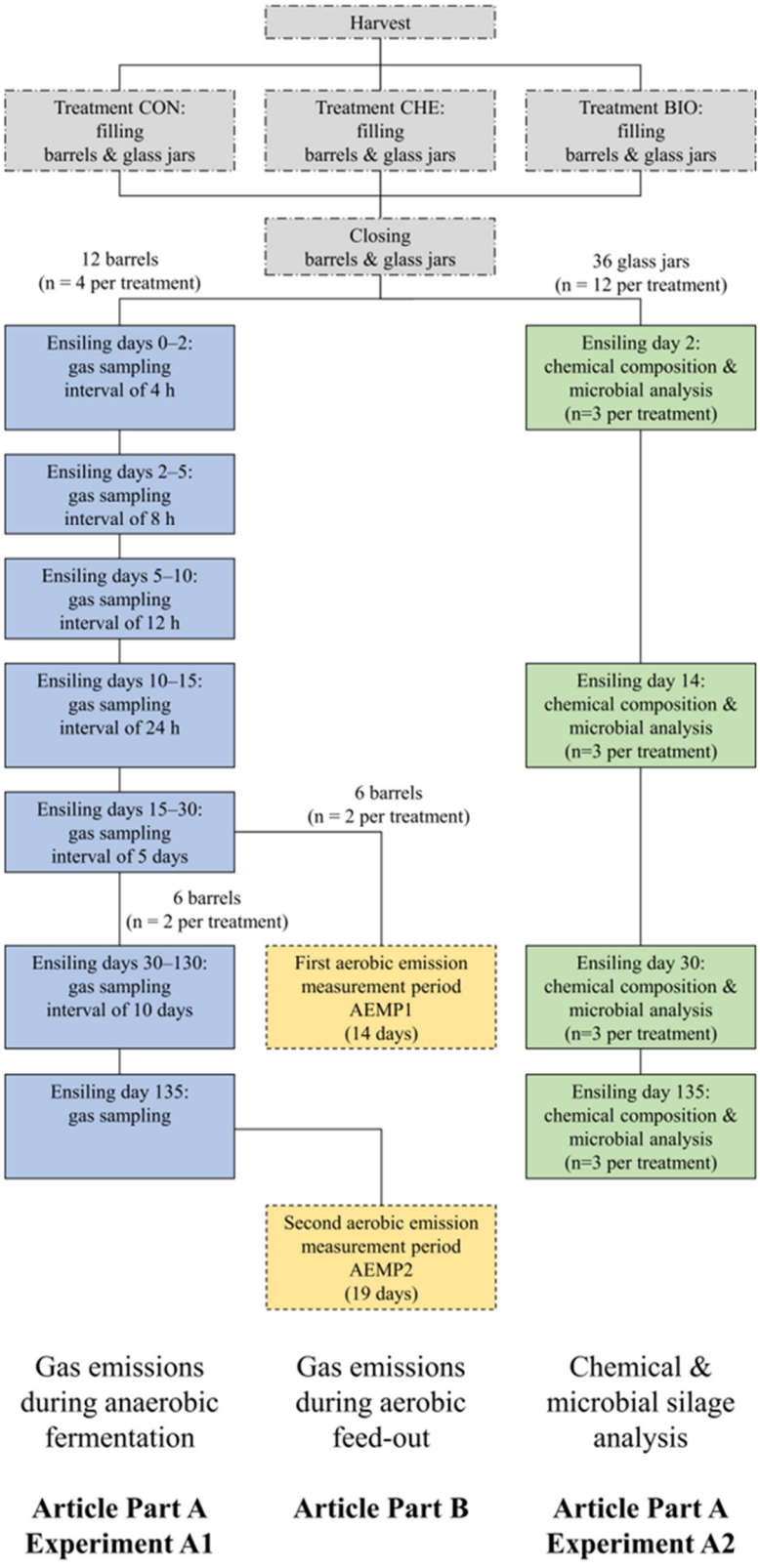

Fig. 5.2 Procedure of the overarching trial and the two consecutive articles. The processing of the treatments (grey boxes) is followed by the gas emission measurements (Article Part A, Experiment A1; blue boxes) and the analyses of chemical and microbial composition (Article Part A, Experiment A2; green boxes) during anaerobic fermentation. After 30 and 135 days of anaerobic storage, two aerobic emission measurement periods (AEMP) follow (Article Part B; yellow boxes).
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

5.2.2 Forage material, silage treatments and silo set-up

The detailed trial methodology and material parameters used during ensiling can be found in Article Part A. The most critical aspects follow shortened.

A self-propelled forage harvester with a corncracker (Claas Jaguar 940, Claas KGaA mbH, Harsewinkel, Germany) harvested maize (*Zea mays*; variant SY Werena, Syngenta Agro GmbH, Frankfurt am Main, Germany) grown at the Campus Frankenforst of the University of Bonn (Königswinter, Germany; 50°42′50.1″N, 7°12′24.9″ E). Randomly selected whole-plant forage material was used. The forage material was split into equal portions for the three silage treatments and then packed into the silos. The control treatment (CON) was not supplemented with a silage additive. The chemical additive treatment (CHE) was supplemented with a dose of 0.5 g of sodium benzoate per kg_{FM} and 0.3 g of potassium sorbate per kg_{FM} using the additive Kofasil Stabil according to the manufacturer's recommendation (Addcon GmbH, Bitterfeld-Wolfen, Germany). The biological additive treatment (BIO) was supplemented with SILA-BAC RAPID REACT Maize Combi (Pioneer Hi-Bred International Inc., Johnston, Iowa, USA). In detail, 1.0×10^8 colony-forming units (CFU) of *Lentilactobacillus buchneri* per kg_{FM} and 1.0×10^7 CFU of *Lactiplantibacillus plantarum* per kg_{FM} were applied according to the manufacturer's recommendation.

High-density polyethylene barrels (34.8 L maximum volume) were used as silos ($n = 4$). For each silo, 10.2 kg_{FM} of silage was added to the barrel. These barrels were the silos used in Experiment A1 (Part A). The barrel-specific silage material was retained within the barrels throughout the anaerobic fermentation and aerobic measurement period. Consequently, constant material was employed for both emission trials. A silage volume of 28.8 L corresponds to a resulting packing density of 150.6 kg_{DM} m^{-3}. The silage porosity was 74.9% [41]. A temperature logger (Testo 174T, Testo SE Co. KGaA, Titisee-Neustadt, Germany) was inserted.

Silage was laid on perforated plastic intermediate plates with 63 drill holes (diameter 10 mm), which were placed on little supports within the plastic barrels (Fig. 5.3). A gas space of 3 L volume (referred to as the floor space) was placed beneath these plates to guarantee that the silage was adequately ventilated. When the barrel cover was put on, a gas space (3 L volume; referred to as the head space) was left above the silage. There were two hose connections on the floor-space level and on the cover surface. These were used to ventilate the barrels (Sections 5.2.4, 5.2.5 and 5.2.6). Each barrel had a total silage face of 0.064 m^2 (head space silage face 0.059 m^2, floor-space silage face 0.005 m^2).

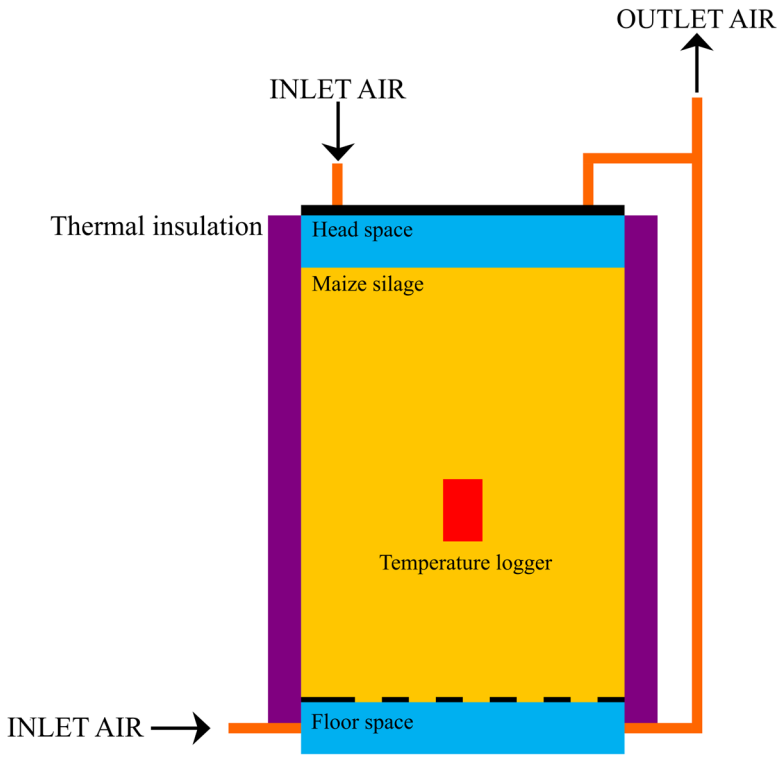

Fig. 5.3 Maize silage barrels used in the emission trials.

The barrels were stored indoors during anaerobic emission measurements and checked regularly (Part A). On ensiling day 30, 6 barrels ($n = 2$ per treatment) were used for the first aerobic emission measurement period (AEMP1, Section 5.2.4). With the remaining 6 barrels, anaerobic emission measurements were carried out until ensiling day 135, the day the second aerobic measurement period (AEMP2) began.

In Experiment A2 (Part A), the silage treatment material was placed in glass jars (maximum volume 1.8 L; J. Weck GmbH & Co. KG, Wehr-Öflingen, Germany) in parallel with the barrel preparation. The jars were filled with 0.602 kg_{FM} up to a volume of 1.65 L. This corresponds to a packing density of 155.1 kg_{DM} m^{-3} and is therefore at a similar level as the barrel silos. The 36 jars ($n = 12$ per treatment) constituted the material for the chemical and microbiological analyses (Section 5.2.3), thus ensuring that the silage barrels remained unaffected throughout the ensiling process.

5.2.3 Analysis of the silage material

Material samples of the fresh material at the harvest day and ensiled material from parallel stored glass jar silos (ensiling days 2, 14, 30 and 135) were used for chemical and microbiological analysis in Experiment A2 (Part A).

Silage quality in Experiment A1 (Part A) was assessed using the maize silage scoring system of the German Agricultural Society [42, 43]. As mentioned in Section 5.2.1, this was used to limit

the examination to a superficial assessment of the material, so that the constant material passed from the fermentation (Part A) to the feed-out emission research (Part B) completely and untouched.

5.2.4 Set-up and procedure of the aerobic emission measurement periods

AEMP1 lasted for 14 days, and AEMP2 lasted for 19 days. AEMP1 used the barrels CON1, CON2, BIO1, BIO2, CHE 2 and CHE3; AEMP2, barrels CON3, CON4, BIO3, BIO4, CHE1 and CHE4.

Two heated and insulated climate chambers ensured constant storage conditions (Section 5.3.1). The first chamber stored the barrels, and the second contained the measurement equipment (Figs. 5.4 and 5.10). The temperatures in the second climate chamber were higher to minimise condensation in the gas sampling bottles and hoses. Three types of temperature loggers, Testo 174T (Testo SE Co. KGaA, Titisee-Neustadt, Germany), Tinytag Plus 2 TGP-4500 and TGP-4505 (Gemini Data Loggers, Ltd., Chichester, United Kingdom), were used to measure ambient air temperatures at intervals of 10 min. A moving average of 36 measured values was subsequently derived to consider short-term temperature fluctuations. The uninsulated barrels were stored within the first climate chamber for 12 h before the AEMP to favour acclimatisation of the silage temperature.

For set-up, each barrel was opened for approximately 2 min. Two trained persons scored the silage (Part A; [42, 43]). Subsequently, the barrel was closed, and the next barrel was checked. Additional temperature sensors (PT100-Sensor, Type FNA30L0500; Logger: Almemo 710; Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany) were positioned between the silage barrel and the insulation for the online temperature assessment. The AEMP began after all the barrels were prepared.

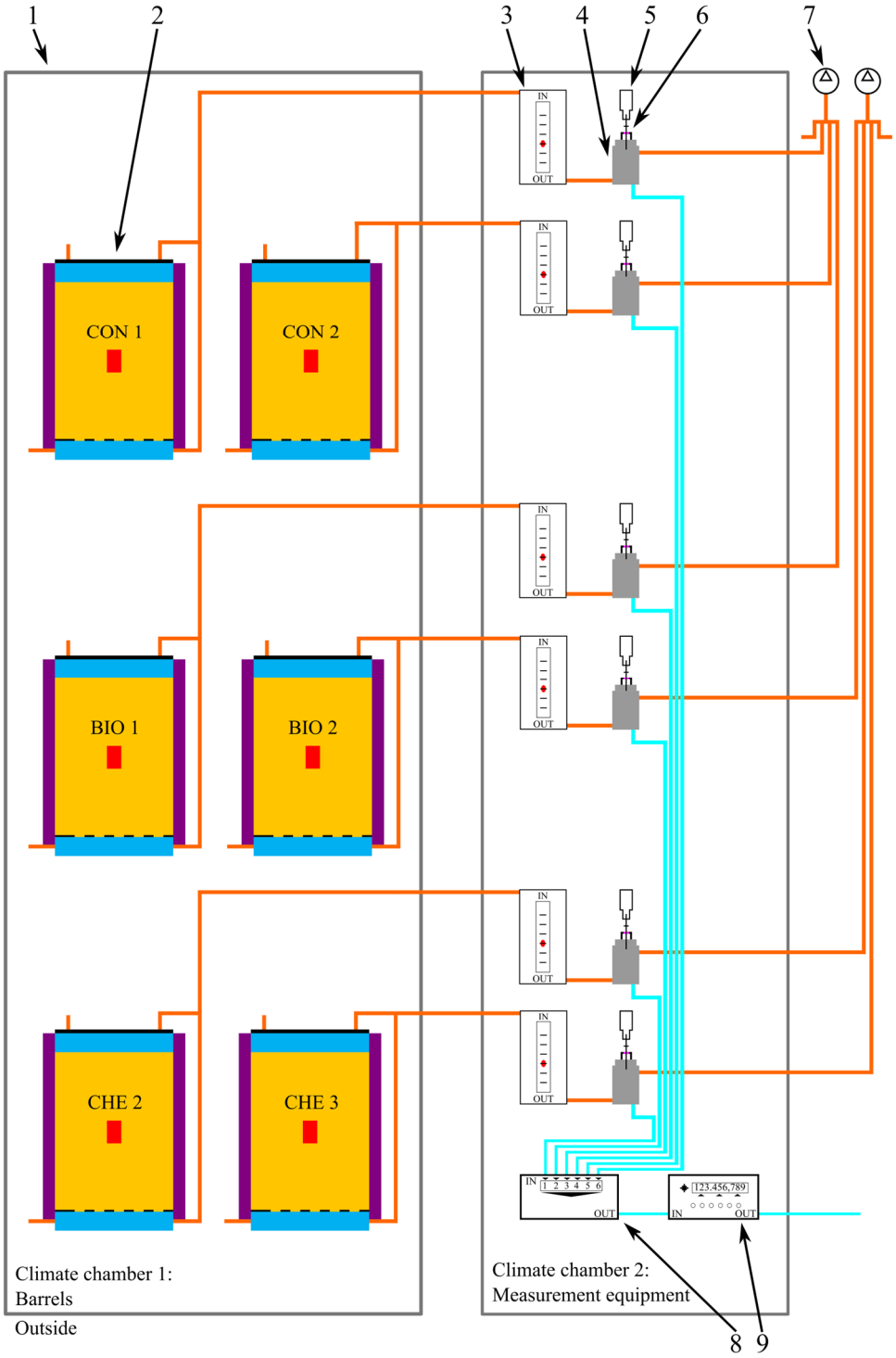

Fig. 5.4 Schematic sketch of the trial set-up for the two aerobic emission measurement periods. Here, the first aerobic emission measurement period.
Legend: 1 = insulated climate chamber, 2 = silage barrel with thermal insulation, 3 = flowmeter, 4 = sampling bottle, 5 = double needle, 6 = sampling vial for gas chromatography, 7 = vacuum pump, 8 = multipoint sampler and doser, 9 = multi-gas analyser (photoacoustic spectroscopy).

Starting with the first barrel, one hose connector at the floor-space level and one in the cover were attached to a Y-hose connector. The others were opened (Figs. 5.3 and 5.4). This should ensure equal air flow rates through the head and floor space, and air flow through the silage

material should be prevented. The barrels were connected to the Y-connectors one by one, considering the measurement interval of the gas analysis (Section 5.2.5). Each Y-connector was connected to a sampling bottle (volume 1 L), one for each silage barrel, where gas sampling was performed (Section 5.2.5) [29, 44]. Each sampling bottle was connected to a flowmeter (measurement range ≤ 10 L min^{-1}; P04/1/102¬ 16CA, Analyt-MTC Messtechnik GmbH, Müllheim, Germany), which ensured an equal air flow rate through each silage barrel during the AEMP. In principle, the air flow was set to 4 L min^{-1} per barrel. However, in AEMP1, the air flow rate was limited to 2 L min^{-1} between 12 and 52 h of storage. In AEMP2, the rate was set to 6 L min^{-1} since 220 h of storage. These modifications depend on the emission mass flow rate and the detection limits of the analysis technology. The flowmeters were connected to two vacuum pumps (3 flow meters per pump; type ME 2C, Vacuubrand GmbH + Co. KG, Wertheim, Germany), which sucked the air through the barrels.

Only polytetrafluoroethylene hoses were used to avoid gas loss through the material. Furthermore, the same hose length for all barrels was used for homogenous air flow.

5.2.5 Measurement of the silage emission mass flow rate

During each AEMP, gas sampling occurred regularly (measurement time points). For both AEMP, the intervals were 15 min between aerobic storage hours 0 and 2, 30 min between hours 2 and 6, 2 h between hours 6 and 12, and 8 h between hours 12 and 92 (Fig. 5.2, Table 5.4). After storage for 92 h, the interval between AEMP1 and AEMP2 differed. The intervals were as follows: for AEMP1, 4 h between hours 92 and 252 and 12 h between hours 264 and 336; for AEMP2, 4 h between hours 92 and 336 and 24 h between hours 360 and 456.

Two methods of gas analysis were applied for each measurement time point. The gas samples used to measure GHG concentrations, including CO_2, CH_4 and N_2O, were filled manually. A double needle was inserted into the sampling bottle's rubber septum, and two vacuumed glass vials (20 mL in volume) were filled. The gas was analysed using a gas chromatograph (GC; electron capture detector and a flame ionisation detector; model 8610C, SRI Instruments, Torrance, CA, USA). This GC analysis indicated detection limits of 50.00 ppm for CO_2, 0.08 ppm for CH_4 and 0.01 ppm for N_2O. This procedure was used in previous studies [2, 29, 44]. The gas concentrations of the inlet air (ambient air) were measured daily using two vials; the mean value was used for the next 24 h. In total, 1964 glass vials were filled.

The ethanol and EA concentrations were continuously analysed using infrared photoacoustic spectroscopy (PAS). A Multi-Gas Analyser INNOVA 1312 and a Multipoint Sampler and Doser INNOVA 1303 (LumaSense Technologies SA, Ballerup, Denmark) were used. The INNOVA 1303 was connected to the six sampling bottles. Gas concentrations of the ambient

air (the inlet air of the barrels) were measured for 48 h before each AEMP, indicating constant values. Therefore, mean values were assumed for calculations (Section 5.2.6). This methodology was used in previous research [21, 29]. The interval between sampling points was approximately 8 min. The continuous concentration values were averaged for the duration between the preceding and the current measurement time points for every time point. Furthermore, the multi-gas analyser also measured CO_2 concentrations. However, these values are only used for determining the dependency between silage temperature and CO_2 emission mass flow (Fig. 5.6) and for methodology comparisons of GC and PAS (Fig. 5.11).

5.2.6 Calculation of gas emissions during aerobic storage

The gas emission mass flow rate for each measurement time point during the AEMP was calculated using Equation 5.1:

Equation 5.1 Calculation of the gas emission mass flow rate.

$$E_{flux} = V_{flux} \times (c_{out} - c_{in}) \quad (5.1)$$

where E_{flux} is the gas emission mass flow rate [$g_{gas}\ h^{-1}$]; V_{flux} is the gas flow rate at this measurement time point [$L\ h^{-1}$]; c_{out} is the gas concentration in the outlet air (= within the sampling bottle of each barrel) at this measuring time point [$g_{gas}\ L^{-1}$]; and c_{in} is the gas concentration in the inlet air (= ambient air) at this measuring time point [$g_{gas}\ L^{-1}$].

The cumulative gas emissions for each silo were calculated using Equation 5.2.

Equation 5.2 Calculation of the cumulative gas emissions.

$$E_{mass,\,j} = \sum_{1}^{j} E_{flux,\,j} \times t_j \quad (5.2)$$

where $E_{mass,\,j}$ is the cumulative gas emission mass until measurement time point j (included) [g_{gas}]; $E_{flux,\,j}$ is the gas emission mass flow rate for measurement time point j [$g_{gas}\ h^{-1}$]; and t_j is the length of measurement interval j [h].

The unmeasured emission quantities during the initial silo opening for silage scoring were calculated using the gas concentrations before the opening (Part A) and the barrel's head space (3 L in volume). These emission quantities were added to the emission quantities during the first measurement time point.

5.2.7 Total emission quantities during anaerobic and aerobic silage storage

The emission quantities of the anaerobic fermentation process (Experiment A1, Part A) and aerobic storage phase (Section 5.2.6) were summed to determine the total emission quantities for each silage treatment using Equation 5.3:

Equation 5.3 Calculation of the total emission quantities.

$$E_{total,\,i,\,j} = M_{gas,\,i} + E_{mass,\,j} \quad (5.3)$$

where $E_{total,\,i,\,j}$ is the total cumulative emission quantity after anaerobic storage length i and aerobic storage length j [g_{gas}]; $M_{gas,\,i}$ is the cumulative emission quantity after anaerobic storage length i [g_{gas}]; and $E_{mass,\,j}$ is the cumulative emission quantity after aerobic storage length j [g_{gas}]. For anaerobic storage length i, the time of maximum gas formation quantity during the anaerobic fermentation process was applied (Part A).

5.2.8 Carbon footprint of maize silage production using silage additives

GHG emissions during silage storage periods were summed with the CF of maize crop production. Data from Feedprint (Version 2020.00, Wageningen UR Livestock Research, Wageningen, The Netherlands) were used [45, 46]. Fresh chopped maize has a CF of 155.95 (kg CO_2eq) t_{DM}^{-1} according to the following assumptions: harvest yield 45.5 t_{FM} ha^{-1}, 33.6% of DM, 170 (kg N) ha^{-1} via manure application, 40 (kg N) ha^{-1} via artificial fertiliser application, 30 (kg P_2O_5) ha^{-1} (= 13.1 (kg P) ha^{-1}) via artificial fertiliser application. The emission factor considers all connected CO_2, CH_4 and N_2O emissions, e.g. for crop production, fertiliser production, and diesel consumption for machinery and transport. This aligns with the magnitude of another report [47].

The sum of emissions during crop production and silage storage (anaerobic fermentation and aerobic storage periods) is the total amount of cumulative CO_2eq emissions per silage mass (excluding emissions during animal or biogas-plant digestion), i.e. for the emission amount for silage production. The emission masses of the various silage treatments were used to assess possible climate- and environment-relevant emission mitigation using SA. The mitigated emissions were compared to the CF of SA production, distribution and application. The following SA CF were applied according to the SA dosage described above (Section 5.2.2) for each kg_{FM}^{-1} silage [48]: 0.0015 (g CO_2) kg_{FM}^{-1} for the biological SA treatment (BIO) and 2.25 (g CO_2) kg_{FM}^{-1} for the chemical SA treatment (CHE, applying a dosage of 2 (kg additive) t_{FM}^{-1}).

The DM losses during the two storage periods were used to calculate the required amount of material harvested [kg_{DM}] to supply a theoretical mass of 1,000 kg_{DM} of feed.

5.2.9 Data processing and statistics

The following conversion ratios were used: 1 ppm CO_2 = 1.83 (mg CO_2) m^{-3}; 1 ppm CH_4 = 0.67 (mg CH_4) m^{-3}; and 1 ppm N_2O = 1.83 (mg N_2O) m^{-3}. The gas quantities formed per silage mass are given concerning the DM mass at the time of ensiling (day 0) [49].

The length of the ASTA lasted until the silage temperature was ≥ 2 K above the ambient air temperature [3, 50]. The GWP was calculated according to the fourth assessment report of the Intergovernmental Panel on Climate Change (IPCC) [51]: $CO_2 = 1$, $CH_4 = 25$ and $N_2O = 298$. The CO_2eq emissions derived in this trial considered only climate-relevant gases, i.e. CH_4 and N_2O; GHG emissions also included CO_2. Negative values indicate DM losses and gas sinks; positive values indicate DM gain and gas sources.

The cumulative gas emissions were compared using mixed ANOVA, with one-way ANOVA for each measurement analysis interval. These intervals differ from the measurement time points (Sections 5.2.5 and 5.2.6). Multiple measurement time points were combined into one measurement analysis interval for a sufficient sample size in the ANOVAs (Table 5.4). For AEMP2, only the measurement time points listed in Table 5.4 were used for statistical analysis. Further data between storage days 10.5 and 19.0 (Section 5.2.5) were considered only for graphical depiction (Fig. 5.15). For each one-way ANOVA, if homogeneity of variance was given, Tukey's HSD test was used for post hoc significance comparison; if not, a Welch ANOVA was followed by a Games-Howell post hoc test. Correlations were analysed using Spearman correlation. In all the analyses, we considered $p < 0.05$ to indicate statistical significance.

Basic data processing and descriptive data analysis were performed using Microsoft Office Excel 2019 (Microsoft Corporation, Redmond, Washington, DC, USA). The statistical analysis was performed using IBM SPSS 26.0 (International Business Machines Corporation Armonk, New York, NY, USA).

5.3 Results

5.3.1 Ambient air and silage temperatures

The silage barrels were stored at 23.7 ± 0.4°C during AEMP1 and 23.7 ± 0.2°C during AEMP2, the measurement equipment at 29.4 ± 1.8°C and 29.1 ± 1.2°C, respectively.

At the time of silo opening prior to AEMP1, the silage temperatures were 22.1 ± 0.1°C for CON barrels, 22.2 ± 0.0°C for BIO barrels and 22.5 ± 0.0°C for CHE barrels; for AEMP2, 23.5 ± 0.2°C, 23.2 ± 0.1°C and 23.3 ± 0.2°C, respectively. Before the heating process, the BIO silage exhibited higher temperatures than CON and CHE silages (Figs. 5.5 and 5.12). In AEMP1, CON barrels were 2 K warmer than the ambient air (2 K Level) after 5.17 ± 0.75 days, BIO after 6.33 ± 0.15 days and CHE after 7.33 ± 0.57 days; thus, ASTA differed significantly ($p < 0.05$). In AEMP2, CON was aerobically stable for 7.75 ± 0.31 days, BIO and CHE for ≥ 19 days.

5.3.2 Composition of the silage

The chemical composition and microbial counts of the silage treatments were analysed in Experiment A2 using the material ensiled within the parallel stored glass jars. The data are shown in Tables 5.5–5.8 and discussed in Part A. At this point, only crucial information will be provided.

The DM concentration of the fresh material (425 ± 6 g kg_{DM}^{-1}, according to [52]) and subsequent silage quality – assessed based on the V-Score method – were high. All the treatments exhibited a rapid pH decrease and a successful ensiling process. BIO showed an increased formation of AA compared to CON and BIO ($p < 0.05$). Before AEMP1, BIO had higher EA concentrations than CON and CHE ($p < 0.05$); before AEMP2, BIO had higher concentrations of ethanol, EA and 1,2-propanediol ($p < 0.05$). The yeast counts decreased during anaerobic storage in all treatments. However, the CON silage ensiled within the glass jars (Experiment A2) exhibited higher yeast counts than the BIO and CHE treatments prior to AEMP1 ($p < 0.05$) and AEMP2 (potentially due to a minor leakage in one of the CON glass jars; Tables 5.5 and 5.6; Part A). The qualitative assessment (without statistical analysis) of the barrel material in Experiment A1 revealed the presence of yeast spots on all treatments' silage faces. For AEMP1, 5%–20% of CON's, 15% of BIO's and 15% of CHE's silage faces were covered with yeast spots; for AEMP2, 15%–30%, < 5%–5%, 5%–15%, respectively (Tables 5.7 and 5.8; Part A).

5.3.3 Emission patterns of greenhouse gases

The period of aerobic storage can be divided into four phases to assess the dynamics of CO_2 emission mass flow and silage temperature (Fig. 5.5; for a modified visualisation, see Fig. 5.13). Phase A lasts for 10 h, and all the treatments show a regressive decrease in the CO_2 emission mass flow immediately after the barrels are opened. Methane emissions occurred within the first hour, and nitrous oxide emissions were detected for the first three hours. No further CH_4 or N_2O emissions were detectable afterwards. After the regressive emission pattern, the silage entered phase B, characterised by constant CO_2 emissions and silage temperatures. At the end of phase B, i.e. after several days of open storage, the aerobic metabolism of microorganisms increases CO_2 emissions and silage temperatures, whereby the former precedes the latter. In the primary period of the heating process (phase C), CON barrels exhibited the highest CO_2 emission mass flow during AEMP1. This was equivalent to 2,409 mg kg_{DM}^{-1} h^{-1}, 0.05 mol kg_{DM}^{-1} h^{-1}, 161,958 mg m^{-2} h^{-1} or 3.68 mol m^{-2} h^{-1}. Since both parameters increase for the same reason, the dependency is shown in Fig. 5.6 (Tables 5.9 and 5.10; for a modified visualisation, see Fig. 5.14). At the end of phase C, the silage temperature and CO_2 emissions reach constant values at high levels for several hours. Subsequently, in phase D, the secondary period of the heating process results in further increases in the temperature and CO_2 mass flow.

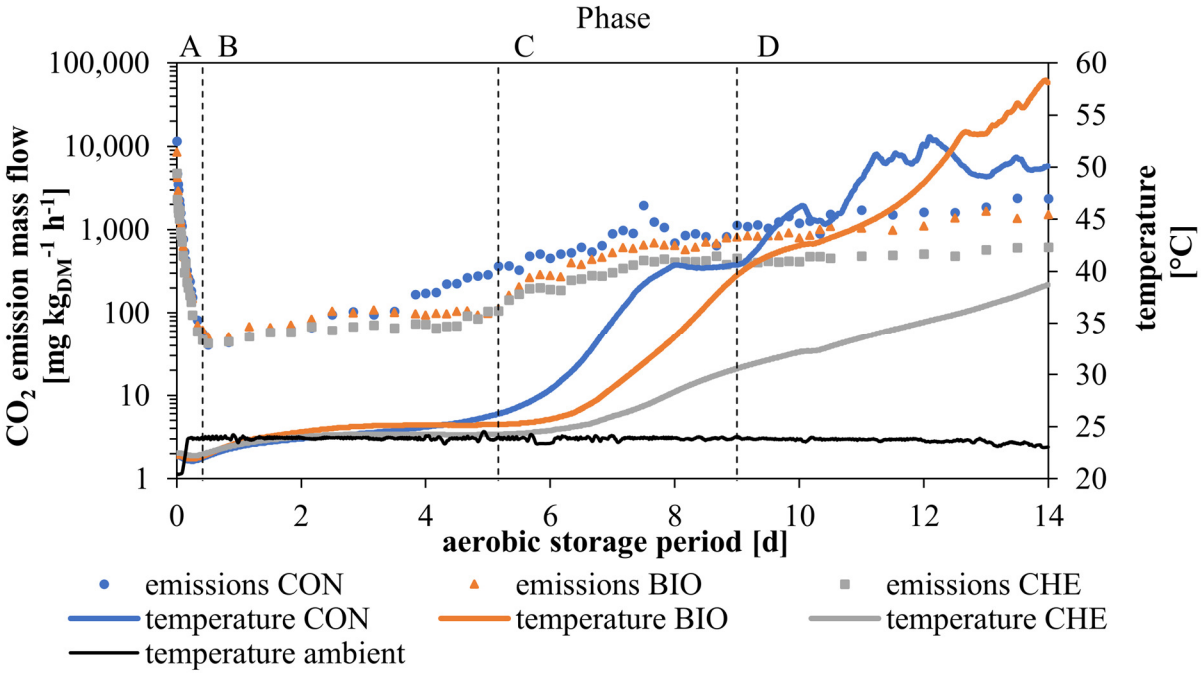

Fig. 5.5 CO_2 emission mass flow, ambient air and silage material temperatures of the silage barrels (mean values) containing each silage treatment during the first aerobic emission measurement period (AEMP1).
The aerobic storage period can be split into four phases, as shown in the example of the CON treatment (Table 5.9). Please note the logarithmic scaling of the left Y-axis (CO_2 emission mass flow) and the non-zero right Y-axis (temperature). The CO_2 emission mass flow is calculated based on the gas concentration analysis via gas chromatography (Sections 5.2.4 and 5.2.5).
Treatments: treatment containing no additive (CON), treatment containing a biological additive (BIO), treatment containing a chemical additive (CHE).

CHE showed higher silage temperatures than CON and BIO within the first 4 h of AEMP1 ($p < 0.05$). BIO had higher temperatures than CON between storage hours 4 and 72 (except for storage hour 56), and had higher temperatures than CHE between storage hour 28 and storage day 14 ($p < 0.05$, except for storage hour 56). The preceding heating process in CON led to significantly higher temperatures than in BIO between storage days 6–8 and 11–12; the opposite occurred on storage day 14. CHE indicates lower cumulative CO_2 emissions throughout AEMP1 than CON and BIO ($p < 0.05$; Section 5.3.5). Furthermore, CON had higher cumulative emissions than BIO between storage hours 1 and 24 ($p < 0.05$); vice versa occurs between storage days 12 to 14. Cumulative CH_4 emissions were lower for CHE than for CON and BIO ($p < 0.05$), and cumulative N_2O emissions were greater for CHE than for BIO. CHE had higher CO_2eq emission quantities than BIO ($p < 0.05$; Table 5.11).

For AEMP2, the silage temperature of CHE was the lowest after hour 72; BIO was warmer than CON between hours 5 and 48. While CON showed a noticeable heating process (ASTA of 7.75 ± 0.31 days; Section 5.3.5), the treatments BIO and CHE had constant temperature levels for the remaining AEMP2. The emission pattern after opening the silos was similar to that of AEMP1

(Figs. 5.12 and 5.13, Table 5.12): emission peaks with subsequent, regressive emission mass flows. For CO_2, CHE had the lowest emission quantities throughout AEMP2 (Section 5.3.5). The cumulative emission quantities were greater for BIO than for CON on the first day. CON had the highest cumulative masses since day 4 ($p < 0.05$). CH_4 emissions occurred within the first 0.5 storage hours, with lower quantities for BIO and CHE than for CON ($p < 0.05$); N_2O emissions occurred within the first 3 storage hours (BIO < CHE < CON, $p < 0.05$). Thus, CHE had the smallest CO_2eq emission quantities (CHE < CON < BIO, $p < 0.05$).

The correlation between CO_2 concentrations measured by GC and those measured by the PAS methodology was high ($r_S = 0.985$, $p < 0.05$), as shown for AEMP1 in Fig. 5.11.

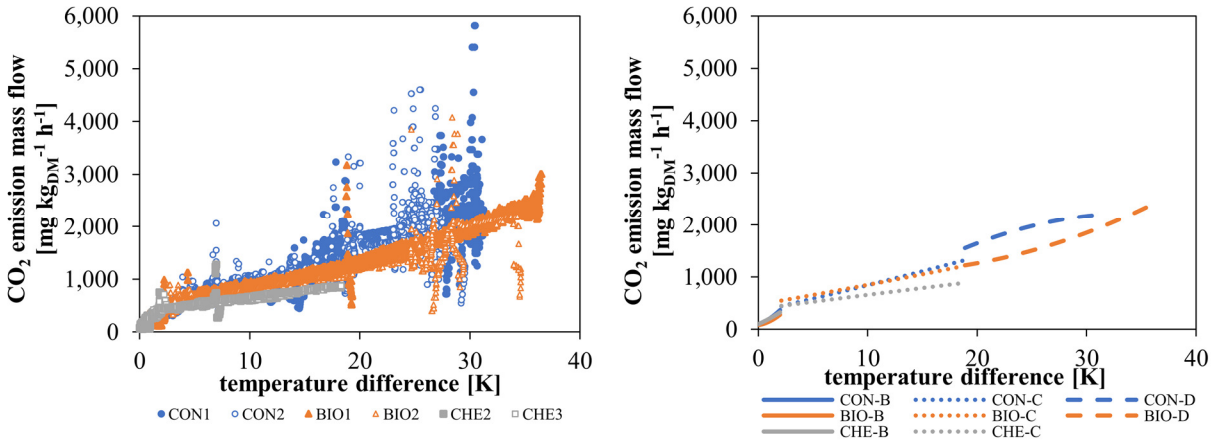

Fig. 5.6 CO_2 emission mass flow during the heating process as a function of the temperature difference between the silage and the ambient air temperature (23.7 ± 0.4°C) during the first aerobic emission measurement period (AEMP1).
Left: CO_2 emission mass flow of individual barrels. Right: regression courses for each treatment within the phases B–D (for instance, CON-B refers to the regression course of CON treatment in phase B; see Figs. 5.5 and 5.14, Tables 5.9 and 5.10). The graphs from phase A were not shown due to the lack of relevance. For simplified visualisation, the graphs of phase B are only shown for temperature differences ≥ 0. For regression equations and r_S, see Table 5.10; all regressions are significant ($p < 0.05$). The CO_2 emission mass flow was calculated via photoacoustic spectrometry via gas concentration analysis.
Treatments: treatment containing no additive (CON), treatment containing a biological additive (BIO), and treatment containing a chemical additive (CHE).

5.3.4 Emission patterns of volatile organic compounds

The ethanol and EA emission mass flow patterns during AEMP1 are similar to the GHG patterns (Fig. 5.7): the initial values show high emission mass flows during the first hours after opening the silos and a regressive course afterwards (phase A). Phase B is also characterised by a regressive pattern. Here, temporal decreases between storage hours 12–52 are due to lower ventilation rates of 2 (L air) min^{-1} instead of 4 (L air) min^{-1}.

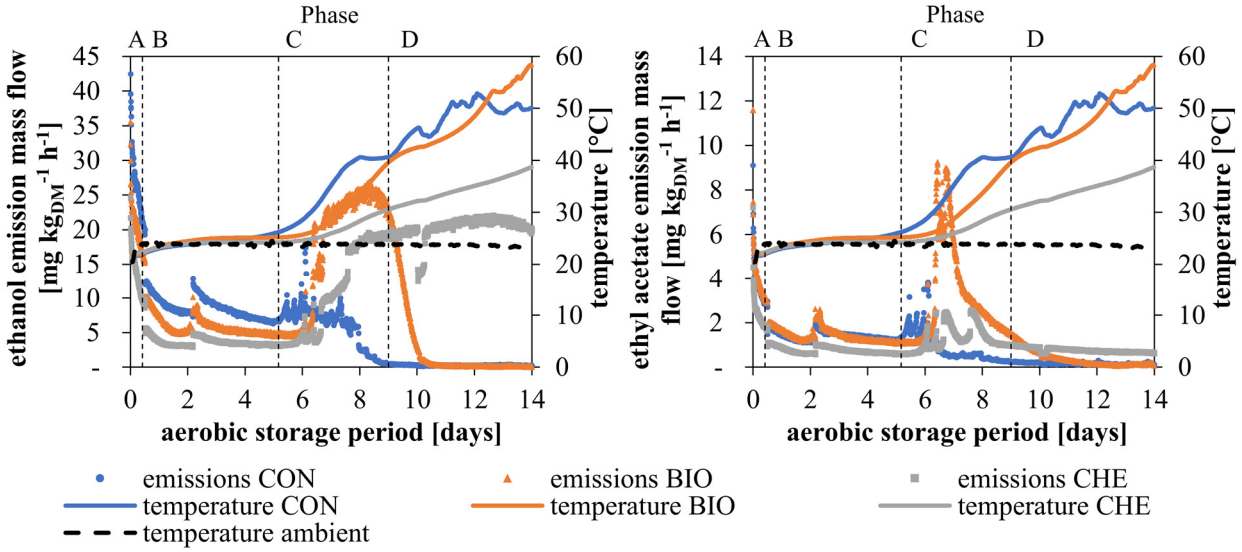

Fig. 5.7 Ethanol and ethyl acetate emissions and ambient air and silage material temperatures of the silage treatments during the first aerobic emission measurement period (AEMP1).
The aerobic storage period can be split into four phases, as shown in the example of the CON treatment (Table 5.9).
Treatments: treatment containing no additive (CON), treatment containing a biological additive (BIO), and treatment containing a chemical additive (CHE).

For ethanol, the mass flow increases during the primary period of the heating process (phase C; Figs. 5.7 and 5.8) and differs noticeably between the treatments. CON silos show a sawtooth wave profile at high levels. The BIO treatment generally emits high quantities of VOC during a long-term emission peak during heating. A less distinct pattern with a sawtooth wave profile is shown for temperature differences < 4 K. The CON2, BIO1, and BIO2 silos exhibit an abrupt decrease in emissions to zero as soon as the temperature difference reaches ≈ 19 K (silage temperatures ≈ 42.7°C). This aligns with the start of phase D. CHE barrels offer a similar pattern, but emission rates remain at high levels. The reduced emissions for CHE2 between 6.7 and 7.3 K (storage day 10) are due to the brief barrel opening used to check for condensation on the covers.

CHE indicates significantly lower cumulative ethanol emissions than CON and BIO between storage hour 1 and day 10 ($p < 0.05$). However, CHE had the highest cumulative ethanol emission quantities on storage day 14. This is based on the constant high emission mass flows since the CHE remains in phase C. CON had higher emissions than BIO between storage hours 1.0–8.0 ($p < 0.05$); the opposite was true between storage days 8.5–14.0.

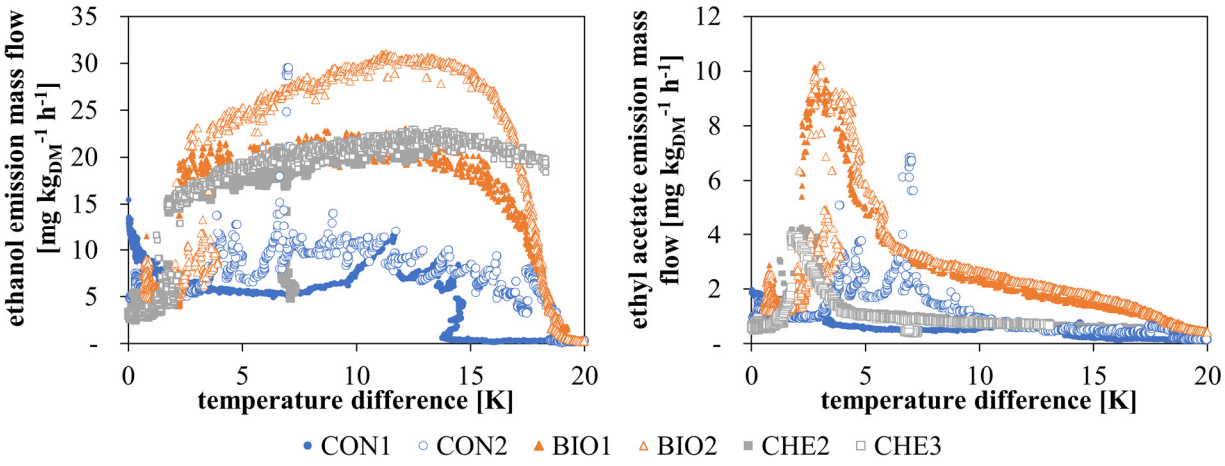

Fig. 5.8 Ethanol and ethyl acetate emissions depending on the temperature difference between the silage and ambient air (23.7 ± 0.4°C) during the first aerobic emission measurement period (AEMP1).
The figures show parts of the data; values before storage hour 48 and above a temperature difference > 20 were not considered due to silage temperature acclimatisation and unnoticeable emission flows. The emission mass flow was calculated using photoacoustic spectrometry based on the gas concentration analysis.
Treatment barrels: treatment containing no additive (CON1, CON2), treatment containing a biological additive (BIO1, BIO2), and treatment containing a chemical additive (CHE2, CHE3).

For EA (Figs. 5.7 and 5.8), while CON1 shows no visible emission peak during phase C, CON2 indicates emission peaks around temperature differences of 4.1–7.0 K. The BIO silos act very homogeneously, with emission peaks between 2–6 K (peaks up to 10.2 mg kg_{DM}^{-1} h^{-1} at 3.0 K) and slowly decaying emissions afterwards. The situation is similar for CHE, but the emission peak occurs earlier (0.8–4.4 K) and less intensely (up to 4.3 mg kg_{DM}^{-1} h^{-1} at 2.2 K). Like for ethanol, EA emissions show a visible drop at temperature differences of 19–20 K (≈ 42°C–43°C silage temperature) at the transition of period C to D, although this change was not as abrupt as that for ethanol.

The emission mass flow courses of AEMP2 are shown in Fig. 5.15 (see also Fig. 5.16 for a modified visualisation of AEMP1 and AEMP2). The regressive emission in phase A aligns with AEMP1. Since no heating is detected, BIO and CHE remain at constant levels. CON indicates a sawtooth pattern during phase C, as observed for AEMP1. While the ethanol emissions of barrel CON4 decreased substantially at a silage temperature of 44.7°C, CON3 exhibited a less distinctive pattern, with noticeable emission mass flows up to 49.4°C. The clear emission threshold between phases C and D (≈ 42°C–43°C) stated for AEMP1 does not apply in this case. For EA, CON indicates no increase in emissions during heating. BIO constantly has the highest emission mass flows.

Table 5.1 Ethanol and ethyl acetate concentrations within the ensiled material and the cumulative emissions during the two aerobic emission measurement periods (AEMP).

Treatment [A]	Anaerobic storage period	Aerobic storage period	Ethanol			Ethyl acetate		
			Material [B,C]	Emissions [B,D]	Ratio [B,E]	Material [B,C]	Emissions [B,D]	Ratio [B,E]
	[d]	[d]	[mg kg_{DM}^{-1}]	[mg kg_{DM}^{-1}]	[%]	[mg kg_{DM}^{-1}]	[mg kg_{DM}^{-1}]	[%]
CON	30	4	**8,253** ± 879	**1,113**^{b} ± 340	**13** ± 2	**175**^{a} ± 12	**168**^{b} ± 20	**93**^{b} ± 5
BIO	30	4	**7,488** ± 1,287	**787**^{b} ± 23	**10** ± 2	**262**^{b} ± 13	**180**^{b} ± 5	**67**^{a} ± 3
CHE	30	4	**5,588** ± 1,361	**485**^{a} ± 44	**9** ± 2	**153**^{a} ± 15	**97**^{a} ± 8	**62**^{a} ± 2
CON	30	14	**8,253** ± 879	**1,841**^{a} ± 346	**22**^{a} ± 1	**175**^{a} ± 12	**295**^{a} ± 15	**170**^{a} ± 6
BIO	30	14	**7,488** ± 1,287	**2,820**^{b} ± 320	**38**^{b} ± 3	**262**^{b} ± 13	**528**^{b} ± 16	**202**^{b} ± 5
CHE	30	14	**5,588** ± 1,361	**3,934**^{c} ± 454	**74**^{b} ± 12	**153**^{a} ± 15	**315**^{a} ± 16	**208**^{b} ± 11
CON	135	4	**7,133**^{a} ± 249	**738**^{b} ± 36	**10**^{a} ± 0	**110**^{a} ± 17	**175**^{b} ± 8	**161**^{a} ± 18
BIO	135	4	**9,985**^{b} ± 219	**1,753**^{c} ± 297	**18**^{b} ± 2	**244**^{b} ± 34	**914**^{c} ± 177	**373**^{b} ± 15
CHE	135	4	**6,313**^{a} ± 660	**489**^{a} ± 23	**8**^{a} ± 1	**84**^{a} ± 16	**139**^{a} ± 14	**167**^{a} ± 18
CON	135	14	**7,133**^{a} ± 249	**2,458**^{b} ± 743	**35**^{ab} ± 8	**110**^{a} ± 17	**360**^{a} ± 36	**340**^{a} ± 22
BIO	135	14	**9,985**^{b} ± 219	**4,469**^{c} ± 810	**46**^{b} ± 7	**244**^{b} ± 34	**2,327**^{b} ± 426	**979**^{b} ± 27
CHE	135	14	**6,313**^{a} ± 660	**1,262**^{a} ± 43	**21**^{a} ± 2	**84**^{a} ± 16	**331**^{a} ± 24	**410**^{a} ± 54

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[B] Significant differences ($p < 0.05$) among the three treatments are indicated by different lowercase letters for each time point.

[C] The results are based on laboratory tests of the silage that was ensiled in the glass jar silos in Experiment A2 (Section 5.2.3, Table 5.6).

[D] The results are based on the tests of AEMP1 and AEMP2 using the silage that was ensiled in the barrel silos.

[E] Ratio of the amount of gas emitted to the amount of substrate stored in the silage material.
Ratio [%] = 100 × $quantity_{emissions}$ [mg kg_{DM}^{-1}] / $concentration_{material}$ [mg kg_{DM}^{-1}]
Significant differences ($p < 0.05$) among the three treatments are indicated by different lowercase letters.

The change in air flow guided through the barrels (from 4 to 6 L min^{-1}) on storage day 9.2 led to a higher emission mass flow. Furthermore, there were short but substantial increases in emission mass flow for BIO and CHE on days 14, 15 and 17, which resulted from modifications of the air flow pattern within the silos (Section 5.4.3).

A comparison of the ethanol concentrations within the material (silage in the glass jars of Experiment A2, Part A) and emission quantities (silage in the barrels) revealed that only parts of the stored alcohol were released (Table 5.1). For AEMP1, the highest ethanol concentrations were observed in the CON group, but this treatment emitted the lowest ethanol concentrations during the 14-day trial; CHE had the opposite pattern. For AEMP2, BIO indicates the highest concentrations in the material, emission quantities, and ratios despite the lack of a heating process. EA shows a deviating emission pattern; here, the escaping gas quantities exceed the original amounts in the material. This trend applies to both AEMP1 and AEMP2, with BIO showing significantly greater emission quantities in the later period (Section 5.4.2).

5.3.5 Total emission quantities during the anaerobic and aerobic storage periods and the carbon footprint of silage additive use

SA positively affected the ASTA and cumulative CO_2 emission quantities during aerobic storage (Fig. 5.9, Tables 5.11 and 5.12). For AEMP1, CON showed the shortest ASTA and CHE the longest ($p < 0.05$). In AEMP2, only CON exhibited a heating process. At all time points, the CO_2 emission quantities in CON were lower in AEMP2 than in AEMP1 ($p < 0.05$). For AEMP1, the cumulative CO_2 emissions of CON were lower than those of BIO and CHE when the 2 K level was reached ($p < 0.05$). Up to the 2 K level, the emissions of CON in AEMP2 did not differ from those of any of the treatments in AEMP1.

For example, a distinction is made between two scenarios in the further article. In the best-case scenario, silage is utilised before the heating process (phase B, aerobic storage period 4 days). In the worst-case scenario, a substantial heating is considered (phase C or D, aerobic storage period 14 days). The majority of total CO_2 emissions are emitted during the aerobic storage period. The shares range from 52% for CHE in AEMP1 (32% in AEMP2) to 57% for BIO (44%) and 58% for CON (45%) in the best-case scenario; for the worst-case scenarios, the shares are 91% (94%), 95% (52%) and 96% (94%).

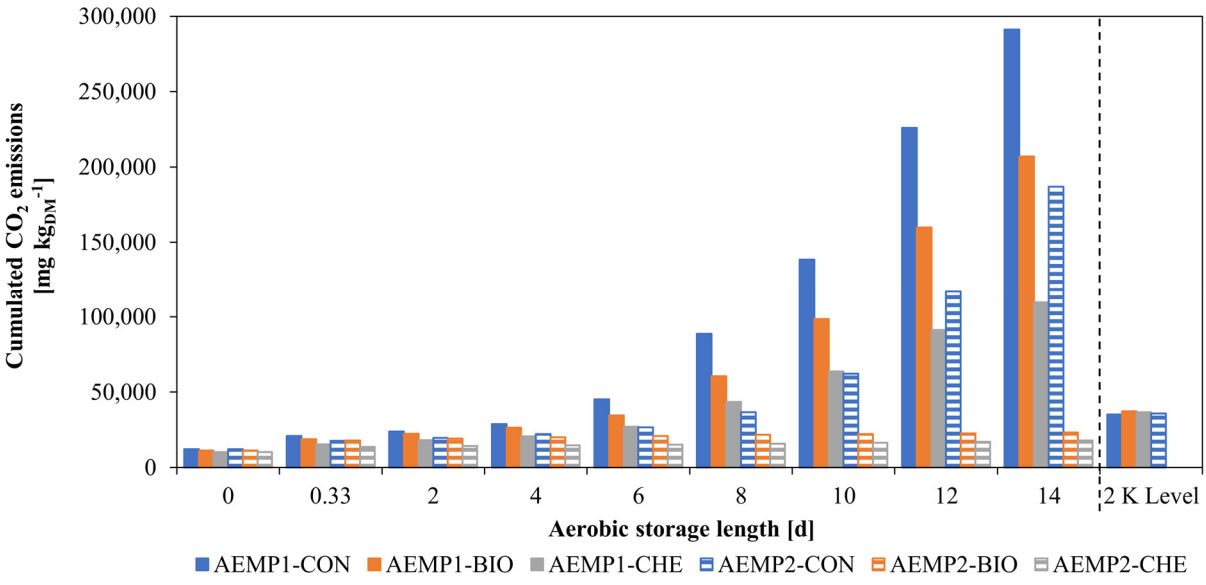

Fig. 5.9 Cumulative CO_2 emissions of the silage treatments during the anaerobic and aerobic storage periods.
AEMP = Aerobic emission measurement period.
An aerobic storage length of 0 refers to the cumulative emissions during the anaerobic storage period (Experiment A1 in Article Part A). The 2 K level indicates the cumulative emissions during the anaerobic and aerobic storage periods until the silage treatment reaches the heating status, i.e. temperatures 2 K above the ambient air temperature. For significant differences between treatments within each AEMP, see Tables 5.11 and 5.12.
Treatments: treatment containing no additive (CON), treatment containing a biological additive (BIO), and treatment containing a chemical additive (CHE).

In contrast, only small parts of climate-relevant CO_2eq emission quantities, i.e. CH_4 and N_2O, are emitted during aerobic storage periods. Overall, CON had the highest shares (26% for AEMP1, 10% for AEMP2), and CHE had the lowest shares (14%, 5%; Tables 5.2 and 5.3). CHE had higher total CO_2eq emissions than CON and BIO during silage storage. However, the share of silage emission quantities ($\leq 0.1\%$) can be neglected compared to the CF of crop production or chemical SA production and application. With this, the total emission quantities of CHE exceed the values of CON in the best-case scenario and at the 2 K level in AEMP1 (Tables 5.2 and 5.3). However, chemical SA use can mitigate total CO_2eq emissions in the worst-case scenarios. For BIO, the smaller emission quantities during silage storage and SA production lead to total emission mitigation in all the scenarios.

Table 5.2 Carbon footprint analysis for the process chain of silage production of trial treatments in the first aerobic emission measurement period (AEMP1). Measured DM losses and CO_2eq emission quantities of the silage treatments during the anaerobic fermentation (30 days, Experiment A1, Article Part A) and AEMP1, the GHG emission quantities during crop and silage additive production, and the total GHG emissions of the process chain, including the reductions due to silage additive use calculated for a target feed mass of 1,000 kg_{DM} maize silage. The CO_2eq emission quantities during silage storage consider CH_4 and N_2O emissions (GWP: CH_4 = 25, N_2O = 298). The values within the brackets consider only the CO_2 emissions; their climate-relevance may be important depending on the contextualization.

Treatment [A]	Aerobic storage period	Anaerobic DM losses	Anaerobic GHG emissions	Aerobic DM losses [B]	Aerobic GHG emissions	Total silage DM losses	Total silage GHG emissions	Harvest material to support 1,000 kg_{DM} feed	Crop production GHG emissions [C]	Additive Production GHG emissions [D]	TOTAL emissions	Ratio silage emissions	Reduction using additives
	[d]	[%]	[g CO_2eq]	[%]	[g CO_2eq]	[%]	[g CO_2eq]	[kg DM]	[g CO_2eq]	[g CO_2eq]	[g CO_2eq]	[%]	[%]
CON	4.00 [E]	-7.2	86	-1.1	30	-8.2	116	1,089	169,845	/	169,961	0.1	/
			(11,984)		*(16,630)*		*(28,614)*				*(198,458)*	*(14.4)*	
BIO	4.00 [E]	-6.7	80	-1.0	17	-7.6	97	1,082	168,744	4	168,844	0.1	-0.7
			(11,307)		*(14,948)*		*(26,255)*				*(195,002)*	*(13.5)*	*(-1.7)*
CHE	4.00 [E]	-5.9	144	-0.7	24	-6.6	168	1,070	166,934	5,667	172,769	0.1	1.7
			(10,037)		*(10,656)*		*(20,693)*				*(193,294)*	*(10.7)*	*(-2.6)*
CON	14.00 [E]	-7.2	86	-18.6	30	-24.4	116	1,323	206,366	/	206,482	0.1	/
			(11,984)		*(279,148)*		*(291,132)*				*(497,498)*	*(58.5)*	
BIO	14.00 [E]	-6.7	80	-13.0	17	-18.8	97	1,232	192,111	4	192,213	0.1	-6.9
			(11,307)		*(195,585)*		*(206,892)*				*(399,008)*	*(51.9)*	*(-19.8)*
CHE	14.00 [E]	-5.9	144	-6.6	24	-12.2	168	1,138	177,527	6,027	183,722	0.1	-11.0
			(10,037)		*(99,526)*		*(109,563)*				*(293,117)*	*(37.4)*	*(-41.1)*
CON	5.17 [F]	-7.2	86	-1.5	30	-8.6	116	1,094	170,578	/	170,694	0.1	/
			(11,984)		*(23,008)*		*(34,992)*				*(205,570)*	*(17.0)*	
BIO	6.33 [F]	-6.7	80	-1.7	17	-8.3	97	1,090	170,004	4	170,105	0.1	-0.3
			(11,307)		*(25,960)*		*(37,266)*				*(207,274)*	*(18.0)*	*(0.8)*
CHE	7.33 [F]	-5.9	144	-1.8	24	-7.6	168	1,082	168,730	5,728	174,626	0.1	2.3
			(10,037)		*(26,505)*		*(36,542)*				*(210,999)*	*(17.3)*	(2.6)

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[B] Calculation based on aerobic GHG emissions [48]: DM losses [g kg_{DM}^{-1}] = CO_2 emission quantities [g kg_{DM}^{-1}] 1.5^{-1}.

[C] Calculation based on Feedprint (2020) [46]: 155.95 (g CO_2eq) (kg_{DM} silage)$^{-1}$, including crop production (e.g. fertiliser, seeds, chemicals, machine use) and transport.

[D] Calculation based on Milimonka et al. (2019) [48]: for BIO, 0.0015 (g CO_2) kg_{FM}^{-1}; for CHE, 2.25 (g CO_2) kg_{FM}^{-1}; DM concentration at harvest, 42.5%.

[E] The storage length of 4.00 days is the assumed best-case scenario, and the storage length of 14.00 days is the worst-case scenario.

[F] Aerobic storage period [d] until the barrels reach the heating status, i.e. a silage temperature 2 K above the ambient air temperature during the feed-out phase.

Table 5.3 Carbon footprint analysis for the process chain of silage production of trial treatments in the second aerobic emission measurement period (AEMP2).
Measured DM losses and CO_2eq emission quantities of the silage treatments during the anaerobic fermentation (135 days, Experiment A1, Article Part A) and AEMP2, the GHG emission quantities during crop and silage additive production, and the total GHG emissions of the process chain, including the reductions due to silage additive use calculated for a target feed mass of 1,000 kg_{DM} maize silage. The CO_2eq emission quantities during silage storage consider CH_4 and N_2O emissions (GWP: CH_4 = 25, N_2O = 298). The values within the brackets consider only the CO_2 emissions; their climate-relevance may be important depending on the contextualisation.

Treatment [A]	Aerobic storage period	Anaerobic DM losses	Anaerobic GHG emissions	Aerobic DM losses [B]	Aerobic GHG emissions	Total silage DM losses	Total silage GHG emissions	Harvest material to support 1,000 kg_{DM} feed	Crop production GHG emissions [C]	Additive Production GHG emissions [D]	TOTAL emissions	Ratio silage emissions	Reduction using additives
	[d]	[%]	[g CO_2eq]	[%]	[g CO_2eq]	[%]	[g CO_2eq]	[kg DM]	[g CO_2eq]	[g CO_2eq]	[g CO_2eq]	[%]	[%]
CON	4.00 [E]	-8.3	86	-0.7	10	-8.9	95	1,089	171,265	/	171,360	0.1	/
			(11,984)		*(9,995)*		*(21,979)*				*(193,244)*	*(11.4)*	
BIO	4.00 [E]	-5.5	65	-0.6	5	-6.1	70	1,065	166,072	4	166,146	0.0	-3.0
			(11,113)		*(8,789)*		*(19,902)*				*(185,978)*	*(10.7)*	*(-3.8)*
CHE	4.00 [E]	-8.3	144	-0.3	7	-8.6	152	1,094	170,668	5,794	176,613	0.1	3.1
			(10,037)		*(4,619)*		*(14,656)*				*(191,117)*	*(7.7)*	*(-1.1)*
CON	14.00 [E]	-8.3	86	-11.7	10	-19.0	95	1,235	192,595	/	192,691	49.3	/
			(11,984)		*(175,018)*		*(187,002)*				*(379,598)*	*(58.5)*	
BIO	14.00 [E]	-5.5	65	-0.8	5	-6.3	70	1,067	166,428	4	166,502	0.0	-13.6
			(11,113)		*(11,981)*		*(23,094)*				*(189,526)*	*(12.2)*	*(-50.1)*
CHE	14.00 [E]	-8.3	144	-0.5	7	-8.8	152	1,097	171,034	5,806	176,992	0.1	-8.1
			(10,037)		*(7,823)*		*(17,860)*				*(194,700)*	*(9.2)*	*(-48.7)*
CON	7.75 [F]	-8.3	86	-1.6	10	-9.8	95	1,108	172,851	/	172,946	0.1	/
			(11,984)		*(23,667)*		*(35,651)*				*(208,502)*	*(17.1)*	
BIO	/	/	/	/	/	/	/	/	/	/	/	/	/
CHE	/	/	/	/	/	/	/	/	/	/	/	/	/

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).
[B] Calculation based on aerobic GHG emissions [48]: DM losses [g kg_{DM}^{-1}] = CO_2 emission quantities [g kg_{DM}^{-1}] 1.5^{-1}.
[C] Calculation based on Feedprint (2020) [46]: 155.95 (g CO_2eq) (kg_{DM} silage)$^{-1}$, including crop production (e.g. fertiliser, seeds, chemicals, machine use) and transport.
[D] Calculation based on Milimonka et al. (2019) [48]: for BIO, 0.0015 (g CO_2) kg_{FM}^{-1}; for CHE, 2.25 (g CO_2) kg_{FM}^{-1}; DM concentration at harvest, 42.5%.
[E] The storage length of 4.00 days is the assumed best-case scenario, and the storage length of 14.00 days is the worst-case scenario.
[F] Aerobic storage period [d] until the barrels reach the heating status, i.e. a silage temperature 2 K above the ambient air temperature during the feed-out phase.

5.4 Discussion

5.4.1 Formation and emission of greenhouse gases

CO_2 emissions are based on aerobic deterioration and microbial respiration. Several reviews concerning the ASTA of silage, including the impact of SA use, have been published [7, 11, 12, 50, 53, 54]. However, previous research has focused on DM and feed quality losses concerning livestock nutrition rather than climate impacts.

The CO_2 emissions in phase A are based on the discharge of the gases. The rate of GHG discharge is affected by gas diffusion based on Fick's law [27, 55]. Nevertheless, it is assumed that additional quantities of CO_2 are emitted after volatilisation from the liquid to the gas phase (Part A; [3]). The asymptote of regressive emission was reached after 8.0–10.0 h in AEMP1 and 4.5–8.0 h in AEMP2. The CO_2 quantities in the barrel's gas space, calculated using the gas space volume and gas concentrations in the head space (Experiment A1, Part A), were emitted after 1.25–3.00 h for AEMP1 or 0.75–2.00 h for AEMP2. These account for 75% ± 6% or 72% ± 12%, respectively, of the total emission quantities during the regressive emission pattern (phase A). Thus, CO_2 volatilisation out of the liquid phase seems to be reasonable. However, gas discharge during silage scoring or emission quantities from silage gas pores and floor space must also be considered. Nevertheless, the emission quantities of phase A were lower in AEMP2 than in AEMP1, aligning with decreasing CO_2 concentrations in the silos (Part A; [56, 57]) and possible CO_2 fixation during anaerobic fermentation (Part A). CO_2 emission quantities during phase A of AEMP1 equalled 50.6%–72.9% of the emission quantities during the ensiling period (Tables 5.2 and 5.3; Experiment A1, Part A); for AEMP2, values were 34.3%–57.6%. Thus, a large portion of the CO_2 produced during anaerobic fermentation can be emitted when silos are opened. However, not all of these gases are discharged in phase A rather than throughout the silo film during anaerobic fermentation [7].

Later, in phase C, microbial respiration, primarily of *Acetobacter* sp. and yeasts, leads to the degradation of lactic acid and carbohydrates to CO_2 and heat [1]. This affects silage temperature and pH [3, 27]. The high ASTA of most trial treatments allows a separation between diffusing and newly formed CO_2 emissions [3, 28]. This pattern was also shown by Shan et al. [3] investigating triticale silage. A low packing density treatment indicated a shorter ASTA due to increased microbial activity with high oxygen availability. This also applies to this trial. Low-density silage increases emission quantities since more material is exposed to oxygen [3]. However, O_2 penetrating the silage face can be respirated rapidly in the upper layers, while deeper layers experience anaerobic conditions [3, 27, 58, 59]. Studies with one silage face are similar to those with commercial silos, but spatial O_2 differences lead to unclear quantities of silage material being

the reason for respiratory CO_2 emissions. In the trial presented here, the authors assumed constant oxygen availability in all silage layers due to two silage faces, high porosity, and high silage core temperatures. With this, silage emissions can be attributed to the total silage mass stored in each silo. Spatial O_2 availability may cause differences between linear [3, 27] and exponential regression equations for CO_2 emission rates during heating. However, the recorded silage temperatures are also influenced by the mass of the silage and the thermal insulation that covers the silos.

BIO and CHE had longer ASTA and lower CO_2 emissions than CON did for both AEMP. Several reviews or meta-analyses [6, 7, 11, 12, 50, 60] have reported the positive impact of microbial inoculants or organic acids. For CHE, potassium sorbate and sodium benzoate effectively inhibit yeast activity [61, 62]. For BIO, AA, which has antifungal properties, is mainly formed by heterofermentative LAB such as *Llb. buchneri* [7, 60, 63]. Longer anaerobic fermentation times led to improved ASTA; BIO had the greatest difference. The anaerobic fermentation length correlated positively with the ASTA for *Llb. buchneri*-treated silage [6].

Yin et al. [64] and Drouin et al. [65] observed changes in the microbiota composition during aerobic storage, but LAB remained the most abundant bacteria for several days [66]. Yeasts and acetic acid bacteria initiate the heating process in phase A [1, 7, 31, 66]. Changes in silage temperature, organic acid degradation and pH increase (phases B and C) promote spoilage microorganisms, including yeasts and moulds [64, 65]. Fungal diversity decreases noticeably in phase B, with accelerated changes in phase C [64, 65]. Changes in phase D seem to be based on mould growth [67]. However, in the present study, no microbial community analysis was performed during or after the aerobic storage phase. The specific periods of heating within each phase could be caused by varying meso- or thermophilic yeasts, moulds or bacteria species with changing temperature (and pH) optima. Further studies should conduct emission and microbial analyses in parallel for more detailed deductions.

All the silage treatments emitted small quantities of CH_4 and N_2O during the AEMP. Maize silage emits less N_2O at the silage face than does lucerne silage [68]. Both substances are formed under anaerobic conditions during fermentation (Part A; [2]); no further quantities are formed during aerobic storage. Furthermore, the first article (Part A) reported the possible solubility of CH_4 and N_2O in the late phase of the anaerobic fermentation process. In the first hour of AEMP1, barrels emitted 37% ± 16% of the CH_4 measured in the gas space of the zero-pressure systems at that time; the percentage of AEMP2 increased to 82% ± 7%; for N_2O, the percentages were 67% ± 8% and 138% ± 34%, respectively. The values indicate higher ratios for AEMP2 based on possible effects of the trial procedure (Section 5.4.4; Part A). Increased N_2O solubility [69, 70]

could lead to prolonged volatilisation. Furthermore, N_2O has a higher gas density than CH_4, which could lead to gas layering and delayed exhaustion. These effects apply mainly to AEMP2, which has higher emission quantities than the present N_2O quantities calculated for the silo gas space.

5.4.2 Formation and emissions of volatile organic compounds

The regressive emission pattern of VOC is in line with that of former trials [21, 27, 29, 34], but the magnitude and speed of decline are smaller than that for GHG emissions. For CON in AEMP1, the regressive pattern extends over phase B and transits directly into increasing mass flow during phase C. This is due to the steady volatilisation of VOC bound in the liquid phase and the gaseous transport of VOC stored in the silos' gas spaces. The former is affected by temperature, air velocity and silage porosity [15, 21, 34]. The impact of temperature changes can be neglected for the first days of aerobic storage due to constant ambient air and silage temperatures. The variations in emission mass flow due to changes in the ventilation rate are shown in Figs. 5.7 and 5.15. The low packing density of silage in this trial enhances volatilisation [21, 33]. Moreover, the amount of ethanol emitted during regressive mass flow patterns seems to be connected to the ethanol levels in the silage's gas and liquid phases. This contradicts the VOC gas emission pattern observed during anaerobic fermentation (Part A).

To date, the pattern of VOC emissions during the heating process of silage has been shown by Shan et al. [27]. In that trial, the ethanol emission rates remained steady during phase C. Nevertheless, the ethanol emission rates increased during phase D when the CO_2 emission rate had already declined. Shan et al. [27] reported that ethanol emission rates correspond to microbial activity in deeper, anaerobic layers. In the trial presented here, however, all silage treatments indicate increasing ethanol emission mass flow rates in phase C. Rising silage temperatures increase the air temperature in the silage face's boundary layer; consequently, the volatilisation rate of liquid ethanol increases. Renewed ethanol formation is not assumed based on aerobic conditions.

CON indicated the earliest increase in the ethanol emission mass flow rate in phase C due to the lowest ASTA. However, when heating was applied for all three treatments in AEMP1 (phase C), BIO and CHE had higher emission rates and quantities than did CON. Therefore, SA may delay ethanol emissions but increase them if heating applies during feed-out phases after short ensiling periods. The pattern of AEMP2 differs due to the low ethanol emission quantities from the CHE material and the constantly high mass flow rates for BIO.

Hafner et al. [34] reported that 10%–80% of the initial ethanol quantities in maize silage is emitted during the first 12 h of aerobic storage (phase A); Montes et al. [21] reported a value of 10%. The trial results presented here indicate that 10% of the initial ethanol quantities were emitted

after approximately 4.0 days. The varying silage masses, 1.2 kg_{FM} [33] and 10.2 kg_{FM} in this trial and silo geometry are assumed to be the most crucial impact factors. However, the silo geometry used here is much more comparable to that of a commercial silo clamp than to that of earlier wind tunnel attempts. Therefore, the former calculation model [34] may overestimate the ratio of ethanol emitted from silos for short aerobic storage periods. Only 22.3%–70.4% of the ethanol quantities were emitted over 14 days of aerobic storage, including silage heating.

At days 14 and 17 of AEMP2, BIO and CHE indicate short but substantial VOC emission mass flow increases. These changes result from modifications of the air flow pattern within the silos ("Section 5.4.4). The low emissions from CON led to the assumption that there was either a) a blockage of air flow due to condensation at the barrel cover or b) no additional ethanol or EA gas within the silage. The barrels' air inlet and outlet hoses were closed so that the air flow was led just through the head space, floor space, or the material's gas pores. The short-term rising emission mass flows of BIO and CHE resulted from the latter, indicating that further VOC gases are stored in the gas pores. There was no increase in ethanol and EA emissions in CON. Therefore, both VOC were already emitted at this point in the trial; this proved that b) was the most likely case. However, the results indicate that the cumulative ethanol gas emission quantities were lower than the ethanol masses within the material for all three treatments. The remaining amounts of the material could lead to subsequent ethanol gas emissions. However, ethanol emission mass flow rates stop between 42 and 49°C (phase D). This pattern differs from previous results [27].

One possible explanation is that parts of the ethanol in the material were emitted via volatilisation, and the microbiological activity metabolised the remainder of the ethanol. For instance, yeasts can metabolise ethanol to EA under aerobic conditions, affecting VOC emission quantities and ratios [38, 39]. Furthermore, other microorganisms, especially bacteria such as *Acetobacter* sp., degrade ethanol for aerobic metabolism [1, 71]. The assumption is that the activity of the various microbiota in the silage material is parallel. Therefore, the authors assumed that the ethanol degradation rate of the microbiota exceeded the volatilisation rate since this specific storage point occurred in phase D. Changes between mesophilic and thermophilic microbiota could be relevant [30]. An increase in microbial diversity and activity in the CON treatment [64, 72] could lead to more ethanol degradation than emission. In BIO and CHE, more ethanol remains in the material until heating begins; therefore, the emission mass flow rates are higher in this phase. Microbial ethanol formation or degradation in specific silo layers seems to affect emission patterns for silages with varying densities [27].

VOC have been discussed as factors influencing the feed intake of ruminants. The odour-intensive ethyl esters, in particular, were discussed to have a negative influence

[36, 37, 73,74,75]. However, preference trials with the addition of EA showed no influence on goats [76, 77], but the volatilisation of VOC should be considered when performing the application. Previous studies focused on the emission pattern during the first hours after opening silos [21, 33, 34]. Only one article reported VOC emissions for a long aerobic storage period [29]. Krommweh et al. [29] measured VOC emissions from grass and lucerne silage at lower ambient air temperatures and without heating. Thus, emission quantities are hardly comparable. Furthermore, no work has yet measured the ratio of initial EA quantities in silage and emissions during aerobic storage. Therefore, this study provides novel insights for silage emissions.

For EA, the emitted quantities exceed the initial quantities within the silage material. This could be explained in two ways: (a) the measured values are incorrect or (b) further masses of EA are formed during aerobic storage and lead to additional emissions. The laboratory analysis procedure is well established and treated as correct since the concentrations match the levels of previous literature [73, 78]. The methodological approach will be discussed Section 5.4.4. Thus, explanation a) seems unlikely. The formation of EA during aerobic silage storage has yet to be examined. As stated above, EA formation by yeasts is primarily relevant under aerobic conditions [38, 39]. The formation of these products is possible via different pathways using sugars, ethanol and AA as substrates. Therefore, EA emissions are generated from preexisting sources and are newly formed. Despite this, AEMP2 showed that the emission quantities were greater than the initial amount in the long-lasting phase B. In order to determine the effect of the newly formed EA on the emission ratios of ethanol (Table 5.1), it is necessary to consider both its emission quantities of EA and the mass fractions of the ethanol components in EA (51.1%). The increase is negligible for all three treatments in AEMP1 and CON and CHE in AEMP2, with an increase of less than 2%. Only BIO in AEMP2 exhibits a markedly elevated ethanol outgassing, with levels reaching 3% to 4% after four days and 9% to 13% after 14 days. In AEMP2, BIO and CHE showed no signs of heating and probably low yeast activity. Other pathways may contribute to the formation of EA, but lactic and acetic acid bacteria are the most likely to be active in phase B. The remaining LAB colonies, especially for treatment BIO, shift into aerobic metabolism, including various enzyme activities [79, 80, 81]. However, to the best of the authors' knowledge, too little research has been conducted concerning silage-specific LAB esterase activity after silo opening. LAB esterase activity results in the formation of EA under anaerobic conditions [82]; a similar pathway may apply to the supply of oxygen. This would align with the stable CO_2 emissions and high relative abundance of LAB in *Llb. buchneri*-treated silage in phase B [64]. Previous studies have reported a relevant abundance of *Acetobacter* sp. in treated and untreated silages, especially in phases A and B [1, 64, 83]. *Acetobacter* sp. forms EA under aerobic conditions due to

alcohol acetyl transferase activity [84, 85]. This enzyme is also generated by yeasts [84, 86]. However, EA emissions decreased during the ongoing heating process, excluding formation pathways by which the microbiota was activated in phase D. Further research is required to investigate the EA and VOC formation in silage during the aerobic feed-out phase. Possible abiotic effects, such as solubility changes due to pH or temperature increase, may also apply.

5.4.3 Carbon footprint and carbon retention efficiency of silage additive use

As stated, CO_2 emissions during silage storage can be considered climate-neutral. Thus, CH_4 and N_2O emissions and potential mitigation by SA can be compared to the CF of SA. Nevertheless, if CO_2 emissions apply, DM losses during silage storage require increased quantities of harvested material, which are connected to climate-relevant GHG emissions [45, 46]. Thus, if SA can reduce DM losses, these losses can directly mitigate climate-neutral CO_2 emissions and indirectly mitigate climate-relevant GHG emissions. However, climate-neutral CO_2 emissions have been reported. These emissions can be compared to those of other stages of the C cycle or with those of other methods of forage conservation. Thus, these emissions help assess the carbon retention efficiency of the C cycle and improve the management of high-value forage in times of resource and climate change awareness.

SA are connected to a specific CF since production, distribution, and application require, among others, fossil fuels. As far as the authors are aware, only Milimonka et al. [48] provided a CF for SA. Personal discussions with industry representatives revealed that these values may deviate: One company stated that the CF of biological additive production is 25 times greater. However, these differences may apply due to varying production steps or the scope of the CF analyses. Biological inoculants require large quantities of nutrients and thermal energy to ensure optimal growth. Organic acids are either directly produced for the SA or by-products of other industrial processes. Determining the CF of the final products used in this trial is challenging due to this factor. To offset its CF, SA must reduce emissions of climate-relevant gases during silage production. However, these calculations should be interpreted with caution and as a first step toward more research, given the limitations of the small database.

The greater CF of chemical SA use is not compensated for by emission reductions in crop production and silage storage in the best-case scenario or at the 2 K level. In these cases, CHE indicates no CO_2eq mitigation potential. However, if substantial DM losses can be inhibited, e.g. in the worst-case scenario of AEMP1, the CHE treatment shows noticeable CO_2eq reductions. Biological SA have a smaller CF than chemical SA. The reduced GHG emissions during silage storage and crop production exceeded the CF of SA use, leading to improvements compared to those of CON for all the scenarios. However, short anaerobic fermentation periods reduce the

effect of heterofermentative LAB [6, 7, 87], indicating reduced mitigation potential in AEMP1. Thus, if harvest and ensiling conditions fulfil the requirements of biological additives, these seem to be the most CO_2-efficient treatments. In these cases, emission reduction in the feed-out phase compensates for the possibly greater DM losses and CO_2 emissions during heterofermentative anaerobic fermentation (Part A; [5]). These findings address open research questions demanding the quantification of losses caused by heterofermentative LAB inoculants [88].

In the best-case scenario, which involved simulating a farm-scale silo with a sufficient feed-out rate, the total emission reduction between all the treatments differed by -3.0% to 3.1%. SA did not improve silage CO_2 efficiency consistently in this scenario, i.e. if the CON treatment indicated no significant losses. A similar pattern applies when the heating status is reached (at the 2 K level). Therefore, SA may not be necessary if high-quality silage management is applied but are essential if heating processes can be avoided, as shown in the worst-case scenario. However, the emission pattern of commercial silage management probably lies between the best- and worst-case scenarios. Borreani et al. [7] state: '*Aerobic deterioration of silages during the feed-out phase is a significant problem for farm profitability and feed quality worldwide* [89, 90].' Thus, the widespread improvement of ASTA, e.g. by using SA, could improve the emission quantities of climate-relevant gases of global silage production. Moreover, a significant deterioration in the worst-case scenario may require discarding the entire feed due to the possibility of mycotoxin formation and critical hygiene quality. However, the effects of SA application on silage nutritional value, animal DM intake and feed efficiency, and milk or methane yield were not considered. These factors may apply and lead to additional effects.

Total VOC emissions during crop production for maize silage can vary widely between 0.3–8.0 (g VOC) kg_{DM}^{-1} [16]. Therefore, the measured ethanol and EA emission amounts during anaerobic and aerobic silage storage periods can add significantly to these VOC emissions, especially considering that only two of the at least 46 VOC identified in silage [16] were measured. The GWP of VOC emissions and subsequent ozone formation were not considered. Previous research has been limited since ozone formation based on VOC can vary widely, e.g. depending on the availability of nitrogen oxides [15, 16, 17]. However, Krueger et al. [14] established factors for such calculations. The total GWP of combined CH_4, N_2O and VOC emissions would increase noticeably, multiplying the measured emission quantities of ethanol and ethanol's share of total VOC emissions (approximately 56%) [35] with the given equal benefit incremental reactivity [14]: climate-relevant emissions would increase up to 114%–376% in the best-case scenario of AEMP1 (115%–706% in AEMP2) and up to 620%–1,132% in the worst-case scenario of AEMP1 (294%–1,793% in AEMP2). For the best-case scenarios, CHE had the lowest increase in both

AEMP; BIO had the highest increase in AEMP2, probably based on the noticeable ethanol formation during the late anaerobic fermentation period. For the worst-case scenario, both SA treatments exhibited greater increases than CON in AEMP1; in AEMP2, CHE exhibited the lowest increase and BIO exhibited the greatest increase. Nevertheless, further quantitative research is necessary, and a variety of climate-related VOC emissions must be considered.

The activity of plant materials and microorganisms leads to considerable conversion of biomass to climate-neutral CO_2 emissions, mainly if long-term heating processes are applied. The CO_2 emission quantities would exceed the GHG emissions of crop production if silage storage was considered a CO_2 source and if the CO_2 binding of photosynthesis was not considered. However, focusing on carbon retention efficiency and adequate silage management, e.g. SA, which are used to reduce heating risk, can decrease emissions. With this, CO_2-based biomass will be used for the original target, e.g. feeding ruminants, and avoidable CO_2 emissions will be reduced. While SA use causes little CO_2 mitigation in the best-case scenario or at the 2 K level, the reduction potential in the worst-case scenario is notable (-19.8% to -50.1%).

Furthermore, the CO_2 emissions of the silage production process chain can be compared to the CO_2 binding capacity of maize crops. In a Swiss study, Maier et al. [91] reported that maize crops fix -19,440 (kg CO_2) ha^{-1} during vegetative growth; this corresponds to -1,005 (kg CO_2) t_{DM}^{-1}, assuming a crop yield of 19.34 t_{DM} ha^{-1}. During anaerobic storage, 0.9%–1.1% of this fixed CO_2 is re-emitted by anaerobic fermentation processes, and 0.4%–1.5% during the aerobic storage period, assuming the best-case scenarios. The latter share may increase to 21.9% for CON, 16.7% for BIO and 9.6% for CHE in the worst-case scenario of AEMP1 (AEMP2: CON 15.1%, BIO 2.2%, CHE 1.6%). Therefore, silage heating can lead to essential losses of bound carbon compounds. In the ongoing C cycle, these losses are unavailable for farm animals or biogas plants. Thus, SA can help improve the carbon retention efficiency. This small-scale calculation could be the starting point for further reliable large-scale trials.

5.4.4 Examination of the methodological procedure

One important factor affecting ASTA is the packing density and subsequent air ingress [7]; this trial's density was significantly less than the recommended values (Part A) provoking heating [92]. This is also the principle used in official German tests to assess ASTA [32]. Therefore, the packing density of the trial set-up is a compromise between established ASTA tests and commercial silo packing densities. However, increased porosity enhances oxygen availability prior anaerobic fermentation (Part A) and oxygen penetration into silage and increases the activity of aerobic yeasts during the feed-out period [30]. The set-up allows the assumptions that (a) CO_2 within the gas pores is rapidly exchanged with oxygen due to diffusion and volume flow [30] and

(b) the whole silage material comes into contact with oxygen during AEMP. With the approach chosen here, the whole silage mass is aerobic, and emission rates per silage mass [kg_{DM}] can be transferred to farm-scale silos. Previous research concentrated on emissions per silage face area [3, 27].

Furthermore, the silage masses within each silo result from the chosen silo volume and packing density. The masses of 10.2 kg_{FM} and 4.4 kg_{DM} per silo are between those of small-scale emission trials [21, 33, 35] and large-scale trials [27, 29]. The former mainly used compacted or loose silage samples of up to 3.41 kg_{FM} [21]. The latter used silage bales. Thus, the silage sample size chosen here can be considered to be at a medium-scale for laboratory silage emission research. The methodological approach of silo ventilation involves a compromise between former wind tunnel tests [21, 33] and barrel trials conducted by the Sino-German working group [2, 3]. Mechanical ventilation using hoses and vacuum pumps enables controlled air flow rates depending on emission rates to reach gas concentrations within the analysers' ranges. Furthermore, a general trial set-up using barrels was necessary to combine anaerobic (Experiment A1, Part A) and aerobic measurement trials.

However, one drawback to the test set-up was condensation on the underside of the cover and in the hoses due to microbial respiration. The maximum air flow rate was limited to 6 L min^{-1} to maintain a farm-relevant wind speed at the silo face. Therefore, water exhaustion was insufficient, and temperature differences between the silage and ambient air led to condensation. To assess condensation, some barrel covers were opened irregularly. GHG and VOC gases could be bound into this water. Thus, condensation could be a mechanism for the sawtooth emission pattern of heated barrels due to the dissolution of gases or the blockade of hoses. The latter could lead to temporary accumulation of gases and subsequent emission flow increases. However, the trial set-up does not explain why condensation should be more frequent in CON than in BIO at equal temperatures in phase C.

Previous studies have concentrated on emissions during anaerobic fermentation [2, 5, 13, 26, 49, 93, 94, 95] or the feed-out period [3, 15, 21, 27, 28, 29, 33, 34, 68]. One of the trials' objectives was constant gas emission measurements without undetected losses at any storage phase. In this regard, this trial represents the most comprehensive silage emission measurements to date. However, in this trial, unmeasured emissions may also apply. The 2-min period of silage quality scoring was the only time without controlled gas collection or air ventilation. However, possible emissions in this phase were calculated using the headspace volume and the gas concentrations within. Nevertheless, CH_4, considering the low gas density, may be emitted in higher quantities than head space quantities may compensate for. Currently, it is unclear whether

gases inside silos exhibit strict layers based on gas density or whether temperature changes and diffusion lead to gas equilibrium.

Former trials showed that silage from laboratory silos, i.e. glass jars or barrels, indicates higher ASTA than farm-scale silos, i.e. silage material taken behind the silo face from commercial silos [58, 96]. Oxygen penetrates the silo up to several metres [7] depending on the packing density, but microbial respiration leads to oxygen depletion near the silo face [27, 30]. However, removing microbial-active material during the feed-out phase regularly increases oxygen penetration. This enhances aerobic microorganisms in deeper layers, leading to low ASTA when these layers become the actual silage face. In contrast, laboratory-scale silos suggest greater gas tightness, and the material is exposed to oxygen for the first time after the silo is opened. Therefore, the aerobic activity and ASTA seemed to differ between the two silo scales. The authors suggest an ASTA of, e.g. 4 days in this laboratory-scale trial equals an ASTA of 2 days (half the aerobic storage length) for farm-scale silo faces. This should be considered by applying the CF reported in this study to commercial silos.

The measurement of the continuous emission of silage in the barrel silos (Experiment A1) required the use of parallel glass jar silos to provide the necessary material for laboratory analysis (Experiment A2). It is recognised that certain material differences between the silos may be attributable to their respective types. Possible influences of the different silo types on the fermentation (especially emissions and microbial community) are discussed in Part A.

The Multipoint Sampler and Doser INNOVA 1303 allows simultaneous gas sampling from six sampling points. Therefore, the total barrel number was limited to twelve for the trial. This may affect the statistical analysis, and gas analysis intervals had to be formed (Part A). It would be advisable to bear this in mind when considering the significance of the results and the conclusions drawn. Thus, the trial reported here should be considered an initial step toward establishing this methodology in silage emission trials but may be repeated with additional silage samples. Furthermore, the gas analysis reported negative values for ethanol and EA concentrations during the first hours of the ensiling process. These errors could be affected by the activated cross and water compensation recommended by former studies [21, 29]. High CO_2 concentrations in humid gas could have led to corrections that were too strong.

The calculation of CF mitigation using SA is based on the presented trial results and literature values (Section 5.2.8). To the authors' knowledge, this is the first time this has been done in this form. However, the results should be supplemented or corrected by future trials. Furthermore, the cited emissions during crop production [45, 46] may vary depending on the farm-specific management. For instance, Jacobs et al. (2017) [97] report CF of maize crop production between

130–171 (kg CO_2eq) t_{DM}^{-1} (mean 148 ± 21 (kg CO_2eq) t_{DM}^{-1}, assuming a crop yield of 19.34 t_{DM} ha^{-1}). Consequently, the potential for savings from using SA can vary, but the methodological approach chosen here enables an initial assessment of the magnitude.

5.4.5 Implications for ensiling management and ensiling research

The factors influencing the course of the fermentation process and the ASTA of silage are numerous and significant. These include the type of material, DM, chopping length and porosity, ambient and silage temperature, epiphytic microbial community, harvesting and silage management, SA and many more. Consequently, this trial cannot represent the heterogeneity of influences. Further trials are essentially required. This applies especially to trials assessing farm-scale emissions of commercial silos. Nevertheless, the meticulous experimental design and the comprehensive measurement data collection provide valuable insights. The data presented here show the great importance of ASTA for silage emission patterns. In the deterioration process, highly digestible components within the forage or silage material are metabolised first [7]. Furthermore, total feed disposal may be necessary if the occurrence of mould and toxins exceeds critical hygienic thresholds. This disrupts the carbon or nitrogen cycle and impairs the sustainability of agricultural processes. However, even small mould infestations can lead to a noticeable decrease in animal DM intake or performance [7, 77, 98, 99]. Therefore, animal farms often prioritise high-quality silage management to ensure their animals' best health and performance. Thus, the emission pattern of the best-case scenario should apply to most of these farms.

In general, the silo face should not be exposed to oxygen for 14 days, as in this trial. However, lab- and farm-scale time differences should be considered. Some trials indicate significant heating after 1–2 days using silage material taken behind the silo face from commercial silos [58, 96]. Furthermore, some farms – especially biogas plants – use enormous silo piles for large-scale operations [21] or prioritise one large silo against several small silos to reduce construction costs. This can result in the emission of significant quantities of CO_2 and VOC, as shown in the worst-case scenario. Therefore, aerobic storage durations for future research should be considered more carefully because they may be too short [21, 33] or too long [29].

The trial results indicate that the small quantities of climate-relevant emissions of CH_4 and N_2O can be neglected compared to the GHG emissions from crop production. According to the literature review, these gases are generated during the first days of the ensiling process under anaerobic conditions. No further quantities are formed during the aerobic storage period because of the high oxygen availability in the uncompacted silage material. Furthermore, unheated maize silo faces showed no CH_4 and small N_2O emissions [68]. However, other trial set-ups with conventional

packing densities involve anaerobic conditions in the deeper layers beyond the silage face [3, 27] if heating applies. This may lead to a pH increase and additional ethanol formation [27, 100]. Under these neutral and anaerobic conditions, additional CH_4 and N_2O may be generated by the various remaining and active microorganisms. This phenomenon should be evaluated in future trials.

Furthermore, minimising DM losses during silage storage is essential but the same applies to lowering feed disposal during harvest or mixing and providing a feed ratio. This finding aligns with former reports emphasising the importance of DM losses for the environmental burden of various ensiling techniques [101]. Thus, improving silage management is crucial, e.g. using specific SA when ASTA is at risk. The CF of SA can be smaller than that of emissions mitigation during silage production, especially for silos with poor management (worst-case scenario). Thus, these SA should be applied more often to improve the agricultural CF. Muck et al. [11] described several objectives of SA use. The authors recommend that reducing emissions of climate- and environment-relevant gases during silage storage should be added to that list.

5.5 Conclusions

The chosen trial set-up was able to measure the GHG, ethanol and ethyl acetate emissions of maize silage during the aerobic storage period. The length and quality of the previous ensiling process and the use of specific silage additives, which prolong the ASTA, affect specific emission patterns. The stable or increasing silage temperatures and CO_2 emission rates during the aerobic storage period help to split the storage period into four phases. The CO_2 emission rate increases noticeably during the heating process (phases C and D). Climate-relevant methane and nitrous oxide emissions occur within the first 3 h of aerobic storage based on the expulsion of quantities formed during anaerobic fermentation. The used SA, i.e. the inoculation with heterofermentative LAB or the addition of sodium benzoate and potassium sorbate, improve ASTA but may increase ethanol and ethyl acetate emission quantities during heating. Ethyl acetate emissions during silage heating were measured for the first time. Furthermore, emission quantities exceed the amounts measured in silage material; the occurrence of aerobic metabolism was derived via literature analysis.

The measurements taken during the anaerobic (see Article Part A) and aerobic storage periods allowed nearly complete collection of emission quantities from the start of the ensiling process until the end of the aerobic feed-out phase. The climate-relevance of CO_2 and VOC was discussed, however, their emissions during the aerobic storage period exceeded that of anaerobic fermentation. The opposite applied for methane and nitrous oxide emissions. However, silage storage has lower CO_2eq emissions than does the carbon footprint of crop and chemical

SA production. However, if the use of specific SA reduces DM losses during silage storage, less harvest material is needed, decreasing crop production-associated GHG emissions. As a result, these silage additives might reduce the carbon footprint of initial silage production and the subsequent production of milk, meat, or biogas-based energy. Nevertheless, additional studies are required to evaluate the carbon footprint of silage production based on different plant materials for a range of management and environmental impacts.

5.6 Supplementary material

Fig. 5.10 Trial set-up for the two aerobic emission measurement periods, here for the first aerobic measurement period (starting at ensiling day 30).
Left: Photo of the 6 barrels, including the thermal insulation and the polytetrafluoroethylene hoses for ventilating the barrels in the first climate chamber. Right: Photo of the sampling bottles, flowmeters, and measuring equipment, e.g. photoacoustic spectrometry and temperature loggers, in the second climate chamber.

Table 5.4 Allocation of actual measurement time points to the measurement analysis intervals for ANOVA analyses of gas emission quantities during the aerobic measurement periods.

Measurement time point [d]	Analysis interval [d]	Measurement time point [d]	Analysis interval [d]	Measurement time point [d]	Analysis interval [d]
0.000	0.021	2.833	3.500	7.833	8.000
0.010	0.021	3.167	3.500	8.000	8.000
0.021	0.021	3.500	3.500	8.167	8.000
0.031	0.042	3.833	4.000	8.333	8.500
0.042	0.042	4.000	4.000	8.500	8.500
0.052	0.042	4.167	4.000	8.667	8.500
0.062	0.083	4.333	4.500	8.833	9.000
0.073	0.083	4.500	4.500	9.000	9.000
0.083	0.083	4.667	4.500	9.167	9.000
0.104	0.083	4.833	5.000	9.333	9.500
0.125	0.167	5.000	5.000	9.500	9.500
0.146	0.167	5.167	5.000	9.667	9.500
0.167	0.167	5.333	5.500	9.833	10.000
0.187	0.208	5.500	5.500	10.000	10.000
0.208	0.208	5.667	5.500	10.167	10.000
0.229	0.208	5.833	6.000	10.333	11.000
0.250	0.333	6.000	6.000	10.500	11.000
0.333	0.333	6.167	6.000	11.000	11.000
0.417	0.333	6.333	6.500	11.500	12.000
0.500	1.000	6.500	6.500	12.000	12.000
0.833	1.000	6.667	6.500	12.500	12.000
1.167	1.000	6.833	7.000	13.000	14.000
1.500	1.000	7.000	7.000	13.500	14.000
1.833	2.000	7.167	7.000	14.000	14.000
2.167	2.000	7.333	7.500	17.000 [A]	19.000
2.500	2.000	7.500	7.500	18.000 [A]	19.000
/	/	7.667	7.500	19.000 [A]	19.000

[A] Only for the second aerobic emission measurement period.

Table 5.5 Chemical composition and energy concentration of fresh maize and ensiled material for the silage treatments in Experiment A2 (unless otherwise stated).

Ensiling time	Treatment[A]	Dry matter[B]	DM losses[C]	pH	Lactic acid	Acetic acid	Propionic acid	Crude ash	Sugar	Starch	Water-soluble carbohydrates	Crude fibre	Crude protein	Utilisable crude protein at the duodenum	Metabolizable energy
[d]		[g kg_{DM}^{-1}]			[g kg_{DM}^{-1}]										[MJ kg_{DM}^{-1}]
0	fresh	**425** ± 6	/	**5.94** ± 0.05	**1.4** ± 0.1	**2.6** ± 0.5	N/D	**30.0** ± 1.7	**56.0** ± 5.3	**353** ± 11	**144** ± 15	**203** ± 4	**65.7** ± 2.1	**128** ± 1	**11.1** ± 0.1
2	CON	**399** ± 2	**-64.2**^{a} ± 0.1	**4.61** ± 0.03	**17.0**^{a} ± 0.5	**7.4** ± 0.6	N/D	**33.0** ± 1.0	**20.7** ± 1.5	**371** ± 14	**34**^{ab} ± 5	**191** ± 5	**70.0** ± 1.0	**131** ± 1	**11.2** ± 0.1
2	BIO	**402** ± 16	**-57.5**^{b} ± 0.1	**4.55** ± 0.04	**19.4**^{b} ± 0.6	**7.4** ± 0.8	N/D	**33.7** ± 1.5	**18.3** ± 4.6	**372** ± 29	**25**^{a} ± 1	**191** ± 12	**71.0** ± 1.0	**132** ± 1	**11.2** ± 0.2
2	CHE	**405** ± 5	**-51.0**^{c} ± 0.1	**4.64** ± 0.05	**16.5**^{a} ± 0.5	**6.9** ± 0.9	N/D	**32.7** ± 2.1	**21.7** ± 2.5	**393** ± 25	**43**^{b} ± 1	**180** ± 11	**70.0** ± 2.6	**133** ± 0	**11.4** ± 0.2
14	CON	**393** ± 5	**-80.3**^{a} ± 0.2	**3.94** ± 0.06	**27.0**^{a} ± 2.2	**8.1** ± 1.0	N/D	**35.0** ± 1.0	**13.0** ± 1.7	**354** ± 24	**5** ± 1	**195** ± 9	**76.0** ± 1.0	**134** ± 2	**11.2** ± 0.2
14	BIO	**395** ± 10	**-76.4**^{b} ± 0.1	**3.98** ± 0.02	**35.9**^{b} ± 1.6	**9.1** ± 1.1	N/D	**36.7** ± 0.6	**10.7** ± 4.0	**337** ± 18	**5** ± 1	**206** ± 9	**78.0** ± 0.0	**133** ± 2	**11.0** ± 0.2
14	CHE	**395** ± 4	**-75.5**^{c} ± 0.2	**3.97** ± 0.01	**39.9**^{b} ± 1.6	**7.7** ± 2.1	N/D	**35.7** ± 0.6	**10.3** ± 2.1	**351** ± 19	**6** ± 1	**197** ± 7	**77.7** ± 0.6	**134** ± 1	**11.1** ± 0.1
30	CON	**397** ± 5	**-71.5**^{a} ± 0.3	**3.92** ± 0.00	**41.1**^{a} ± 3.0	**9.2**^{ab} ± 0.6	N/D	**35.7** ± 1.2	**12.7** ± 2.1	**356** ± 24	**6**^{ab} ± 1	**193** ± 12	**78.7** ± 1.5	**135** ± 2	**11.2** ± 0.2
30	BIO	**400** ± 7	**-66.5**^{b} ± 0.2	**3.95** ± 0.02	**36.3**^{a} ± 1.1	**11.4**^{b} ± 0.4	**0.16** ± 0.02	**35.7** ± 0.6	**13.0** ± 1.7	**358** ± 17	**5**^{a} ± 0	**193** ± 6	**77.7** ± 0.6	**135** ± 1	**11.2** ± 0.1
30	CHE	**402** ± 7	**-59.1**^{c} ± 0.2	**3.91** ± 0.00	**49.2**^{b} ± 1.7	**8.7**^{a} ± 1.5	N/D	**35.7** ± 0.6	**12.3** ± 2.3	**366** ± 8	**6**^{b} ± 0	**189** ± 4	**77.7** ± 0.6	**135** ± 1	**11.2** ± 0.1
135	CON	**393** ± 14	**-83.3**^{a} ± 0.1	**3.91**^{a} ± 0.07	**52.2**^{c} ± 2.2	**11.6**^{a} ± 0.6	**0.09** ± 0.15	**37.7** ± 1.5	**16.0**^{b} ± 3.0	**329** ± 33	**11**^{b} ± 1	**200** ± 14	**81.3** ± 1.2	**135** ± 2	**11.1** ± 0.2
135	BIO	**407** ± 2	**-55.4**^{b} ± 0.4	**4.29**^{b} ± 0.02	**15.9**^{a} ± 1.3	**30.3**^{b} ± 2.4	**1.85** ± 0.23	**36.7** ± 0.6	**4.0**^{a} ± 1.7	**374** ± 5	**3**^{a} ± 0	**187** ± 2	**82.0** ± 1.0	**138** ± 1	**11.3** ± 0.0
135	CHE	**392** ± 8	**-83.4**^{a} ± 0.3	**3.92**^{a} ± 0.06	**45.1**^{b} ± 0.8	**11.7**^{a} ± 0.9	**0.** N/D	**37.0** ± 1.0	**16.0**^{b} ± 3.0	**346** ± 20	**13**^{b} ± 1	**191** ± 10	**81.0** ± 1.0	**136** ± 2	**11.2** ± 0.2

DM = Dry matter (concentration), N/D = Not detectable.

Treatments with different superscript lowercase letters within a parameter and ensiling day differ significantly (Tukey's-HSD or Games-Howell tests, $p < 0.05$)

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[B] Corrected dry matter based on Weißbach and Strubelt [50].

[C] Based on the dry matter weight and losses of the silage barrels (Experiment A1, n = 4 for ensiling days 2–30, n = 2 for ensiling day 135). Losses concerning the silage mass filled into the silos on the harvest day (day 0).

Table 5.6 Chemical composition, the V-Score, and microbial counts of fresh maize and ensiled material for the silage treatments in Experiment A2.

Ensiling time	Treatment [A]	Methanol	Ethanol	Propanol	1,2-Propanediol	2-Butanol	2,3-Butanediol	Ethyl acetate	Ethyl lactate	V-Score	Total bacterial counts	Lactic acid bacteria	Yeasts	Moulds
[d]		[g kg_{DM}^{-1}]									[log_{10} CFU g_{FM}^{-1}]			
0	fresh	**0.04** ± 0.01	**0.00** ± 0.00	N/D	**0.09** ± 0.00	N/D	**0.09** ± 0.00	N/D	N/D	**100** ± 0	**8.20** ± 7.93	**6.64** ± 5.56	**7.48** ± 7.43	**6.63** ± 6.18
2	CON	**0.07** ± 0.01	**5.35** ± 0.80	N/D	**0.10** ± 0.00	N/D	**0.22** ± 0.03	**0.12**[a] ± 0.02	**0.01** ± 0.01	**99** ± 0	**9.43** ± 8.06	**9.29** ± 8.18	**6.56** ± 6.65	**6.04** ± 6.28
2	BIO	**0.07** ± 0.01	**6.62** ± 1.36	N/D	**0.10** ± 0.00	N/D	**0.24** ± 0.02	**0.16**[b] ± 0.02	**0.02** ± 0.00	**99** ± 0	**9.34** ± 8.95	**9.43** ± 8.82	**6.10** ± 5.06	**3.17** ± 3.13
2	CHE	**0.07** ± 0.01	**4.28** ± 0.37	N/D	**0.10** ± 0.00	N/D	**0.12** ± 0.10	**0.08**[a] ± 0.01	**0.01** ± 0.01	**99** ± 0	**9.36** ± 8.86	**9.36** ± 8.80	**6.52** ± 6.64	**3.03** ± 2.55
14	CON	**0.08** ± 0.00	**7.14** ± 1.14	N/D	**0.10**[a] ± 0.00	**0.05**[b] ± 0.00	**0.24** ± 0.04	**0.14** ± 0.03	**0.07** ± 0.00	**99** ± 0	**8.83** ± 7.30	**8.71** ± 8.36	**5.29**[b] ± 4.67	**1.52** ± 1.76
14	BIO	**0.10** ± 0.02	**7.12** ± 0.87	**0.3** ± 0.0	**0.42**[b] ± 0.01	**0.03**[a] ± 0.00	**0.23** ± 0.04	**0.11** ± 0.02	**0.07** ± 0.00	**99** ± 0	**9.15** ± 8.42	**8.76** ± 8.46	**5.09**[b] ± 4.18	**1.52** ± 1.76
14	CHE	**0.08** ± 0.01	**5.18** ± 1.09	N/D	**0.10**[a] ± 0.00	**0.02**[a] ± 0.00	**0.11** ± 0.09	**0.08** ± 0.02	**0.06** ± 0.02	**99** ± 1	**8.88** ± 7.98	**8.63** ± 8.14	**4.33**[a] ± 3.18	N/D
30	CON	**0.12** ± 0.01	**8.25** ± 0.88	**0.1** ± 0.1	**0.10** ± 0.00	**0.11**[b] ± 0.02	**0.24**[b] ± 0.06	**0.17**[a] ± 0.01	**0.12** ± 0.01	**99**[ab] ± 0	**8.00** ± 6.93	**7.97**[a] ± 7.21	**4.49**[b] ± 3.49	N/D
30	BIO	**0.15** ± 0.02	**7.49** ± 1.29	**1.8** ± 0.1	**1.63** ± 0.11	**0.06**[b] ± 0.01	**0.15**[a] ± 0.01	**0.26**[b] ± 0.01	**0.10** ± 0.01	**98**[a] ± 0	**9.29** ± 8.89	**8.62**[b] ± 8.08	**3.16**[a] ± 3.23	N/D
30	CHE	**0.10** ± 0.03	**5.59** ± 1.36	N/D	N/D	**0.03**[a] ± 0.00	**0.15**[a] ± 0.01	**0.15**[a] ± 0.01	**0.10** ± 0.01	**99**[b] ± 1	**8.16** ± 8.23	**7.56**[a] ± 7.26	**2.99**[a] ± 3.12	N/D
135	CON	**0.25** ± 0.03	**7.13**[a] ± 0.25	**1.4**[a] ± 0.3	**0.17**[a] ± 0.06	**0.22**[b] ± 0.06	**0.30**[ac] ± 0.02	**0.11**[a] ± 0.02	**0.17**[b] ± 0.02	**96**[b] ± 0	**7.83**[a] ± 7.95	**7.82**[a] ± 7.96	**6.70** ± 6.94	N/D
135	BIO	**0.20** ± 0.02	**9.98**[b] ± 0.22	**16.0**[b] ± 0.5	**0.34**[b] ± 0.02	**0.58**[c] ± 0.03	**0.13**[ab] ± 0.00	**0.24**[b] ± 0.03	**0.09**[a] ± 0.00	**90**[a] ± 1	**9.05**[b] ± 8.52	**9.05**[b] ± 8.06	N/D	N/D
135	CHE	**0.21** ± 0.02	**6.31**[a] ± 0.66	**1.1**[a] ± 0.1	**0.19**[a] ± 0.04	**0.07**[a] ± 0.02	**0.05**[a] ± 0.09	**0.08**[a] ± 0.02	**0.14**[b] ± 0.01	**97**[b] ± 1	**7.16**[ab] ± 6.36	**7.08**[a] ± 6.00	N/D	N/D

CFU = Colony-forming units, DM = Dry matter, FM = Fresh matter, N/D = Not detectable.

Treatments with different superscript lowercase letters within a parameter and ensiling day differ significantly (Tukey's-HSD or Games-Howell tests, $p < 0.05$).

[A] Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

Table 5.7 Silo face characteristics for the barrel silos and silage treatments in Experiment A1 at silo opening at ensiling day 30 (right before the first aerobic emission measurement period started).

	Parameter		Quality point deduction	Treatment					
				CON		BIO		CHE	
				CON1	CON2	BIO1	BIO2	CHE2	CHE3
Sensory test [A]	Smell	Pleasantly acidic, aromatic, bread-like	0	X	X			X	X
		Slightly alcoholic or slight acetic acid odour	1			X	X		
		Strong alcoholic or roasted smell	3						
		Musty or slight smell of butyric acid	5						
		Disgusting, rotten smell, yeasty	7						
	Structure	Unchanged (like the original material)	0	X	X	X	X	X	X
		Easily attacked, plant parts friable	1						
		Vigorously attacked, greasy, slimy	2						
		Rotten	4						
	Colour	Colour similar to the original material	0	X	X	X	X	X	X
		Colour little changed	1						
		Colour strongly changed	2						
	Moulds	Visible mould infestation: Do not feed silage!	7	N/D	N/D	N/D	N/D	N/D	N/D
	Total	Quality (Mean ± SD)		Very good (**0.00** ± 0.00)		Very good (**1.00** ± 0.00)		Very good (**0.00** ± 0.00)	
Qualitative observation [B]	Moulds	Scale		N/D	N/D	N/D	0.50	N/D	0.50
	Yeasts	Scale		1.00	2.50	2.00	2.00	2.00	2.00

N/D = Not detectable, SD = Standard deviation.

Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] Based on the official procedure for silage scoring from the German Agricultural Society [55, 56].

[B] Qualitative, subjective observation via a scale from 0 (very good) to 5 (very bad): N/D = no mould/yeast spots; 0.50 = occasional mould/yeast spots, approx. < 5% of the surface; 1.00 = occasional mould/yeast spots, approx. 5% of the surface; 2.00 = small yeast nest, approx. 15% of the silo face, 2.50 = small yeast nest, approx. 20% of the silo face; 3.50 = multiplied yeast nests, approx. 30% of the silo face.

Table 5.8 Silo face characteristics for the barrel silos and silage treatments in Experiment A1 at silo opening at ensiling day 135 (right before the second aerobic emission measurement period started).

	Parameter		Quality point deduction	Treatment					
				CON		BIO		CHE	
				CON3	CON4	BIO3	BIO4	CHE1	CHE4
Sensory test [A]	Smell	Pleasantly acidic, aromatic, bread-like	0	X	X				
		Slightly alcoholic or slight acetic acid odour	1			X	X	X	X
		Strong alcoholic or roasted smell	3						
		Musty or slight smell of butyric acid	5						
		Disgusting, rotten smell, yeasty	7						
	Structure	Unchanged (like the original material)	0	X	X	X	X	X	X
		Easily attacked, plant parts friable	1						
		Vigorously attacked, greasy, slimy	2						
		Rotten	4						
	Colour	Colour similar to the original material	0	X	X	X	X	X	X
		Colour little changed	1						
		Colour strongly changed	2						
	Moulds	Visible mould infestation: Do not feed silage!	7	N/D	N/D	N/D	N/D	N/D	N/D
	Total	Quality (Mean ± SD)		Very good (**0.00** ± 0.00)		Very good (**1.00** ± 0.00)		Very good (**1.00** ± 0.00)	
Qualitative observation [B]	Moulds	Scale		N/D	1.00	N/D	N/D	N/D	N/D
	Yeasts	Scale		3.50	2.00	1.00	0.50	1.00	2.00

N/D = Not detectable, SD = Standard deviation.

Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] Based on the official procedure for silage scoring from the German Agricultural Society [55, 56].

[B] Qualitative, subjective observation via a scale from 0 (very good) to 5 (very bad): N/D= no mould/yeast spots; 0.50 = occasional mould/yeast spots, approx. < 5% of the surface; 1.00 = occasional mould/yeast spots, approx. 5% of the surface; 2.00 = small yeast nest, approx. 15% of the silo face, 2.50 = small yeast nest, approx. 20% of the silo face; 3.50 = multiplied yeast nests, approx. 30% of the silo face.

Table 5.9 Classification and range of different phases of aerobically stored silage material, and duration of phases and silage temperatures for the different silage treatments examined. The phases are limited to the first 14 days of AEMP2.

AEMP	Phase	Classification	Range [A]	CON			BIO			CHE		
				Duration	Silage temperature		Duration	Silage temperature		Duration	Silage temperature	
					Mean	Range		Mean	Range		Mean	Range
				[h]	[°C]	[°C]	[h]	[°C]	[°C]	[h]	[°C]	[°C]
1	A	Regressive outgassing	h0–h10	0–10	21.8	21.7–22.1	0–10	22.0	21.9–22.2	0–10	22.3	22.2–22.5
1	B	Aerobic stability	$t_{si} \leq$ 2 K Level	10–124	24.2	22.0–26.2	10–152	24.7	22.2–26.2	10–176	24.3	22.4–26.6
1	C	Primary period of heating process	2 K Level < $t_{si} \approx$ 40–43°C	124–216	34.7	26.2–40.6	152–246	35.2	26.2–42.7	176–336	33.0	26.6–38.7
1	D	Secondary period of heating process	$t_{si} \leq$ 40–43°C	216–336	48.2	40.6–52.9	246–336	49.7	42.7–58.4	/	/	/
2	A	Regressive outgassing	h0–h10	0–8	23.1	22.9–23.5	0–8	23.3	23.2–23.5	0–8	23.2	23.2–23.3
2	B	Aerobic stability	$t_{si} \leq$ 2 K Level	10–179	24.3	22.9–25.7	10–336	24.1	23.5–24.5	10–336	23.5	23.2–23.7
2	C	Primary period of heating process	2 K Level < $t_{si} \approx$ 40–43°C	179–282	32.3	25.7–41.7	/	/	/	/	/	/
2	D	Secondary period of heating process	$t_{si} \leq$ 40–43°C	282–336	47.3	41.7–53.7	/	/	/	/	/	/

AEMP = aerobic emission measurement period, h = hour of storage, t_{si} = silage temperature.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).
[A] The 2 K Level indicates the aerobic storage period until the silage treatment is aerobically unstable, i.e. temperatures 2 K above ambient air temperature.

Table 5.10 Regression and r_S for the correlations between carbon dioxide (CO_2) emission mass flow [(mg CO_2) kg_{DM}^{-1}] and the time after silo opening (phase A) and the temperature difference between the silage material and the ambient air temperature (phases B–D) during the aerobic emission measurement periods.
Please consider Table 5.9 for more information concerning the phases. The phases are limited to the first 14 days of AEMP2. All regressions stated are significant ($p < 0.05$).

AEMP	Phase	Classification	CON		BIO		CHE	
			Regression	r_S	**Regression**	r_S	**Regression**	r_S
1	A [A]	Regressive outgassing	$3{,}044.8\ e^{-10.34\ t}$	0.645	$2{,}549.5\ e^{-9.411\ t}$	0.641	$1{,}922.9\ e^{-9.461\ t}$	0.730
1	B [B]	Aerobic stability	$29.984\ x^2 + 77.129\ x + 92.813$	0.890	$32.319\ x^2 + 35.215\ x + 77.539$	0.655	$10.052\ x^2 + 90.877\ x + 96.205$	0.835
1	C [B]	Primary period of heating process	$0.1859\ x^2 + 48.369\ x + 337.45$	0.835	$0.1714\ x^2 + 35.953\ x + 469.27$	0.920	$-0.0495\ x^2 + 27.366\ x + 386.03$	0.968
1	D [B]	Secondary period of reheating process	$-2.7444\ x^2 + 190.03\ x - 1{,}060$	0.567	$2.0519\ x^2 - 43.921\ x + 1{,}317.6$	0.804	/	/
2	A [A]	Regressive outgassing	$1{,}882.9\ e^{-0.442\ t}$	0.763	$2{,}443.1\ e^{-0.465\ t}$	0.391	$1{,}259.9\ e^{-0.449\ t}$	0.598
2	B [B]	Aerobic stability	$9.6654\ x^2 + 49.042\ x + 73.331$	0.828	$62.667\ x^2 - 40.409\ x + 28.467$	0.385	$148.98\ x^2 + 72.573\ x + 29.563$	-0.207
2	C [B]	Primary period of heating process	$-1.5831\ x^2 + 94.466\ x + 117.61$	0.883	/	/	/	/
2	D [B]	Secondary period of reheating process	$2.6208\ x^2 - 48.419\ x + 1{,}400.2$	0.773	/	/	/	/

AEMP = aerobic emission measurement period.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] The independent variable for phase A is the time after silo opening [h]. Please note: The regression equations may underestimate the CO_2 emission mass flow for the first two hours after opening in AEMP1 and for the first 0.5 hours in AEMP2.

[B] The independent variable for phases B–D is the relative silage temperature, i.e. the difference between silage and ambient air temperature [K].

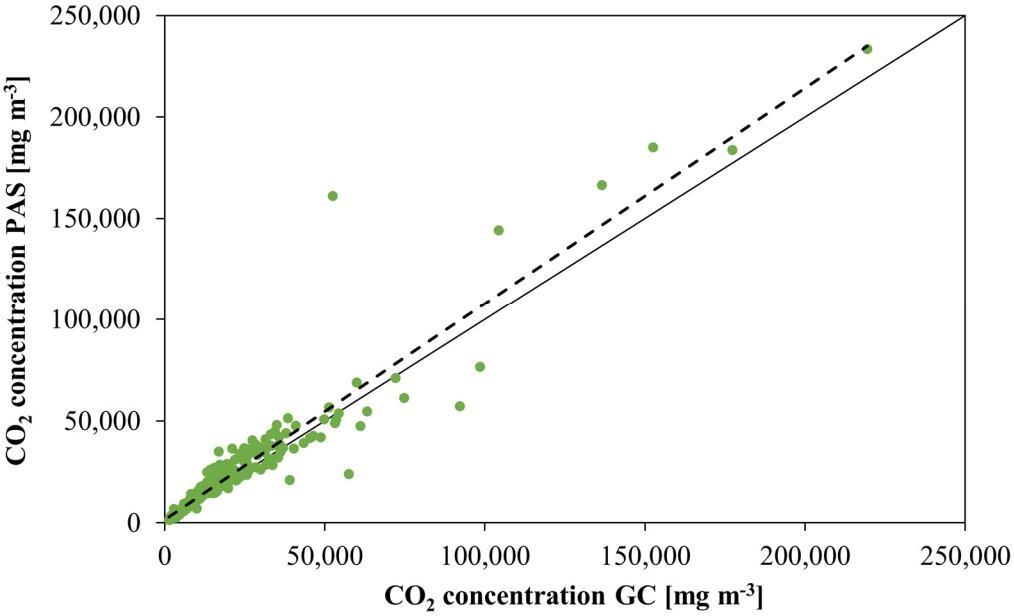

Fig. 5.11 Correlation between CO_2 concentrations measured within the exhaust air during the first aerobic emission measurement period (barrel opening on ensiling day 30) using the GC or PAS measurement methodology and technology (Sections 5.2.4 and 5.2.5).
The regression parameters are: $y = 1.0646 \times x + 1{,}333.2$, $r_S = 0.985$, $p < 0.05$.

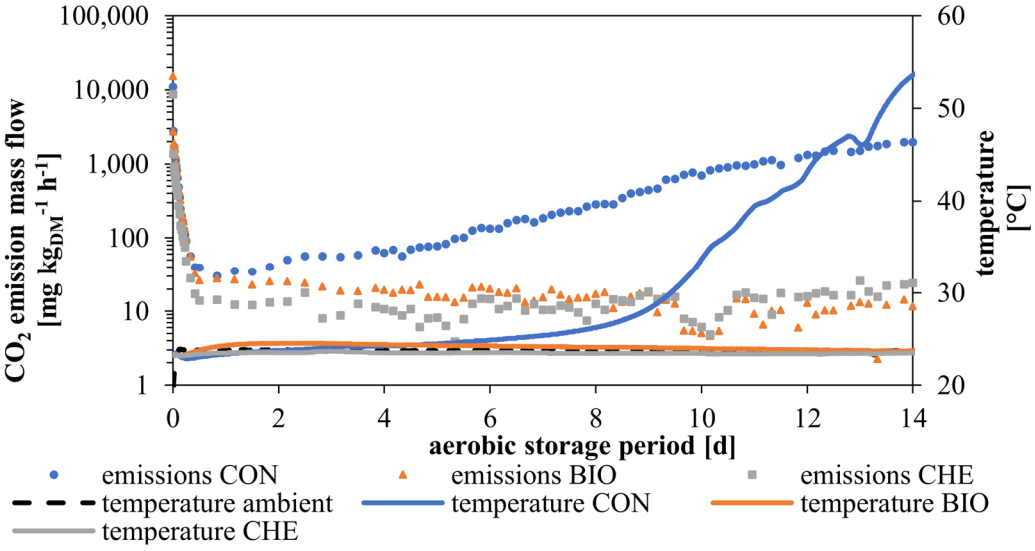

Fig. 5.12 CO_2 emission mass flow, ambient air and silage material temperatures of the silage barrels (mean values) containing each silage treatment during the second aerobic emission measurement period (opening on ensiling 135).
Please note the logarithmic scaling of the left Y-axis (CO_2 emission mass flow) and the non-zero right Y-axis (temperature). The CO_2 emission mass flow is calculated based on the gas concentration analysis via gas chromatography (Sections 5.2.4 and 5.2.5).
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

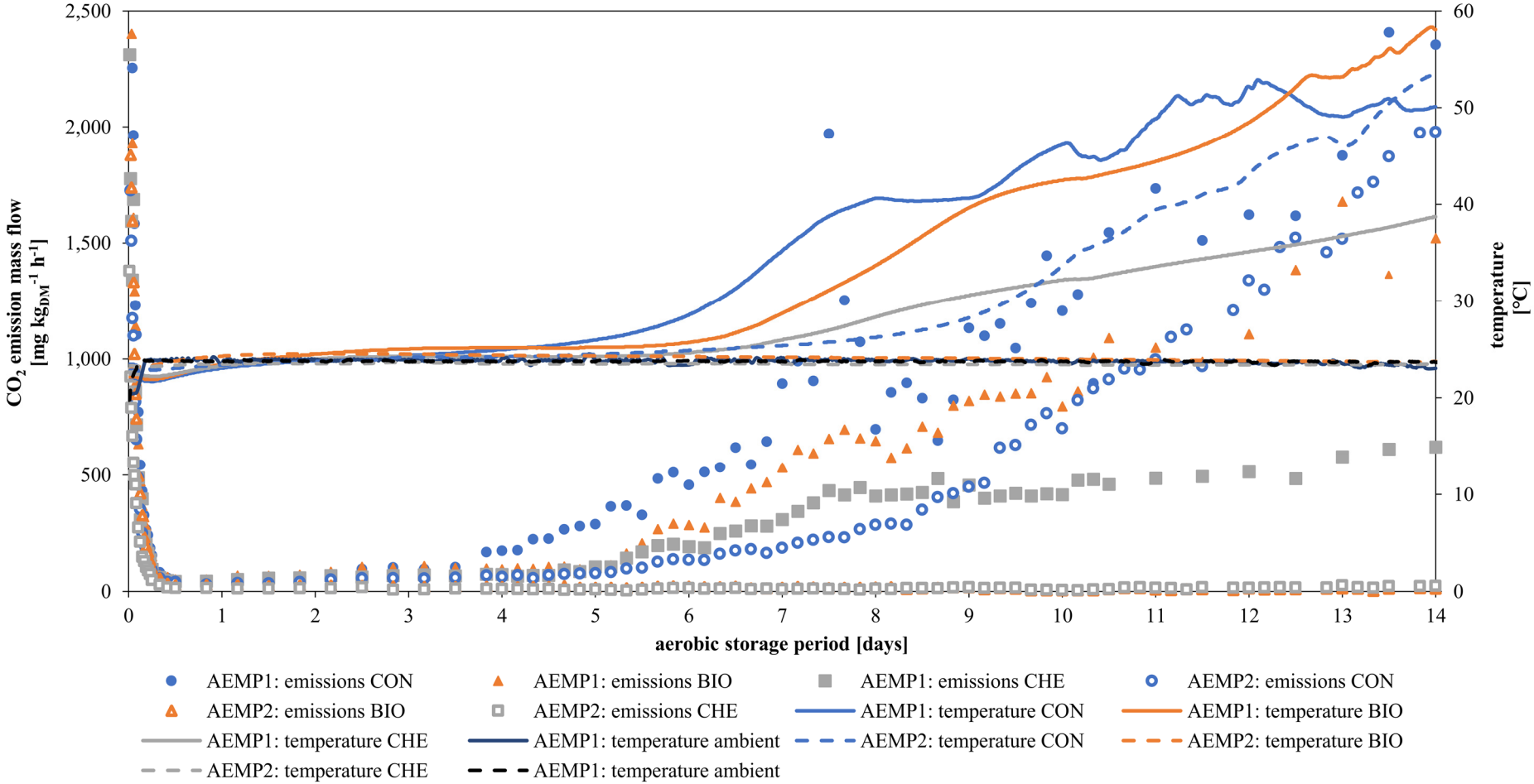

Fig. 5.13 CO_2 emission mass flow, ambient air and silage material temperatures of the silage barrels (mean values) containing each silage treatment during both aerobic emission measurement periods (AEMP1 and AEMP2).
AEMP = aerobic emission measurement period.
For instance, AEMP1: emissions CON refers to the emission mass flow of CON treatment in AEMP1.The CO_2 emission mass flow is calculated based on the gas concentration analysis via gas chromatography (Sections 5.2.4 and 5.2.5).
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

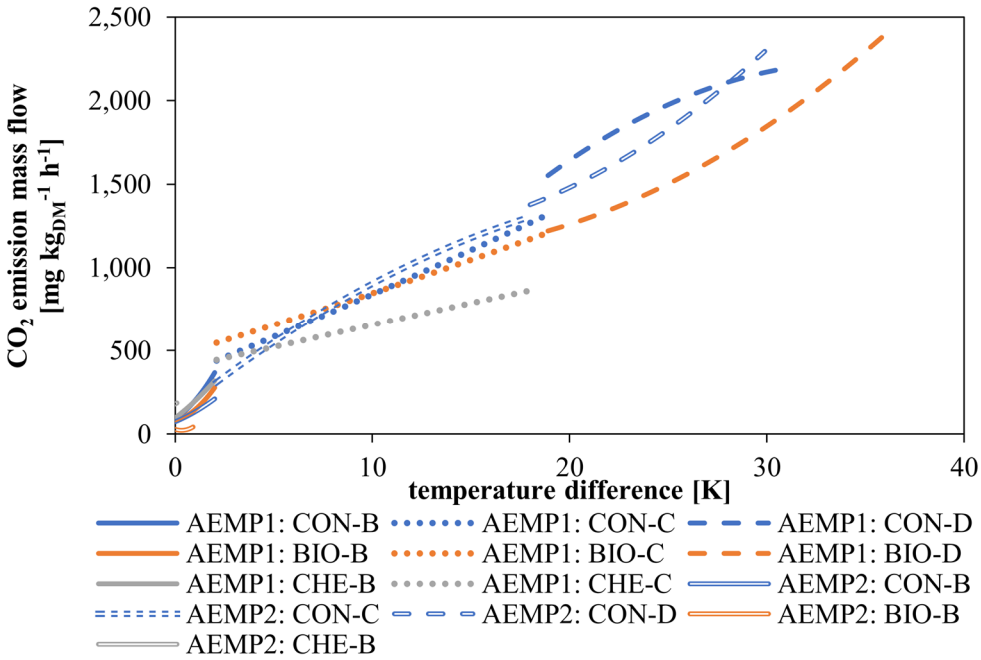

Fig. 5.14 CO_2 emission mass flow during the heating process as a function of the temperature difference between the silage and the ambient air temperature (23.7 ± 0.4°C) during AEMP1 and AEMP2 (23.7 ± 0.2°C, respectively).
AEMP = aerobic emission measurement period. Regression courses for each treatment within the phases B–D (for instance, AEMP1: CON-B refers to the regression course of CON treatment in phase B in AEMP1; see Figs. 5.5, 5.6, 5.12 and 5.13, Tables 5.9 and 5.10). The graphs from phase A were not shown, the same applies to AEMP2: BIO-C, AEMP2: BIO-D, AEMP2: CHE-C and AEMP2: CHE-D. For simplified visualisation, the graphs of phase B are only shown for temperature differences ≥ 0. For regression equations and r_S, see Tables 5.9 and 5.10; all regressions are significant ($p < 0.05$). The CO_2 emission mass flow was calculated via photoacoustic spectrometry via gas concentration analysis.
Treatments: treatment containing no additive (CON), treatment containing a biological additive (BIO), and treatment containing a chemical additive (CHE).

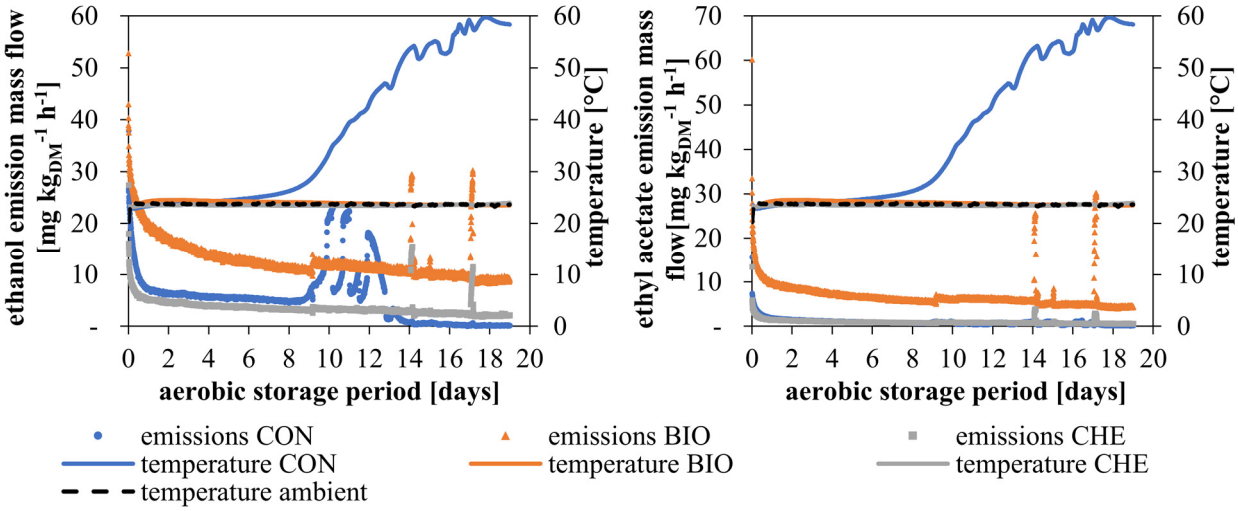

Fig. 5.15 Ethanol and ethyl acetate emissions and ambient air and silage material temperatures of the silage treatments during the second aerobic emission measurement period (opening on ensiling day 135).
Treatments: treatment containing no additive (CON), treatment containing a biological additive (BIO), and treatment containing a chemical additive (CHE).

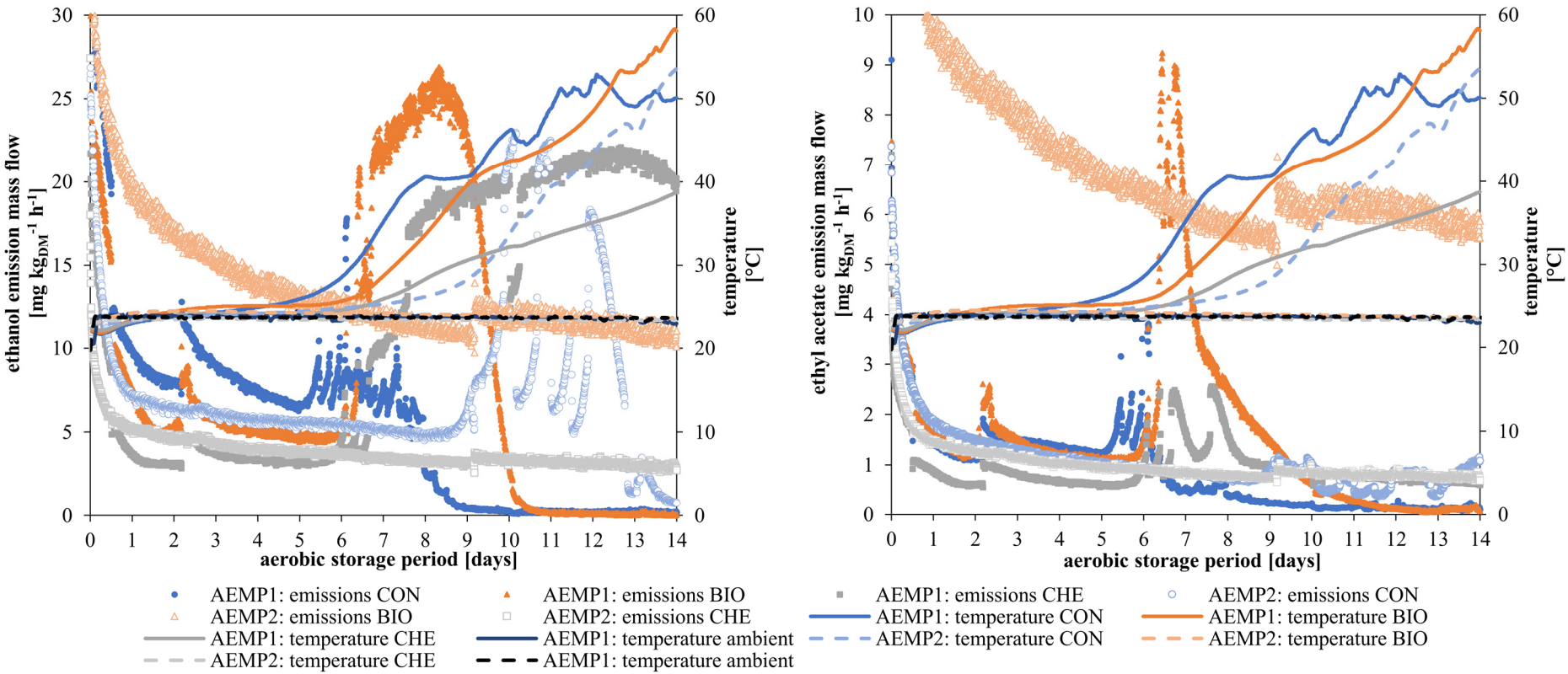

Fig. 5.16 Ethanol and ethyl acetate emission mass flow, ambient air and silage material temperatures of the silage barrels (mean values) containing each silage treatment during both aerobic emission measurement periods (AEMP1 and AEMP2).
AEMP = aerobic emission measurement period.
For instance, AEMP1: emissions CON refers to the emission mass flow of CON treatment in AEMP1.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

Table 5.11 Cumulative CO_2, CO_2eq, ethanol and ethyl acetate emissions during the first aerobic emission measurement period (silos opened at ensiling day 30).

Treatment	Storage period	Maximum absolute silage temperature	Maximum relative silage temperature	Cumulative gas emissions			
				CO_2	CO_2eq [A]	Ethanol	Ethyl acetate
	[d]	[°C]	[K]	[mg kg_{DM}^{-1}]			
CON	0.33	**21.8**^{a} ± 0.1	**-2.1**^{a} ± 0.2	**8,767**^{c} ± 742	**30.5**^{ab} ± 10.5	**271**^{c} ± 45	**37.8**^{b} ± 6.0
BIO	0.33	**22.0**^{ab} ± 0.1	**-1.9**^{ab} ± 0.2	**7,391**^{b} ± 151	**16.7**^{a} ± 1.6	**208**^{b} ± 30	**40.7**^{b} ± 5.5
CHE	0.33	**22.2**^{b} ± 0.2	**-1.6**^{b} ± 0.2	**5,127**^{a} ± 272	**23.9**^{b} ± 0.7	**138**^{a} ± 19	**24.2**^{a} ± 3.5
CON	2.00	**23.9**^{a} ± 0.3	**0.0**^{a} ± 0.3	**11,768**^{b} ± 1,450	**30.5**^{ab} ± 10.5	**769**^{b} ± 231	**110.0**^{b} ± 12.3
BIO	2.00	**24.6**^{b} ± 0.2	**0.7**^{b} ± 0.2	**10,895**^{b} ± 739	**16.7**^{a} ± 1.6	**561**^{b} ± 57	**122.9**^{b} ± 13.9
CHE	2.00	**24.1**^{a} ± 0.1	**0.2**^{a} ± 0.1	**7,891**^{a} ± 538	**23.9**^{b} ± 0.7	**337**^{a} ± 44	**66.1**^{a} ± 8.4
CON	4.00	**25.0**^{ab} ± 0.6	**1.0**^{ab} ± 0.6	**16,630**^{b} ± 3,332	**30.5**^{ab} ± 10.5	**1,113**^{b} ± 340	**168.3**^{b} ± 20.2
BIO	4.00	**25.2**^{b} ± 0.2	**1.2**^{b} ± 0.2	**14,948**^{b} ± 357	**16.7**^{a} ± 1.6	**787**^{b} ± 23	**179.6**^{b} ± 4.6
CHE	4.00	**24.3**^{a} ± 0.1	**0.3**^{a} ± 0.1	**10,656**^{a} ± 699	**23.9**^{b} ± 0.7	**485**^{a} ± 44	**97.0**^{a} ± 7.5
CON	6.00	**28.6**^{c} ± 2.0	**5.1**^{c} ± 1.9	**33,231**^{b} ± 10,188	**30.5**^{ab} ± 10.5	**1,472**^{c} ± 334	**240.7**^{b} ± 11.3
BIO	6.00	**25.7**^{b} ± 0.2	**2.2**^{b} ± 0.1	**23,086**^{b} ± 1,374	**16.7**^{a} ± 1.6	**1,023**^{b} ± 24	**237.3**^{b} ± 12.6
CHE	6.00	**24.6**^{a} ± 0.4	**1.1**^{a} ± 0.4	**16,942**^{a} ± 2,730	**23.9**^{b} ± 0.7	**650**^{a} ± 63	**129.6**^{a} ± 11.2
CON	8.00	**40.4**^{c} ± 2.3	**16.5**^{c} ± 2.3	**76,566**^{b} ± 25,258	**30.5**^{ab} ± 10.5	**1,789**^{b} ± 316	**271.2**^{b} ± 6.5
BIO	8.00	**33.7**^{b} ± 0.9	**9.8**^{b} ± 0.9	**49,073**^{b} ± 2,214	**16.7**^{a} ± 1.6	**1,998**^{b} ± 139	**446.7**^{c} ± 8.7
CHE	8.00	**28.3**^{a} ± 1.6	**4.5**^{a} ± 1.6	**33,325**^{a} ± 5,338	**23.9**^{b} ± 0.7	**1,193**^{a} ± 277	**204.8**^{a} ± 20.6
CON	10.00	**45.6**^{b} ± 6.9	**21.8**^{b} ± 6.9	**126,613**^{b} ± 36,585	**30.5**^{ab} ± 10.5	**1,821**^{a} ± 337	**282.9**^{b} ± 10.2
BIO	10.00	**42.4**^{b} ± 0.3	**18.6**^{b} ± 0.3	**87,075**^{b} ± 4,045	**16.7**^{a} ± 1.6	**2,803**^{b} ± 316	**510.8**^{c} ± 11.9
CHE	10.00	**32.1**^{a} ± 1.6	**8.3**^{a} ± 1.6	**53,723**^{a} ± 6,484	**23.9**^{b} ± 0.7	**2,103**^{a} ± 364	**251.3**^{a} ± 19.3
CON	12.00	**51.3**^{b} ± 2.4	**27.7**^{b} ± 2.4	**213,929**^{c} ± 36,987	**30.5**^{ab} ± 10.5	**1,833**^{a} ± 344	**291.1**^{a} ± 13.3
BIO	12.00	**48.9**^{b} ± 3.1	**25.2**^{b} ± 3.2	**148,575**^{b} ± 16,549	**16.7**^{a} ± 1.6	**2,818**^{b} ± 320	**524.4**^{b} ± 16.1
CHE	12.00	**35.1**^{a} ± 1.9	**11.4**^{a} ± 2.0	**81,189**^{a} ± 8,466	**23.9**^{b} ± 0.7	**3,258**^{b} ± 492	**293.7**^{a} ± 18.9
CON	14.00	**50.0**^{b} ± 3.2	**26.7**^{b} ± 3.2	**279,148**^{c} ± 22,949	**30.5**^{ab} ± 10.5	**1,841**^{a} ± 346	**295.3**^{a} ± 15.3
BIO	14.00	**55.8**^{c} ± 4.5	**32.5**^{c} ± 4.6	**195,585**^{b} ± 22,208	**16.7**^{a} ± 1.6	**2,820**^{b} ± 320	**527.7**^{b} ± 15.7
CHE	14.00	**37.7**^{a} ± 2.7	**14.4**^{a} ± 2.8	**99,526**^{a} ± 9,333	**23.9**^{b} ± 0.7	**3,934**^{c} ± 454	**315.2**^{a} ± 15.9
CON [B]	**5.17**^{a} ± 0.75	**26.2** ± 0.4	**2.5** ± 0.6	**23,008**^{a} ± 1,428	**30.5**^{ab} ± 10.5	**1,310**^{ab} ± 484	**204.0**^{a} ± 45.9
BIO [B]	**6.33**^{b} ± 0.15	**26.3** ± 0.4	**2.4** ± 0.4	**25,960**^{b} ± 1,761	**16.7**^{a} ± 1.6	**1,107**^{b} ± 55	**270.4**^{b} ± 25.3
CHE [B]	**7.33**^{c} ± 0.57	**26.5** ± 0.5	**2.6** ± 0.4	**26,505**^{b} ± 1,468	**23.9**^{b} ± 0.7	**896**^{a} ± 62	**181.9**^{a} ± 9.1

Significant differences ($p < 0.05$) among the three treatments are indicated by different lowercase letters.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] CO_2eq emissions consider only the climate-relevant CH_4 and N_2O emissions.
Cumulative methane emission quantities (emissions within the first hour after opening):
CON $9.26^{b} \pm 3.50$ µg kg_{DM}^{-1}, BIO $4.52^{ab} \pm 0.49$ µg kg_{DM}^{-1}, CHE $1.70^{a} \pm 2.69$ µg kg_{DM}^{-1}.
Cumulative nitrous oxide emission quantities (emissions within the first three hours after opening):
CON $101.5^{ab} \pm 34.9$ µg kg_{DM}^{-1}, BIO $55.5^{a} \pm 5.1$ µg kg_{DM}^{-1}, CHE $80.0^{b} \pm 5.2$ µg kg_{DM}^{-1}.
Global warming potentials: $CH_4 = 25$, $N_2O = 298$.

[B] Time point when silage temperature was 2 K above ambient air temperature (≅ length of aerobic stability [d]). The lengths for the specific barrels were: CON1 5.59 days, CON2 4.26 days, BIO1 6.15 days, BIO2 6.08 days, CHE2 7.68 days and CHE3 6.63 days. For the statistical analysis of temperatures and emissions, the values of the following three measurement time points were used as the data basis.

Table 5.12 Cumulative CO_2, CO_2eq, ethanol and ethyl acetate emissions during the second aerobic emission measurement period (silos opened at ensiling day 135).

Treatment	Storage period	Maximum absolute silage temperature	Maximum relative silage temperature	Cumulative gas emissions			
				CO_2	CO_2eq [A]	Ethanol	Ethyl acetate
	[d]	[°C]	[K]	[mg kg_{DM}^{-1}]			
CON	0.33	**22.9**^{a} ± 0.1	**-0.9**^{a} ± 0.1	**5,588**^{b} ± 79	**9.6**^{c} ± 0.3	**143**^{b} ± 26	**35.7**^{b} ± 5.7
BIO	0.33	**23.5**^{b} ± 0.2	**-0.4**^{b} ± 0.2	**6,676**^{c} ± 278	**4.8**^{a} ± 0.7	**231**^{c} ± 57	**137.1**^{c} ± 30.7
CHE	0.33	**23.3**^{b} ± 0.2	**-0.6**^{b} ± 0.2	**3,490**^{a} ± 60	**7.2**^{b} ± 0.2	**72**^{a} ± 17	**24.6**^{a} ± 4.5
CON	2.00	**23.8**^{a} ± 0.3	**0.0**^{a} ± 0.3	**7,606**^{b} ± 398	**9.6**^{c} ± 0.3	**471**^{b} ± 56	**117.8**^{b} ± 10.6
BIO	2.00	**24.5**^{b} ± 0.1	**0.7**^{b} ± 0.1	**7,973**^{b} ± 459	**4.8**^{a} ± 0.7	**1,082**^{c} ± 212	**564.4**^{c} ± 119.0
CHE	2.00	**23.5**^{a} ± 0.0	**-0.2**^{a} ± 0.1	**4,173**^{a} ± 208	**7.2**^{b} ± 0.2	**299**^{a} ± 36	**88.9**^{a} ± 12.5
CON	4.00	**24.2**^{b} ± 0.4	**0.5**^{b} ± 0.4	**9,995**^{c} ± 235	**9.6**^{c} ± 0.3	**738**^{b} ± 36	**174.6**^{b} ± 8.4
BIO	4.00	**24.4**^{b} ± 0.1	**0.7**^{b} ± 0.1	**8,789**^{b} ± 543	**4.8**^{a} ± 0.7	**1,753**^{c} ± 297	**913.8**^{c} ± 177.0
CHE	4.00	**23.5**^{a} ± 0.0	**-0.2**^{a} ± 0.0	**4,619**^{a} ± 275	**7.2**^{b} ± 0.2	**489**^{a} ± 23	**139.2**^{a} ± 13.6
CON	6.00	**24.9**^{c} ± 0.3	**1.1**^{c} ± 0.3	**14,574**^{c} ± 1,250	**9.6**^{c} ± 0.3	**1,006**^{b} ± 30	**224.2**^{b} ± 12.7
BIO	6.00	**24.2**^{b} ± 0.1	**0.4**^{b} ± 0.1	**9,655**^{b} ± 654	**4.8**^{a} ± 0.7	**2,384**^{c} ± 411	**1,241.6**^{c} ± 243.0
CHE	6.00	**23.5**^{a} ± 0.0	**-0.3**^{a} ± 0.0	**5,069**^{a} ± 447	**7.2**^{b} ± 0.2	**672**^{a} ± 26	**185.6**^{a} ± 16.8
CON	8.00	**26.2**^{c} ± 0.2	**2.5**^{c} ± 0.2	**24,668**^{c} ± 5,588	**9.6**^{c} ± 0.3	**1,253**^{b} ± 20	**264.2**^{b} ± 15.4
BIO	8.00	**24.1**^{b} ± 0.0	**0.4**^{b} ± 0.1	**10,443**^{b} ± 711	**4.8**^{a} ± 0.7	**2,953**^{c} ± 512	**1,533.9**^{c} ± 297.6
CHE	8.00	**23.4**^{a} ± 0.0	**-0.3**^{a} ± 0.1	**5,609**^{a} ± 687	**7.2**^{b} ± 0.2	**838**^{a} ± 27	**226.0**^{a} ± 19.0
CON	10.00	**33.6**^{c} ± 2.1	**9.9**^{c} ± 2.0	**50,249**^{c} ± 15,450	**9.6**^{c} ± 0.3	**1,628**^{b} ± 186	**305.1**^{a} ± 30.3
BIO	10.00	**24.0**^{b} ± 0.0	**0.3**^{b} ± 0.1	**11,007**^{b} ± 834	**4.8**^{a} ± 0.7	**3,504**^{c} ± 620	**1,815.9**^{b} ± 351.9
CHE	10.00	**23.4**^{a} ± 0.1	**-0.3**^{a} ± 0.1	**6,217**^{a} ± 876	**7.2**^{b} ± 0.2	**997**^{a} ± 29	**264.6**^{a} ± 20.7
CON	12.00	**43.4**^{c} ± 2.6	**19.6**^{c} ± 2.6	**104,843**^{c} ± 30,166	**9.6**^{c} ± 0.3	**2,211**^{b} ± 611	**334.0**^{a} ± 35.1
BIO	12.00	**23.8**^{b} ± 0.1	**0.0**^{b} ± 0.1	**11,496**^{b} ± 966	**4.8**^{a} ± 0.7	**4,067**^{c} ± 736	**2,112.1**^{b} ± 400.1
CHE	12.00	**23.4**^{a} ± 0.1	**-0.4**^{a} ± 0.1	**6,943**^{a} ± 1,251	**7.2**^{b} ± 0.2	**1,152**^{a} ± 44	**303.2**^{a} ± 23.2
CON	14.00	**50.0**^{c} ± 4.2	**26.3**^{c} ± 4.2	**175,018**^{c} ± 57,279	**9.6**^{c} ± 0.3	**2,458**^{b} ± 743	**360.1**^{a} ± 35.9
BIO	14.00	**23.7**^{b} ± 0.1	**0.0**^{b} ± 0.1	**11,981**^{b} ± 1,077	**4.8**^{a} ± 0.7	**4,469**^{c} ± 810	**2,326.8**^{b} ± 425.6
CHE	14.00	**23.4**^{a} ± 0.2	**-0.3**^{a} ± 0.2	**7,823**^{a} ± 1,739	**7.2**^{b} ± 0.2	**1,262**^{a} ± 43	**331.0**^{a} ± 23.7
CON [B]	**7.75** ± 0.31	**26.0** ± 0.2	**2.3** ± 0.2	**23,667** ± 6,662	**9.6** ± 0.3	**1,225** ± 26	**260.0** ± 20.0
BIO [B]	/	/	/	/	/	/	/
CHE [B]	/	/	/	/	/	/	/

Significant differences ($p < 0.05$) among the three treatments are indicated by different lowercase letters.
Treatments: treatment containing no additive (CON), treatment containing biological additive (BIO), and treatment containing chemical additive (CHE).

[A] CO_2eq emissions consider only the climate-relevant CH_4 and N_2O emissions.
Cumulative methane emission quantities (emissions within the first 0.5 hours after opening): CON 9.36^{b} ± 1.05 µg kg_{DM}^{-1}, BIO 5.80^{b} ± 0.88 µg kg_{DM}^{-1}, CHE 6.50^{a} ± 0.71 µg kg_{DM}^{-1}.
Cumulative nitrous oxide emission quantities (emissions within the first hour after opening): CON 31.4^{c} ± 1.0 µg kg_{DM}^{-1}, BIO 15.5^{a} ± 2.3 µg kg_{DM}^{-1}, CHE 23.5^{b} ± 0.7 µg kg_{DM}^{-1}.
Global warming potentials: CH_4 = 25, N_2O = 298.

[B] Time point when silage temperature was 2 K above ambient air temperature (≅ length of aerobic stability [d]).
The lengths for the specific barrels were: CON3 7.68 days and CON4 7.27 days; BIO and CHE > 19 days.
For the statistical analysis of temperatures and emissions, the values of the following three measurement time points were used as the data basis.

5.7 Supplementary information

List of abbreviations

AA	Acetic acid
AEMP	Aerobic emission measurement period
ANOVA	Analysis of variance
ASTA	Aerobic stability
BIO	Treatment containing biological additive
C	Carbon
CF	Carbon footprint
CFU	Colony-forming units
CH_4	Methane
CHE	Treatment containing chemical additive
CO_2	Carbon dioxide
CO_2eq	CO_2 equivalent
CON	Treatment containing no additive
DM	Dry matter
EA	Ethyl acetate
FM	Fresh matter
GC	Gas chromatography
GHG	Greenhouse gas(es)
GWP	Global warming potential(s)
IPCC	Intergovernmental panel on climate change
kg_{DM}	Mass of dry matter material in kg
kg_{FM}	Mass of fresh matter material in kg
LAB	Lactic acid bacteria
LCA	Life cycle assessment
N_2O	Nitrous oxide
PAS	Photoacoustic spectroscopy
r_S	Spearman's correlation coefficient
SA	Silage additive(s)
SD	Standard deviation
VOC	Volatile organic compound(s)

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Authors' contributions

HFD: Project administration, supervision, resources, conceptualisation, methodology, investigation, data curation, formal analysis, validation, visualisation, writing – original draft, writing – review & editing. G-CM: Project administration, supervision, resources, conceptualisation, methodology, investigation, validation, writing – review & editing. MT: Resources, methodology, validation, writing – review & editing. WB: Funding acquisition, project administration, supervision, resources, writing – review & editing. All the authors read and approved the final manuscript.

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Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available due to further use of the data for the PhD thesis of Hauke F. Deeken but are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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6 General discussion and conclusions

The three studies in this thesis investigated the formation, emission and fixation of gases from the moment of silo closure to silage removal during feed-out. The studies answer research questions 1–5. The combination of the studies and a literature review serves to complement the research into gas dynamics, from field to barn, and provides answers to questions 6 and 7.

6.1 Answers to the research questions 1–5

1 When are which climate- and environment-relevant gases and gas quantities formed during fermentation?

Studies 1 and 2 investigated the temporal variation in GHG (CO_2, CH_4 and N_2O) formation during the fermentation of grass, lucerne and maize silage. The methodological approach was to measure the gas concentrations within the head space of the silos. For Study 1, this approach limited the information on the time of gas formation. This aligns with previous studies (e.g. Li et al., 2017; Peterson et al., 1958; Wang and Burris, 1960). In Study 2, silos were connected to gas bags to determine the concentrations and amounts of the gases formed. This methodology aligns with Knicky et al. (2014). To the author's knowledge, Studies 1 and 2 set new standards concerning the scope of gas sampling. The combination of the long test period, the short intervals between the individual measuring points and the wide range of gases analysed provided one of the most comprehensive data sets to date.

All variants exhibit significant CO_2 formation within the first two days after silo sealing due to plant material, EB and yeast activity. CH_4 concentrations exhibit local maxima between the ensiling hours 16.2–144.0 and N_2O concentrations between hours 38.3–94.5. The formation pathways of these gases are based mainly on the metabolism of EB and archaea (Studies 1 and 2). Additional CH_4 can be formed during malfermentation.

Study 2 also comprised the formation of ethanol and EA. This was investigated for the first time in silage emission research. Previous studies measured material concentrations (e.g. Weiß et al., 2020) or ethanol gas formation of silage-related MO in broth (Shan et al., 2021c). Ethanol gas quantities peaked at ensiling day 4.67 ± 0.38 and were formed primarily by EB and yeasts. EA peaked between ensiling days 5–30 and was formed by LAB, AAB and yeasts. Additional EA can be formed in the ongoing fermentation based on LAB_{he} and AAB activity.

Further information can be found in Section 6.2.2, which also considers other gases.

2 To what extent do the factors of plant type, DM, and management (e.g. packing density and use of SA) affect gas formation? What emissions occur under more challenging conditions, such as malfermentation and increased risk of aerobic deterioration?

The presented data indicates that low DM led to a significantly faster increase in CO_2 concentrations regardless of the plant type (Study 1; cf. Gomes et al., 2019). Furthermore, low DM led to higher initial CH_4 concentrations (ensiling hours 6–27) but decreased concentrations afterwards (ensiling hours 31–106) (Study 1). Higher DM led to later but increased N_2O concentration peaks. Grass silage indicated increased initial CH_4 and N_2O concentrations compared to lucerne. However, gas concentrations are affected by an outward-directed CO_2 gas flow (Studies 1–2). The malfermentation of lucerne silage resulted in an increase in the pH and formation of methane (CH_4). This was due to the degradation of LA, the formation of butyric acid and the formation of NH_3 (cf. Pahlow et al., 2003). This pattern has been measured for the first time. Unfortunately, the amount of CH_4 formed is not known. However, CH_4 formation may also occur in commercial silos under adverse conditions (e.g. low DM, low FC, high clostridial counts). In these situations, best practice (see Section 6.2.2) shall be applied to mitigate emissions.

SA did not consistently reduce CH_4, N_2O, ethanol or EA emissions during fermentation (Study 2). BIO reduced CH_4 and N_2O, and CHE reduced ethanol and increased N_2O emissions.

Increased porosity (e.g. high DM, low packing effort) led to increased O_2 supply after silo closure and increased EB and yeast activity. This affected DML and CO_2 formation (Study 2; Shan et al., 2021b). High porosity and increased counts of these MO affected the ASTA (Study 3). Thus, DML and CO_2 emissions during the feed-out phase were probably substantially increased. In contrast, climate-relevant CH_4 and N_2O emissions during the feed-out phase were unaffected based on outgassing of previously formed quantities. However, increased DML led to rising emission quantities, indirectly connected to silage production, e.g. during crop production (Studies 1 and 3).

SA delayed the heating but increased the emissions of ethanol and EA during feed-out.

3 Which emission quantities apply in the fermentation and which in the aerobic feed-out phase? Which ratio is derived for continuous silage material? When should action be taken to reduce emissions?

CH_4 and N_2O are formed in the first days of fermentation. The data show that larger parts are emitted during fermentation, smaller parts after the silo is opened (Studies 2 and 3). SA can affect emission quantities: the BIO treatment indicated the lowest values (Study 3). Increases in CH_4 and N_2O concentrations occur earlier in the low DM variants. However, it's still unclear whether this is due to earlier formation or influenced by gas depletion. Mitigation strategies should generally be applied during the early fermentation phase (Sections 6.2.1 and 6.2.2).

VOC emissions during the feed-out phase exceed those during fermentation. This is due to the continuous volatilisation of the substances present in the liquid phase or additional microbial VOC formation. Shan et al. (2023) showed the new formation of ethanol in anaerobic layers of heating silage. Study 3 shows increased EA formation for stable and heating material.

CO_2 emissions during feed-out exceed those produced during fermentation. DML affects the indirect emissions associated with silage production (Study 3; cf. Emery and Mosier, 2012). Based on the studies, management decisions should focus on reducing DML to improve sustainability.

4 Does silage fix gases in modified trial set-ups using gas-proof materials? When are which quantities fixed? Can silage be a CO_2 sink?

Previous studies partially used silicone, known as CO_2-diffusable (Study 2; Bueno et al., 2020; Restelatto et al., 2019; Schmidt et al., 2018). The trial set-up in Study 2 focused on the reduction of methodological errors affecting gas tightness negatively. Nevertheless, all gases indicated decreasing quantities within the zero-pressure systems (silos plus gas bags) observed in Study 2. Several biological, physical and chemical pathways were discussed (Section 4.4.5). However, further interdisciplinary research is required to prove or refute the proposed explanations.

Despite the reduction in gas volume, the net balance of GHG and VOC formed and fixed during fermentation is positive. Thus, the open research question of whether silage can act as a carbon sink (Schmidt and Vigne, 2023) is answered and negated for the first time in Study 2. Emissions during the feed-out phase are added to this. Thus, silage storage must be seen more as contributing to climate-relevant emissions and less as sequestering CO_2 or other gases.

5 If the use of SA mitigates silage emissions during fermentation and/or the feed-out phase, what's the balance of silage-related mitigations and the CF of SA themselves?

As the best-case scenario in Study 3 shows, SA use does not lead to relevant reductions in climate-relevant emissions (Section 5.4.4). Consequently, the CF of SA production and application in this scenario cannot be compensated by reduced emissions from silage storage.

The situation is different if using SA leads to a reduction in DML (worst-case scenario, see Section 5.4.4). In this case, the savings in indirectly associated CO_2eq emissions – during crop production (Emery, 2013; cf. Jacobs et al., 2017; Wilkinson and Garnsworthy, 2021) – can offset the CF of the SA. Similarly, if a malfermentation would lead to substantial DML (Study 1), and management decisions, e.g. the use of SA, would prevent clostridial activity. From an ecological point of view, the use of SA is therefore positive if high DML can be avoided. The empirical studies confirm the first approaches in this context (Milimonka et al., 2019) and complement earlier objectives of SA use (Muck et al., 2018).

6.2 Advanced findings

The more complex research questions 6 and 7 are discussed in separate sections below.

6.2.1 Research of gas dynamics during the ensiling process

The presented studies complement the silage emission research conducted over the last decades. Based on the current state of research, the findings are combined to answer research question 6 and to derive consequences for the process chain of silage provision.

6 Which gases are formed, emitted or fixed by which MO in which pathways? What phases of gas dynamics can be deduced?

The gas dynamics of silage material during storage periods exhibit high levels of variability, both spatially and temporally, in the pathways employed (Studies 1–3; cf. Daniel et al., 2015; Hafner et al., 2010; Shan et al., 2023; Shan et al., 2021b). The environmental conditions and microbiota show noticeable interactions. As shown, the formation or fixation of detectable gases may help assess the occurrence of specific microbiota metabolism. Thus, gas measurements can help to explain microbial activity in the phases of the ensiling process (see Section 2.3).

Table 6.1 displays a deliberately clear phase structure of gas dynamics during silage provision. It shows the essential formation and fixation pathways. Modifications of the existing phases were used to explain various phenomena in more detail (cf. Ávila and Carvalho, 2020; Shan et al., 2023). Table 6.1 is based on current state of knowledge, complementing Studies 1–3 and previous reports. To the author's knowledge, this level of compact summarisation of emission pathways is innovative. However, Table 6.1 focusses on gas emissions and does not consider other emissions like effluents or the formation of hygienic-relevant substrates such as mycotoxins (e.g. Day and Liscansky, 1987; Gallo et al., 2023; Queiroz et al., 2018; Weiß, 2017; Wróbel et al., 2023). Furthermore, many formation and degradation pathways are based on a combination of different research areas without direct evidence in silage. At this point, there is a need for interdisciplinary research into parallel gas measurement and analyses of the microbial community. First approaches have been made in this regard (Chen et al., 2021). Still, the heterogeneity of the pathways and conditions (e.g. plant species, DM, epiphytic MO) show that a more reliable empiric database is necessary. Thus, an appeal is made to adapt or supplement the facts presented.

Similar to previous models, the transition between the phases may be blurred based on the temporal-spatial differences of environmental conditions (e.g. O_2 supply), silage characteristics (e.g. DM) and resulting MO activity. As a result, the strict phase separation applies more to a specific part of the silage mass; other parts may be in different phases at any given time. Fig. 6.1 indicates the implementation of the various phases into the process chain of silage provision.

Table 6.1 Phases of gas dynamics during silage provision, including major formation and fixation pathways. This table should be seen as a first step, as more evidence is needed in the silage context and other pathways may apply. The findings are based on the studies' results and cited reports.

Phase	Event	Parameter	Oxygen supply		Bacteria					Eucaryota		Other pathways		
			aerobic	anaerobic	LAB_{ho}	LAB_{he}	AAB	EB	Clostridia	Yeasts	Moulds	Plant cell respiration	Archaea	Chemo-physical
1 [A]	Harvest & Transport	Plant cut off, transport vehicle	X		CO_2	CO_2	CO_2	CO_2		CO_2	CO_2	**CO_2**		
2 [B]	Silo filling & compaction	Material (un-)compacted	X	(x)	CO_2	CO_2 E	CO_2	CO_2 **E** N_2O NH_3	CO_2	CO_2 E EA	CO_2	**CO_2**	CH_4	
3	Silo sealed	Wrapping film covers the silo	(x)	X	CO_2	CO_2 E	CO_2	**CO_2** **E** N_2O NH_3	CO_2	**CO_2** **E** EA	CO_2	CO_2	CH_4	
4	Primary hetero-fermentative fermentation	pH above limit of undesired MO (≈ pH ≥ 5.0)		X		**CO_2** E CO	CO_2 EA CO	CO_2 E N_2O NH_3 CO **NO_2**	CO_2 CO	CO_2 E EA	CO_2		CH_4	
5	LAB homo-fermentation	pH decrease (≈ pH < 5.0)		X		**CO_2** E								**NO**
6 [C]	Gas fixation	Gas quanitity decrease		X	CO_2	**CO_2** E CO	CO_2	CO	CO	E			CH_4 CO	CO_2 N_2O CH_4 NH_3
7 [C]	Secondary hetero-fermentative fermentation	Mainly activity of LAB_{he}		X		**CO_2** E EA	EA							
8 [C]	Malfermentation	Clostridia activity		X				CO_2	CO_2 **NH_3** CO		CO_2		CH_4	
9 [A,B]	Feed-out, stable temperature	Silo opened, Δt < 2 K	X		CO_2	CO_2 EA	CO_2 EA	CO_2		CO_2	CO_2			
10 [A,B,C]	Initiation of heating	AAB initiates reheating	X	(x)	CO_2	CO_2 EA	**CO_2** EA	CO_2		CO_2 EA	CO_2			
11 [A,B,C]	Primary phase of heating	Yeasts degradate silage, Δt > 2 K	X	(x)		EA CO_2		E		**CO_2** E EA	CO_2			
12 [A,B,C]	Secondary phase of heating	Moulds degradate silage	X	(x)		EA CO_2		E		CO_2 E	**CO_2**			

Contribution to formation
- Small share
- Medium share
- Large share

[A] = Outside the silo (e.g. transport vehicles, feed-mixer wagon or feed trough), AAB = Acetic acid bacteria, [B] = Inside the silo, [C] = Optional phase, CH_4 = Methane, CO = Carbon monoxide, CO_2 = Carbon Dioxide, Δt = Temperature difference (silage - ambient air), E = Ethanol, EA = Ethyl acetate, EB = Enterobacteria, LAB_{he} = Heterofermentative lactic acid bacteria, LAB_{ho} = Homofermentative lactic acid bacteria, NH_3 = Ammonia, N_2O = Nitrous oxide, NO = Nitric oxide

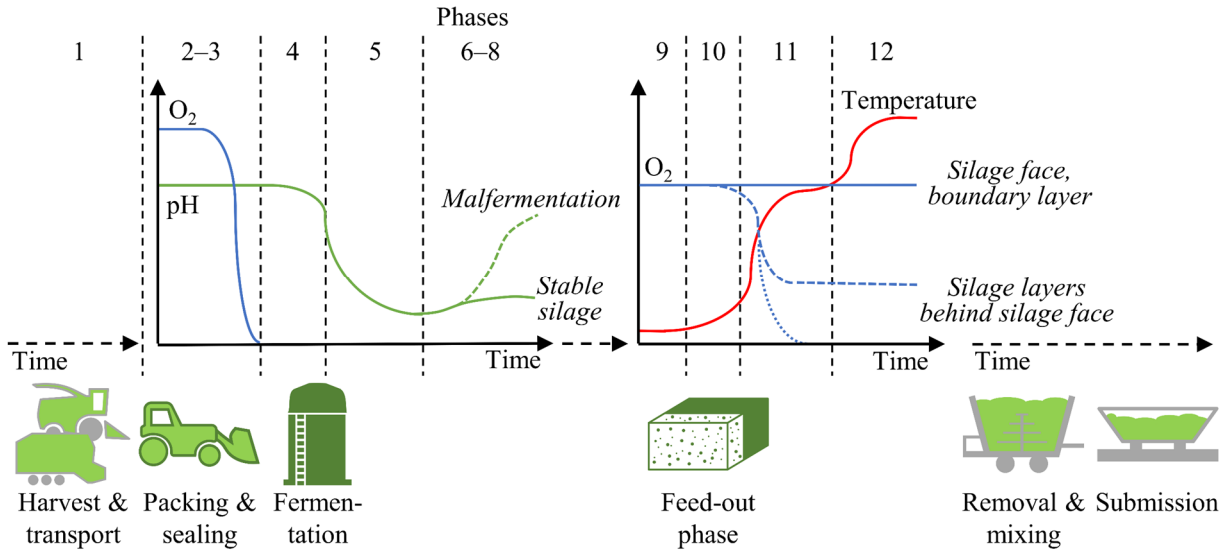

Fig. 6.1 Phases of gas dynamics in the process chain of silage provision. O_2 = Oxygen.

Furthermore, only parts of the gases are depicted. In particular, the remaining 44 VOC (Hafner et al., 2018) require further research. The apparent differences between ethanol and EA formation patterns (Study 3; Shan et al., 2023) show that there may also be heterogeneous formation patterns for the other VOC. In addition, management impacts, such as the use of SA, do not appear to affect the formation of VOC consistently. Despite the principles of Fick's and Henry's law, the SA in Studies 2 and 3 could not demonstrate a uniform reduction in gas formation. This contradicts earlier studies of the VOC concentrations in SA-treated silage (Hafner et al., 2015).

Plant respiration can begin after cutting during Phase 1. Grass or lucerne silage may experience respiration losses during wilting (cf. Macdonald and Clark, 1987; Savoie et al., 2011); maize silage is directly filled into transport vehicles. Chopping and compaction enhance microbial activity, DML, and heat and CO_2 formation. Aerobic conditions will generally be maintained until the material is sealed in Phases 1–3. In this time, silage temperature can increase in commercial silos up to 11.67 K (Seglar, 2003; McCullough, 1984, cited by Schroeder, 2004). These values are considerably higher than the calculated values of respiratory heating in models of O_2 consumption of common silage characteristics (Lindgren, 1999). During filling, O_2 penetrates the uppermost layers of the silo. This can be intensified in bunker silos by the vehicles packing the silo: the compaction and expansion of the material during the transfer can lead to air being sucked in and penetrating (sponge effect). This increases the length of O_2 exposure (Phase 2). However, this may differ for other silos. Silo bags are sealed during filling and bales directly after filling. This shortens the time of exposure to O_2. The same applies to tower silos: the material is conveyed with air by the blower, but the O_2 is quickly respired in the silo. However, the filling rates of these types of silos can be lower than those of bunker silos. Laboratory-scale trials measured more minor

temperature differences (< 4 K) (Study 2; Li et al., 2017). This may be affected by the smaller silage masses or by rapid O_2 consumption (Li et al., 2017; Wang and Burris, 1960). Further trials, the results of which have not yet been published, have demonstrated that laboratory silos (1.5 L glass jars) exhibit anaerobic conditions between 0.5 and 1.0 hours after closure. The rapid filling of laboratory silos differs from commercial silos. Nevertheless, on farms, a combination of aerobic and anaerobic conditions can occur in certain parts of the crop material before the silo is sealed: anaerobic conditions may occur in the deeper layers of the self-compacted silage material or perhaps even in the transport vehicles (Phase 1). The latter should only occur during long dwell times, e.g. during long-distance transport or delays in unloading. As a result, parallel respiration, fermentation, and proteolysis during these phases could lead to emissions that may go undetected.

Phases 3–4 are crucial for the formation of CH_4 and N_2O due to the dominant activity of EB (see Studies 1–2). Therefore, efforts to reduce the formation should focus on these phases (see Section 6.2.2). Additionally, carbon monoxide (CO) and NO_2 are produced during this period. CO can cause health problems and may lead – similar to VOC – to O_3 formation (cf. Zhao et al., 2021). CO is formed in the initial days after silo closure, but the amount of formed quantities is still unknown (Zhao et al., 2021). One possible explanation is that in a non-silage context, some species of proteobacteria and EB have been shown to form CO in mainly anaerobic conditions (Hayashi et al., 1985), single species of firmicutes in aerobic conditions (Engel et al., 1972). However, the possible formation pathways of CO in silage have yet to be detected, but the mentioned phyla were detected in maize silage (e.g. Xu et al., 2021). The formed CO may be oxidised to CO_2, H_2, and AA by, for instance, EB, clostridia or methanogenic archaea using CO dehydrogenase (cf. Davidova et al., 1994; Diekert et al., 1986; Lee et al., 2018). The H_2 may be relevant for subsequent methanogenesis (Study 2). CO dehydrogenase activity depends on the pH, for instance, with an optimum between 6.7–12.0 for specific archaea, but low activity down to pH 3 is possible (Davidova et al., 1994; DeMoll et al., 1987; Grahame and Stadtman, 1987). The literature review clearly shows the knowledge gap. There is only one study on CO emissions from silage and it is unclear whether the above explanations apply to the heterogeneous fermentation processes. Further investigations are required, as the CO dehydrogenase-based CO metabolism, which is partly cross-species, could also be important for CO_2 fixation if the Wood-Ljungdahl pathway (reductive acetyl-coenzyme A pathway) applies in Phase 6 (cf. Berg, 2011; Diekert et al., 1986; Ragsdale and Pierce, 2008; Vigne, 2022). The detection of specific gases in the various phases could therefore provide information on the metabolism pathways that occur.

In parallel, the formation of NO_2 is directly connected to NO_3^- degradation (Study 1). EB generally degrade NO_3^- to NO_2^- and subsequently to N_2O and NH_3 through denitrification, but

this process only applies for pH > 4.5–5.0 (Pahlow et al., 2003). LAB expresses nitrate- and nitrite-reductase enzymes (Rooke and Hatfield, 2003). Due to the ongoing decrease in pH in silage, NO_2^- is unstable when the pH is below 4.5 and is chemically decomposed into NO and NO_3. Sufficient quantities of NO_3^- favours clostridia suppression (cf. Study 1; Kaiser and Weiß, 2007; Weiß, 2001; Wilkinson, 1999). In detail, the inhibitory effect is based on the subsequently formed NO affecting the adenosine triphosphate production of clostridia (Spoelstra, 1985, 1983; Woods et al., 1981). However, if NO is released into the air, it reacts with O_2 to form, among others, NO_2 and N_2O (Pahlow et al., 2003).

Subsequently (Phases 5 and 7), LAB are the most active MO at pH < 4.5–5.0. Seglar (2003) reports the change from thermophilic to mesophilic LAB between phases P3 and P4. As discussed above, this may be of a higher relevance for commercial compared to laboratory silos. Thus, microbial activity and gas formation may vary in the ratio and timing between the presented Studies 1–3 and practical agriculture. Still, the primary microbiological metabolism should be comparable (cf. Krueger et al., 2022). Future research should aim to improve the knowledge transfer between laboratory and commercial silos.

Phase 6 requires further investigation. In particular, research attention should be given to the gas fixation pathways, with a focus on CO_2. Several non-silage studies have addressed possible pathways (e.g. Berg, 2011; Davidova et al., 1994; Diekert et al., 1986; Hayashi et al., 1985; Ragsdale and Pierce, 2008). Some of these pathways have previously been connected to the context of silage (Study 2; Chen et al., 2021; Krueger et al., 2022; Schmidt et al., 2023; Schmidt et al., 2018; Schmidt and Vigne, 2023; Vigne et al., 2019). A comprehensive overview is given by Vigne (2022). Previous approaches have primarily focused on the reductive acetyl-coenzyme A pathway. However, Study 2 highlighted the importance of bicarbonates and carbonic acid during CO_2 fixation, which may enhance other microbial reactions such as the formation of pyrimidine and arginine (Arsène-Ploetze et al., 2006; Arsène-Ploetze and Bringel, 2004). Furthermore, the Arnon-Buchanan cycle (reductive citric acid cycle or reductive tricarboxylic acid cycle) may be another pathway of autotrophic CO_2 fixation (Berg, 2011). In this cycle, CO_2 and bicarbonate are used for the formation of acetyl-coenzyme A, oxaloacetate and phosphoenolpyruvate. This cycle, which can vary slightly between species, has been detected in, among others, mesophilic, aerobic bacteria such as proteobacteria (cf. Berg, 2011). These bacteria may be one of the most abundant phyla within the first 30 days of silage fermentation (Chen et al., 2021). Nevertheless, research has not yet been able to prove this in the silage context. One approach was proposed by Chen et al. (2021), who report negative correlations between the relative abundance of *Serratia* (-0.66), *Sphingobacterium* (-0.58) and *Sphingomonas*

(-0.29) and CO_2 production of rice straw silage. This may be based on the CO_2 fixation by these bacteria. Future studies should concentrate on the empirical evidence of the MO and metabolic pathways involved under the heterogeneous conditions of versatile silage fermentation.

Further research is also needed in Phase 8 especially in the formation of CH_4 during malfermentation or the variety of the 46 VOC. The mutual interaction between microbial metabolism and characteristics of the formed gases, e.g. the antimicrobial and fungal effect of EA (see Studies 2–3), may also be interesting.

The duration of ASTA (Phase 9) varies depending on, among others, DM, porosity, MO community, ambient and initial silage temperatures, the structure of the silage face, wind impact, SA use or other treatments of the silage face. Furthermore, the ASTA of silage and feed diets, which may implement silage, may vary noticeably between laboratory and farm conditions (Kung, 2010). The start of DML, CO_2 emissions and heating are based on AAB respiration (Phase 10) (Merry and Davies, 1999). Subsequently, yeasts precede moulds (Phase 11), but later on, moulds suppress yeasts (Phase 12) (Merry and Davies, 1999). Furthermore, *Bacillus* strains are becoming increasingly relevant in the 40–80°C temperature range, as yeasts are inhibited at high temperatures (Lindgren, 1999; Lindgren et al., 1985). In contrast, Pitt et al. (1991) differ between the four groups of mesophilic and thermophilic yeasts and moulds.

The amount of O_2 that can enter the silo is limited by ambient conditions, silage characteristics and physical laws. The available O_2 is rapidly respired in the layers closest to the silo face, with anaerobic conditions in deeper layers (Shan et al., 2023). The rate of microbial respiration [(g O_2) h^{-1} kg_{DM}^{-1}] defines the depth of aerobic and anaerobic layers. During the ongoing heating process, the respiration rate is assumed to increase rapidly due to the O_2 supply (cf. Pitt and Muck, 1993; Shan et al., 2021b). Thus, the depth of the aerobic layer decreases, and anaerobic conditions are restored beyond it. Within the anaerobic layers, the increased numbers of yeasts may lead to additional ethanol formation (Shan et al., 2023), and other MO may produce additional climate or environmental gases, for instance, CH_4 (Study 3). The ethanol formed may be partially metabolised to EA in the aerobic layers near the silo face (Study 3). In general, ethanol degradation was reported to precede LA and AA degradation (Spoelstra et al., 1988). However, the restoration of anaerobic conditions is cancelled when the face layer is removed. O_2 again penetrates deep into the silo and increases the activity of the facultative aerobic MO in the deeper layers until the already raised CFU of the facultative aerobic MO leads to accelerated respiration losses at the silo face. It can be assumed that this fact, in addition to the different gas tightness (cf. Xiccato et al., 1994), is one of the main reasons for the differences in ASTA between commercial and laboratory silo material, which is exposed to O_2 for the first time when the silo is opened.

The varying microbial activity in Phases 11–12 may lead to a clear separation – indicated by two separated phases of temperature increase (e.g. Merry and Davies, 1999; Shan et al., 2023; Shan et al., 2021b; Sun et al., 2017; Weseh, 2013) – or may show a smooth transition between these phases (Shan et al., 2021a; Shan et al., 2021b; Shan et al., 2019; Sun et al., 2017). Within the silo, the heat is transferred based on conduction and convection; at the silo face or other external barriers, heat dissipates via radiation and convection. These rates depend on silage and silo characteristics, especially porosity, DM or construction and sealing material of the silo. In addition, the specific heat capacity of the silage material [$Wh\ kg^{-1}\ K^{-1}$] may differ. Sun et al. (2017) and Pereira et al. (2019) exhibit the varying heating pattern in silos.

To summarize current knowledge, crucial phases for silage emissions are shown in Table 6.2.

Table 6.2 Relevance of the phases for silage emissions.

Phase	Event	Importance for DML and gas emissions		
		DML & CO_2	**CH_4 & N_2O**	**VOC**
1	Harvest & Transport	Medium	Low	Medium
2	Silo filling & compaction	High	Low	High
3	Silo sealed	High	High	High
4	Primary heterofermentative fermentation	High	Medium	High
5	LAB homofermentation	Low	Low	Low
6	Gas fixation	/	/	/
7	Secondary heterofermentative fermentation	High	–	High
8	Malfermentation	High	High	Medium*
9	Feed-out, stable temperature	Low	–	Medium
10	Initiation of heating	Medium	–	High
11	Primary phase of heating	High	Medium*	High
12	Secondary phase of heating	High	Medium*	High

– = no relevance, / = not considered, * = assumed, CH_4 = Methane, CO_2 = Carbon Dioxide, DML = Dry matter losses, N_2O = Nitrous oxide, VOC = Volatile organic compounds.

Microbial metabolism and subsequent emissions can continue during mixing and submission (cf. Bonifacio et al., 2017; Kung et al., 1998; Seppälä et al., 2013). Mixing distributes the MO to all particles and provides additional O_2 to promote deterioration. In addition, water addition may increase the risk of deterioration (Eastridge, 2006; Felton and DeVries, 2010), and other feed components offer additional substrates for microbial metabolism. The components' different buffer capacities and pH values affect the pH-dependent mitigation of aerobic deterioration by organic acids (cf. reported studies in Kung, 2023). Furthermore, silage with high activity of aerobic MO impairs and SA like organic acid enhance ASTA of the feed ratios (unpublished findings reported in Kung, 2023; Seppälä et al., 2013). During silage submission, precipitation, animal saliva and solar radiation can affect the DM of the forage and MO metabolism.

6.2.2 Implications for silage research and farm management

The studies and the scope of the discussion show that the emission behaviour of silage is complex and influenced by several multidisciplinary factors. Based on the principles of risk assessment, a ranking of hazarding factors is possible (Lindgren, 1999). The relevance of these factors for silage emissions and the effort – i.e. changes in management, cost and labour input or expenditure with environmental consequences – required to change them directly or to implement management decisions that affect them can vary considerably (Fig. 6.2).

7 Which recommendations can be formulated for silage research and commercial silage management to reduce environmental impacts?

The new field of gas fixation needs further trials using various trial set-ups. So far, it cannot be ruled out that gas leaks occurred during (some of) the tests and that the assumptions of gas fixation are based on methodological errors. Further tests with gas-tight silos must be carried out and evaluated with detailed MO analyses and interdisciplinary discussion. Understanding the complex interlinked pathways requires additional effort. Any findings may be helpful for future silage research. In addition, insights into the ensiling of agricultural feedstuffs can contribute to progress in bioprocessing research.

Studies 1–3 and previous research demonstrate the dynamic patterns of substrate concentrations within the material (e.g. Weiß et al., 2020) or gas quantities stored within the silo (e.g. McEniry et al., 2011; Weinberg and Ashbell, 1994). However, some emission models assume constant substance quantities within the material – especially VOC concentrations – for the period of emission prediction (e.g. Bonifacio et al., 2017; El-Mashad et al., 2010; Hafner et al., 2012). While empirical and estimated data align concerning gas formation during fermentation (Study 2, Hafner et al., 2012), variation may occur in the dynamic VOC emissions during the feed-out phase based on additional substrate formation or degradation (cf. Shan et al., 2023). For instance, the ethanol metabolism to EA is assumed to affect gas emissions and challenge the assumption of constant material concentrations (Study 3). It is recommended that established models be modified or new models be developed to account for the highly dynamic processes of substrate formation and degradation affecting the emission rates. This applies particularly to GHG in Phases 3–4 and VOC during Phases 10–12. Furthermore, models should consider long-term feed-out periods of practical relevance. The development of emission models for the first hours after silo opening by El-Mashad et al. (2010) or Hafner et al. (2012) is valuable. Still, it does not represent the entire range of commercial silo emissions. Further empirical research is necessary to provide reliable data for the next generation of models.

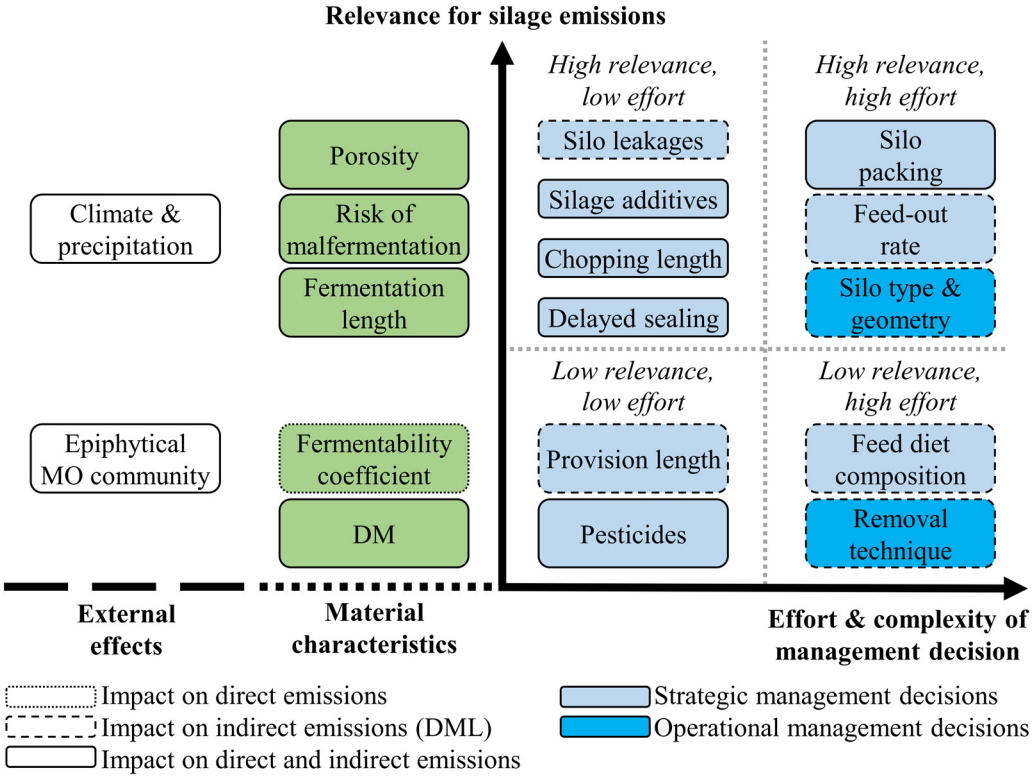

Fig. 6.2 The relevance of various parameters for environmental impacts of the silage provision process chain. The external effects and material characteristics may be affected due to management decisions. These can be differentiated according to their relevance, as well as the effort and complexity (ecological, economic or organisational) of their implementation.
DM = Dry matter (concentration), DML = Dry matter losses.
The presented findings are based on the studies' results and the findings of earlier published reports cited within this dissertation.

The current state of knowledge shows that the phases of gas formation can be very short and dependent on many factors. Earlier studies – which have adapted their methodology to different research foci and provide important findings (Schmidt et al., 2012; Schmidt et al., 2011; Zhao et al., 2021) – have sometimes made simplified statements about, e.g. the DM or the time of gas extraction. However, if gas formation and its processes are considered, this information is relevant. Therefore, future studies should examine and present the experiments in more detail for the greatest gain in knowledge and simplified transfer. Additionally, transferring findings from laboratory-scale trials to farm-scale remains challenging. Future trials should use methodologies similar to those used for commercial silos, as the varying silo set-ups – such as the remaining gases in the head and floor space (Study 2) – differ from those found in commercial silos. In general, research into grass silage requires more effort than research into maize silage. This is due to the fact that silages of grasses or legumes show a higher heterogeneity in the forage material or the fermentation process than maize. This is a consequence of the increased biodiversity in grassland.

VOC emissions may significantly impact the silage provision's carbon and environmental footprint (Study 3). These emissions must be contextualised with those during crop production,

animal digestion, or animal husbandry (cf. Hafner et al., 2018). Modern sensors may aid in assessing the quantities of the 46 VOC. This can be used to improve silage management.

Emissions are affected by multifactorial and interdisciplinary parameters, including material characteristics and management decisions during the process chain of silage production (Fig. 6.2). The emissions are primarily influenced by parameters that are also regarded as crucial for other pivotal levels of quality assessment, such as hygiene, feed value, or feed preference. This has the advantage that a conflict of interest between the objectives can usually be ruled out. However, there may be conflicting objectives if the scope of analysis is changed. In ruminant diets, for instance, a minimum chopping length is aimed for in order to ensure a sufficient structural effect, rumination activity and the resulting salivation. In contrast, short chopping lengths help to ensure optimum compaction. Nevertheless, several recommendations are well-documented in silage management. These include, among others:

- DM harvest material (Kung et al., 2018; Seglar, 2003; Spiekers, 2012):
  e.g. for grass 25%–40%, for lucerne 30%–35%, for maize 30%–37%
- Chopping length (Spiekers, 2012):
  < 40 mm for grass silage, 4–8 mm for dairy maize silage
- Porosity (Honig, 1987; Maack and Wyss, 2012): minimum density [$kg_{DM}\ m^{-3}$],
  e.g. for grass 3,5 × DM + 90, for lucerne 2,143 × DM + 137, for maize 8,0 × DM + 6
- Fermentation coefficient (FC) (Schmidt et al., 1971, cited by Weißbach and Honig, 1996):
  FC > 35, with FC = DM [%] + 8 × WSC × $(\text{Buffer capacity})^{-1}$
- Storage before sealing (cf. Kim and Adesogan, 2006; Nia and Wittenberg, 2000):
  rapid sealing is favourable, maximum ≤ 10 h
- Minimum fermentation length (Borreani et al., 2018; Pahlow and Hünting, 2012):
  > 42–56 days, for optimal LAB_{he} activity: ≥ 45–60 d
- Microbial community in maize silage (Kung, 2023; Wilkinson and Muck, 2019):
  e.g. yeast counts < 10^5–10^6 CFU g_{FM}^{-1}, heating starts at 10^7 CFU g_{FM}^{-1}
- Feed-out rate (Maack and Wyss, 2012; cf. Muck et al., 2003; Pitt and Muck, 1993):
  in winter 1.5 m $(\text{week})^{-1}$, in summer 2.5 m $(\text{week})^{-1}$

Based on current knowledge, these parameters are not well suited for use as HACCP limits. In practical situations, it is sometimes not possible to analyse or evaluate these parameters at the time of short-term decision-making. Furthermore, the emission pattern is not immediately critically affected when one or more limit values are undercut or exceeded. For instance, Studies 2 and 3 show that high DM and porosity values may increase DML during feed-out. However,

with appropriate management, e.g. long ensiling length or use of SA, extreme DML may not occur. Consequently, these parameters can be used as an aid for decision-making and process evaluation, but they do not fulfil the requirements of the definition according to HACCP. The National Advisory Committee on Microbiological Criteria for Foods (1998) states: '*Critical limits should not be confused with operational limits*'. This aligns with the science investigating implementing generalised strategies or principles in agriculture: applying standardised procedures in the highly dynamic environment of agriculture, e.g. silage production may be challenging (cf. Daydé et al., 2014; Wirén-Lehr, 2001). The time-, location- and case-specific requirements of each process chain (e.g. geographical and legal requirements, technical equipment), the unpredictable external influences of the environment (e.g. weather, machine defects, human error) or biological influences (e.g. epiphytic MO community, FC) are mostly unknown at the time of decision-making. The dynamic networking and influence of the individual parameters on each other, plus the farmer's personality (e.g. experience, influence of peers, willingness to take risks), also influence the decision-making process. Many farmers' decisions are more influenced by rationality – or economic constraints – than by the objective of omniscient optimisation (Daydé et al., 2014). Nevertheless, recommendations and guidelines are necessary for farmers to improve silage management on a wider scale, despite the fact that these may not always be feasible in dynamic practical conditions. Consequently, a more farm-specific and goal-oriented strategy is recommended (Wirén-Lehr, 2001), which situates decision-making in the context of previous and possible future decisions (Daydé et al., 2014). This is done for two scenarios of particular emission risks, based on Studies 1–3 (Fig. 6.3).

The limits mentioned above and the discussions in Studies 1–3 can be employed to respond to the yes/no questions. Nevertheless, as these parameters are typically not quantifiable in practice at the time of the decision, an assessment based on best practice principles is typically unavoidable, relying on the experience and judgement of the individuals involved. Nevertheless, even with considerable experience, it can be challenging for individuals to assess the consequences of a decision and to identify the optimal timing for action (Daydé et al., 2014; Steckel, 2018). This involves striking a balance between the earliest (optimal impact) and the latest (most extensive information base). Currently, the assessment of situations and decision-making regarding silage emissions is largely defined by non-quantifiable and interdependent influencing factors. Consequently, decision-making processes based on the 'fuzzy logic' model are more applicable here than clear HACCP limits (cf. Center and Verma, 1998).

Scenario: Risk of malfermentation, e.g. lucerne silage

Favourable plant characteristics (yes → / no ↓)	Professional machine settings (yes → / no ↓)	Sufficient fermentation coefficient (yes → / no ↓)	Stabile, low pH, no butyric acid (yes → / no ↓)	Low risk
Effect for mal-fermentation	Low DM, low WSC, high protein	High ash concentration & buffer capacity	High Protein, low WSC, slow pH decrease	Enhanced (proteolytic) clostridia activity
Operational decisions for risk reduction	Ensure optimal harvest timing	Ensure optimal settings	SA use: homof. LAB and/or WSC (e.g. melasses)	Subsequent SA use, re-ensiling of forage
Strategic decisions for risk reduction	Adjust crop management (fertiliser, species composition)	Repair or exchange machines, rolling the pastures	Improve crop & harvest management, SA use	Improve crop & harvest management, SA use

Scenario: Risk of aerobic deterioration, e.g. maize silage

Favourable harvest conditions (yes → / no ↓)	Rapid harvest & transport (yes → / no ↓)	Sufficient compaction (yes → / no ↓)	Rapid, total silo sealing (yes → / no ↓)	Low risk
Effect for short ASTA & high losses	High DM, high porosity	Long O_2 exposition, increased EB, yeast & mould counts	High porosity, increased O_2 ingress at feed-out	Long O_2 exposition, increased EB, yeast & mould CFU
Operational decisions for risk reduction	Decrease chopping length, increase compaction effort	Subsequent SA use, increase feed-out rate	Increase compaction effort, subsequent SA use	Ensuring gas-tightness, SA use, increase feed-out rate
Strategic decisions for risk reduction	SA use, silo with favourable geometry	SA use, improvement of harvest management	SA use, improvement of harvest management	Modification of silo or silo sealing material

Fig. 6.3 Risk analysis and management recommendations to minimise environmental impacts within the process chain of silage provision for two examples.
CFU = Colony forming unit, DM = Dry matter (concentration), EB = Enterobacteria, LAB_{ho} = Homofermentative lactic acid bacteria, O_2 = Oxygen, SA = Silage additive, WSC = Water-soluble carbohydrates.
The presented causes and recommendations are based on the study results and the findings of earlier published reports cited within this dissertation.

In addition, new systems that improve ad hoc decision-making can significantly support farmers. For instance, online, technology-based methods to quantify the plant characteristics would help find the best harvesting time (Oliveira et al., 2020; Vyas and Adesogan, 2023). Increased harvest and transport rates can lead to challenges in ensuring adequate silage compaction and porosity (Leurs, 2006). Nevertheless, the density determination methods used in practice are primarily designed for applications during the feed-out phase and are only limited suitable during the filling of the silo (Büscher et al., 2013; Latsch and Sauter, 2011; Li et al., 2016; Maack et al., 2023). Only a few methods, such as radiometric or microwave density determination (Fürll et al., 2008; Mumme and Katzameyer, 2008), volume determination using laser theodolites in

combination with weighing of the deposited mass (Hoffmann et al., 2014), sinking a density meter into the silo (Thünen et al., 2019) or satellite-supported position determination of the compaction work (Hoffmann et al., 2014) are possible during silo packing for online density estimation (cf. Vyas and Adesogan, 2023). Other online sensors measure the gas atmosphere within the silo to detect possible air leakages (Bauerdick et al., 2022). Thus, sensor- and software-based assistance systems may prove beneficial in the future (cf. Deeken, 2022; Vyas and Adesogan, 2023). However, these methods are not yet widely employed in practice.

Furthermore, a survey of 148 commercial farmers and contractors shows that only 22% of respondents prioritised the 'compacting' element alongside the 'chopping' and 'transporting' elements (Steckel, 2018). However, the state of knowledge clearly shows that silage compaction and porosity is of fundamental importance for O_2 penetration into the silo and thus for aerobic deterioration and DML (Study 3). Prioritising the compaction effort would therefore be helpful from an emission reduction perspective but would conflict with the economic objectives of management. If compaction dictates the speed of the harvesting chain, this could mean that other vehicles have to wait. However, the high costs of an idle harvesting chain are high and encourage farmers to complete the harvest as quickly as possible. This can lead to lower silage density. At the same time, at least 25 factors affect the mechanical and procedural processes of the harvesting chain (Deeken, 2022; Steckel, 2018). Technical damages, incorrect machinery settings, or human errors may also affect material losses during the harvesting and transport phases (cf. Deeken, 2022; Muck et al., 2003). Thus, harvesting technology and management should be adapted to the prevailing conditions in order to get the forage into the silo efficiently. The input parameters used, e.g. time period from harvest to silo sealing and losses during the harvest chain, are factors influencing the CF of silage production.

After harvest, prolonged oxygenation during harvest and silo filling can enhance aerobic EB and yeast growth. Once sealed, the fermentation process and the formation of CO_2 and organic acids are accelerated by low DM (Study 1; Gomes et al., 2019). Earlier CO_2 formation promotes anaerobic conditions and H_2, which affects methanogenesis, as well as NO, N_2O, and NH_3 formation by EB (cf. Lee et al., 2018). Therefore, a rapid formation of acids is required to undercut the pH optima of EB. However, achieving a substantial pH decrease in low DM and legume silages requires higher quantities of acids (Study 1; cf. Spiekers, 2012; Whittenbury et al., 1967). Therefore, finding the optimal DM at harvest may require a compromise. Additionally, the formation of NH_3 can increase the buffering capacity of the silage material, which promotes stable pH values. However, this can lead to prolonged clostridial activity, which can be suppressed by increased concentrations of NO_3^- (see Section 6.2.1). The presence of NO_3^-, though, also promotes

the formation of N_2O and NH_3 which may affect both the amount of emissions and the rate of pH drop. Fast acidification is necessary for clostridial suppression, but can also lead to the formation of NO, which is a safety issue (Pahlow et al., 2003). To reduce the risk of clostridial malfermentation and N metabolism, it is important to mitigate EB activity from the outset by sealing the silo as quickly and effectively as possible. To ensure optimal silage management, it is important to minimize the time between the depletion of O_2 and the decrease in pH. This is also crucial for high WSC silages, as prolonged O_2 supply can increase EB and yeast activity, which can have a noticeable impact on ethanol and ester formation (Brüning et al., 2018b; Weiß et al., 2022), and DML as shown in Studies 2 and 3.

Excess DML and emissions particularly due to aerobic deterioration have to be avoided at (nearly) all costs during feed-out. This may be a barely new finding (cf. Emery, 2013), but assessing silage's CF (Study 3) enhances previous importance. For this, management may utilize two principles: a) aerobic deterioration may be minimized and ASTA improved by reduction of the aerobic MO activity. The following aspects are helpful in this context, among others, low porosity and O_2 penetration based on improved compaction effort, an optimal harvest timing and short chopping lengths, rapid silo sealing to minimize aerobe MO growth or MO inhibition due to SA use. These parameters and their impact on ASTA are primarily determined during the Phases 1–4, thus before aerobic deterioration occurs. The second principle is that b) the feed-out rate may be adapted to the ASTA, i.e. the silage has to be utilized before aerobic deterioration occurs (Phases 9–12). For short-term adjustments, a feed diet modification, the sale of silage or strategic modifications of the silo geometry may help, but are restricted due to limitations in terms of animal nutrition or profitability. The subsequent application of SA, such as organic acids, on the silage face is possible, but is also often limited in its effect.

Furthermore, Vyas and Adesogan (2023) stated a possible long-term objective: '*Future research should develop technologies that allow online monitoring of greenhouse gases and VOC concentrations in silage using face shavers*'. For mid-term development, the online thermography of the silo face could help to prioritise feed-out areas with high MO activity (cf. Vyas and Adesogan, 2023). This technology may also help to assess deterioration during feed-out or provision of feed in the trough (Felton and DeVries, 2010; Türkgeldi et al., 2023). Seppälä et al. (2013) point out that basic hygiene, like cleaning the mixer wagon or trough, is essential to mitigate MO contamination. Furthermore, additives, especially for improving ASTA of feed diets, may help but do not compensate for feeds of low quality considering the changing DM and pH of the mixed diets (cf. Kung, 2023; Seppälä et al., 2013). Thus, management has to apply the above recommendations in all process stages to improve operational and strategic

decisions (Fig. 6.3). For strategic decisions, the economic and ecologic implications of silo construction or machinery use should also be considered.

SA can be used as an operational or strategic action. In certain regions, the widespread use of SA is common in order to ensure the safety of the silage – due to a lack of information and the feasibility of optimal management options (Lindgren, 1999). Farmers should evaluate the risk of malfermentation or DML while planning the harvest process. If such risks are imminent – and other best practice principles are insufficient to minimise this risk decisively – the use of SA should be seriously considered. This can positively affect emissions and feed value (cf. Randby and Bakken, 2021) and prevent a complete feed loss. The SA must be selected according to their direction of action (cf. Kalzendorf and Staudacher, 2012; Nußbaum, 2013). Short-term application of SA may be possible if the harvesting and storage process is not going well. Subsequent treatment of the silo face with acids is also possible. However, this operational control of symptoms should result in critical adjustments to the following strategic harvest management. If no above-average DML and emissions are to be expected, the use of SA appears to be neither advantageous nor disadvantageous regarding climate protection (Study 3). Consequently, appropriate SA use should not be mandatory, but widespread use can be the most reliable action to significantly reduce the risks of – sometimes unintentional and uncontrollable – mismanagement (cf. Lindgren, 1999).

6.2.3 Relevance of silage emissions and environmental impacts

The amount of globally produced silage in agriculture has increased over the years and may increase further (cf. Rotz et al., 2024; Weinberg and Ashbell, 2003; Wilkins, 2005; Xu et al., 2021). Ensiling is also used for biogas production (cf. Jacobs et al., 2017), using food waste as animal feed (cf. Jones et al., 2021), fermentation of fish or insect larvae (cf. Arruda et al., 2007; Hadj Saadoun et al., 2020; Yunilas et al., 2023), or biorefinery by using extracts from grass silage (cf. McEniy and O' Kiely, 2014; Rinne, 2024). In ruminant nutrition, the change from grazed to conserved forage led, among others, to increased machinery and energy use or VOC emissions during feed production (cf. Rotz et al., 2024). Based on the model of Bonifacio et al. (2017), total VOC emissions of the US dairy industry are estimated to have increased by 53% between 1971 and 2020 (Rotz et al., 2024). CO_2eq emissions during silage storage are considered small compared to the indirect emissions of crop production (Study 3). Schmidt et al. (2012; 2011) stated that silage emissions are also small compared to the emissions of livestock husbandry itself. This argument is underlined by Rotz et al. (2021), who said that most US dairy farms' GHG emissions are based on enteric fermentation or manure storage and application (approximately 69%). Nevertheless, the study also reports that 29% of the GHG emissions are from the cumulated sectors of cropland (soil N_2O emissions), resource production (inputs like fuel, electricity, seeds) and anthropogenic

CO_2 emissions (fuel combustion, fertiliser decomposition), and are thus partly related to the production of on- and off-farm feed. Therefore, increases in efficiency, i.e. low DML and emissions, during silage storage lead to a leverage effect to reduce the GHG emissions mentioned.

The data presented in Study 3 allow the proportion of climate-relevant silage emissions in the CF of FPCM. However, these are the results of a single experiment conducted under specific conditions. Thus, generalisation to other conditions must be made with caution. Nevertheless, for an initial approach, a feed intake of 1,000 ± 302 (g_{FM} maize silage) (kg FPCM)$^{-1}$ and a CF of 1,330 (g CO_2eq) (kg FPCM)$^{-1}$ were assumed based on the results of Cortés et al. (2021). Thus, 0.023–0.056 (g CO_2eq) (kg FPCM)$^{-1}$ were emitted assuming a silage DM of 33.3% and the CH_4 and N_2O emissions during silage storage (Studies 2 and 3). This equates to 0.002%–0.004% of the specified CF. Consequently, the shares can be considered negligible. The future research and management effort to minimise CH_4 and N_2O emissions during silage storage may be only limited worth pursuing. If the climate-irrelevant CO_2 emissions were also considered, the shares would increase by a multiple to 0.469%–0.660% in the best-case and 2.747%–7.291% in the worst-case scenario of AEMP1 (AEMP2: 0.371%–0.553% and 0.451%–4.684%, respectively). Thus, avoidable CO_2 emissions during aerobic deterioration can have a noticeably impact. Looking at the differences in magnitude between direct and indirect CO_2eq emissions from silage production (Studies 2 and 3), it is clear that it is eminently important to reduce indirect emissions. The proportion of indirect and direct CO_2 emissions associated with silage provision is estimated to be 4.2%–4.3% in the best-case scenario and 4.6%–5.2% in the worst-case scenario of AEMP1 (AEMP2: 4.2%–4.4% and 4.2%–4.8%, respectively). However, it should be noted that the total CF mentioned by Cortés et al. (2021) already includes an estimation of the CF associated with on-farm and off-farm feed provision. It is therefore reasonable to assume that the actual proportion may be somewhat higher. Nevertheless, the data demonstrate only minor discrepancies between the best-case and the worst-case scenarios. Despite this, it is more crucial to reduce the DML than to reduce CO_2eq emissions during silage storage. This is often difficult in the practical context, but the mentioned best practice principles or SA use may help if applicable.

The conservation method affects the characteristics of preserved feed and the animals' feed intake or performance parameters. Silage and hay may differ when using the same parent plant material (Böttger et al., 2019; Haselmann et al., 2020; Wilkinson et al., 2003). Some studies have also reported that SA use result in a quality increase. However, an overview of previous studies by Yitbarek and Tamir (2014) indicates that any effects were not consistently observed in all trials. The same applies to experiences in commercial farming, some of which report positive effects of SA on, for instance, silage digestibility, maintenance of protein quality or animal performance.

However, generalised proof of these effects is often difficult due to the multifactorial interactions. At the same time, SA contribute to quality preservation by protecting against deterioration. Spoilt material negatively influences the preference and quantity of feed intake (e.g. Brüning et al., 2018a; Gerlach et al., 2013). Malfermentation can also lead to similar effects (Gerlach et al., 2014). Thus, it can be argued that SA can also contribute to beneficial outcomes (Keady and Murphy, 1998; Rossi et al., 2023). SA use may help to regulate the otherwise uncontrolled spontaneous fermentation process (Pahlow et al., 2003) and to minimise the risk of silage deterioration. Furthermore, a higher quality of the silage may influence the subsequent design of the feed diet (e.g. substitution with concentrate feeds), the contribution of the individual components and dietary characteristics to enteric methane formation (e.g. Åby et al., 2019; Beauchemin et al., 2008; Gerber et al., 2013; Kebreab et al., 2023) or the total CF of the diet (cf. Díaz de Otálora et al., 2024; Wilkinson and Garnsworthy, 2021, 2017). Nevertheless, a comprehensive analysis of these factors is beyond the scope of this thesis, given their impact on a multitude of other variables, including rumen health, animal health and welfare, nutrient excretion, and milk composition.

Suppose the focus is broadened from carbon to the environmental footprint. In that case, Rotz et al. (2021) report that 67% of total fossil energy consumption and 97% of blue (ground and surface) water consumption are connected to feed provision at US dairy farms. At this point, supplementing ecological and economically costly off-farm feed with high-quality silage may be beneficial. Therefore, improving resource retention during the process chain of silage provision is a crucial step to enhance ecological efficiency. A similar issue is reported by Bacenetti and Fusi (2015), showing noticeable sensitivity of, for instance, particulate matter emissions or eutrophication effects of varying ensiling techniques: the impact is directly proportional to DML. In this subject, silo bags seem to be promising compared to bunker silos showing lower losses (Bacenetti and Fusi, 2015; Randby and Bakken, 2021). However, implementing practices has to be discussed case-by-case for each farm or region. Ensiling in silage bags is also promising based on the reduced concrete work compared to bunker silos (Bacenetti and Fusi, 2015). However, German legislation concerning blue water protection, for instance, requires sealed surfaces for most silos (cf. Asen et al., 2019; AwSV, 2020; DWA-A 792, 2018; WHG, 2023). This is linked to further concrete work, and the associated environmental impacts must be considered in the comparisons. Moreover, the ecological burdens of conventional plastic films (cf. Kono, 2023; Levitan and Barros, 2003) have to be considered but may be improved by progress in the challenging recycling processes (Kono, 2023; Kyrikou and Briassoulis, 2007) or by using biodegradable bio-plastics (Tabacco et al., 2020). These issues show that the ecological assessment of strategic and operational decision-making to improve the environmental footprint is complex

and potentially contradictory. Furthermore, solutions must be economical for long-term success and sustainability (cf. Rotz et al., 2021). Other environmental impacts of crop cultivation and silage productionshould be considered, although they fall outside the scope of this thesis. These include – with particular reference to the case of maize production – the impact on biodiversity of flora and fauna (e.g. Norris et al., 2016; Sauerbrei et al., 2014; Schulz et al., 2020), erosion potential (e.g. Mann et al., 2002; Vogel et al., 2016), C losses of ecosystems (e.g. Gamble et al., 2021) or external costs of crop production (e.g. Li et al., 2023).

Previous research enhances the knowledge of the resource efficiency in silage production. It highlights the importance of DML in reducing the ecological footprint. The methodology used in Studies 2 and 3 could be also applied to investigate grass silage. Grass silage production can be essential for utilising farmland, which is unsuitable for crop production, as forage grassland to produce feed and subsequent human-digestible food. However, the composition of grasslands can vary greatly, which complicates emission research, farm management and hence mitigation recommendations. However, knowledge gained from past and future research can be compared with other conservation methods, such as hay production. Thus, assuming the same CO_2 fixation and CO_2eq emissions during parent plant cultivation and growth, both methods can be evaluated in terms of their resource retention efficiency and CF. The individual steps of the process technology during harvesting, e.g. conditioning and tedding, may differ, leading to increased losses or machinery use and CO_2eq emissions in hay production (cf. Collins and Moore, 2017; Wilkinson, 2015). Aerobic respiration during the varying wilting periods may affect DML and resource retention (cf. Collins, 1995; Collins and Moore, 2017; Muck et al., 2003). Respiratory CO_2 emissions could be calculated based on DML (cf. Study 3; Milimonka et al., 2019). While CH_4 and N_2O should not be formed during aerobic wilting, grass cultivation and hay drying can be associated with VOC emissions, as reviewed by Hafner et al. (2018). Subsequently, GHG and VOC emission quantities can be compared between the conservation methods (cf. Emery, 2013), considering the feed values of the final products. From this, it can be deduced whether transferring C-based parent plant biomass to the animal is more ecologically efficient in silage or other preservation forms such as hay production. Even if the CO_2 emissions are climate-neutral per se, it seems sensible that the harvested parent plant crop – which bound a certain amount of CO_2 – is converted into animal feed with minimal CO_2 emissions. Any differences between the conservation methods should be considered if the feed is to have the optimum net CO_2 balance. This could be a further evaluation aspect in future agriculture if the environmental impact of diets (cf. Wilkinson and Garnsworthy, 2021) is included in utility value analyses. Any synergies or conflicting economic, nutritional, or animal welfare objectives must be discussed.

6.3 Outlook

Based on the information presented, some focus areas for future action can be derived for the various stakeholders.

Scientists should conduct research in two directions. First, the understanding of gas formation and the role of different microbial species needs to be further investigated. However, due to the multifactorial influences on gas formation (Studies 1–3), applied microbiology should be supported by other disciplines. These include physics to provide appropriate sensor systems, or economics to assess minimisation of emissions and losses for economic viability. All disciplinary approaches need to be implemented in the context of the silage production process chain in collaboration with the agricultural sciences. Secondly, knowledge transfer between research and practice needs to be encouraged. Priority should be given to the further development of measurement set-ups, such as those designed to simulate varying feed-out rates and measurements on commercial silos. As outlined above (Section 6.2.2), the measures described to minimise DML and losses are already known from best practice principles. It is important to bring this knowledge back to the forefront of farmers' minds. A sharpened critical eye and guidance on recommended actions (see Fig. 6.3) can improve decision-making during harvesting and ensiling. In addition, sensors and measurement methods should be developed to support decision-making. For example, a sensor assessing the silage density would help to regulate the amount of compaction required.

Reducing emissions from silage provision is a desirable goal from an environmental and climate change perspective. However, this goal is more important to policymakers and society than to individual farmers. Unless they are given a business-relevant (economic) reason to act, they can and will prioritise other objectives, such as minimising harvester and silo costs. Financial incentives, such as extra income for implementing measures or penalties for high DML, could be effective in ensuring that reducing emissions is financially beneficial.

Farmers should always implement best practice principles to the best of their ability. In addition, SA should be used consistently from an environmental point of view. According to current knowledge (Study 3), these improve the CF of the silage. In addition, other positive effects on animal husbandry are possible, e.g. the feed value of the silage or an increase in animal performance. The short period of time from harvest to silo sealing influences the course of fermentation and ASTA. These in turn affect the quality and emission behaviour. All possible measures should therefore be taken during the short window of opportunity to control spontaneous fermentation as far as possible. This is the basis for high forage quality, low silage DML and increased profitability throughout the year.

6.4 Conclusions

During silage production, climate and environmental relevant gases are formed, including CO_2, CH_4, N_2O, CO, NO, ethanol, and ethyl acetate. This thesis provides new insights into silage emission research. For this purpose, extensive experiments were carried out which set new standards in terms of the investigation period, the intervals between the individual measurement times and the gas analysis. In addition, the emissions of continuous silage material were recorded without interruption from silo closure to aerobic spoilage for the first time. This made it possible to balance the emissions during the 12 newly defined phases of gas emission production during silage provision (in particular phases of anaerobic fermentation and aerobic deterioration). The extensive data sets provided new insights into this interdisciplinary field of research, as the emission sources of silage production are diverse.

The direct emissions of climate-relevant substances (especially CH_4 and N_2O) during the storage of silage have almost no impact on the carbon footprint of agricultural products such as milk. It is more important to minimise dry matter losses during silage production and in particular during silage storage periods. Thus, malfermentation by clostridia and aerobic deterioration by yeasts and moulds should be avoided. This leads to efficient utilisation of the harvested crop and reduces indirect emissions.

The use of case-specific silage additives can reduce the risk of increased dry matter losses. However, they are not a mandatory requirement, as the emission behaviour of silage is not positively influenced if it is managed appropriately. Nevertheless, if malfermentation or aerobic deterioration is avoided, additives improve the carbon footprint of silage production. Additives can therefore be considered an essential part of the management and decision-making process for silage production, particularly in cases where harvesting conditions are difficult. Silage additives should thus be used more regularly in global silage production. However, it is essential that best practice principles are implemented prior to their use. Ad-hoc decisions need to be made on a case-by-case basis. In this thesis, decision-making tools are presented for specific scenarios.

Further research is required to reduce dry matter losses and climate and environmental relevant emissions. More interdisciplinary collaboration is needed to quantify and evaluate the effects of the various topics on silage emissions more comprehensively. These include, among others: mechanical engineering, harvest and storage chain management, silage material science, plant botany, applied microbiology and biochemistry, animal nutrition and agricultural practice.

This thesis indicates potential avenues. Consequently, the environmental impact of silage can be reduced as a vital component in modern agriculture, in accordance with the 12th United Nations sustainable development goal ‘Sustainable consumption and production’.

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