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Chopped *Miscanthus* biomass for soil organic matter build-up and evaluation of nitrogen and carbon dynamics in arable farming systems

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Abstract

Arable farming practices affect soil C and N dynamics and thus have a crucial impact on global climate change and on aquatic and terrestrial ecosystems. Soil nutrient cycling is driven by soil microbial biomass, making knowledge of C and N pathways a key tool for the sustainable management of cropping systems. The cultivation of the perennial plant *Miscanthus x giganteus* (*Mis*) combines the benefits of ecosystem services for each cultivated area with the production of an additional carbon pool, that is used as a fertiliser for soil C sequestration, thus acting as a C sink. The hypothesis of this thesis is that the integration of C-rich *Mis* biomass into arable management strategies will stimulate soil microbial biomass (SMB) resulting in microbial N immobilisation and soil organic matter (SOM) build-up. Therefore, two different N-containing and C-rich agricultural fertilisers were produced from chopped *Mis* biomass. One was produced by mixing cattle slurry (CS) with *Mis* biomass (CS-*Mis*) and the other by using chopped *Mis* biomass as a bedding material for cattle for the production of cattle manure (CM-*Mis*). Both were then implemented in experiments conducted under greenhouse conditions by cultivation of perennial ryegrass (*Lolium perenne* L.) and under field conditions in a crop rotation with winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris* L.) and winter wheat (*Triticum aestivum* L.). The other treatments tested were a mixture of CS and wheat straw (CS-WS) and a cattle manure from shredded WS bedding (CM-WS) to test WS as a common biomass. A pure CS was also tested as a reference treatment for the two mixtures (CS-*Mis*, CS-WS). The influence of the above mentioned fertilisers on soil (soil inorganic N, soil microbial biomass C and N) and plant parameters (plant N uptake, yield and quality parameters) were continuously determined during the experiments by standard soil and plant analyses. The results indicated that SMB make use of *Mis* as a C source. *Mis* biomass contributes to SMB build-up and thus C sequestration at least as WS. This also resulted in N immobilisation, which had a mostly negative impact on yield and quality parameters and led to a reduction in nitrate leaching. In addition, N uptake was determined using a drone-based sensor and the implementation of digital elements in arable farming was assessed. With increasing knowledge of C and N fluxes and the metabolism of SMB, there is great potential to improve the sustainability of arable farming through their management. *Mis* biomass can be used as a tool for sustainable C and N management in arable farming systems.

Kurzfassung

Die Art der ackerbaulichen Bewirtschaftung hat einen erheblichen Einfluss auf die C- und N-Dynamik im Boden. Damit spielt sie eine wichtige Rolle für den globalen Klimawandel sowie für aquatische und terrestrische Ökosysteme. Da die Nährstoffkreisläufe durch die Bodenmikroorganismen (SMB) gesteuert werden, ist das Verständnis ihrer Stoffwechselprozesse ein wesentliches Instrument für die nachhaltige Ausrichtung von Anbausystemen. Der Anbau der mehrjährigen Pflanze *Miscanthus x giganteus* (*Mis*) verbindet die Bereitstellung von Ökosystemdienstleistungen auf den jeweiligen Anbauflächen mit der Erzeugung einer zusätzlichen C-Senke. In dieser Arbeit wird die Hypothese aufgestellt, dass die Integration von C-reicher *Mis*-Biomasse in ackerbauliche Bewirtschaftungsstrategien mikrobielle Umsetzungsprozesse gezielt fördert und dadurch eine mikrobielle N-Immobilisierung und einen Aufbau organischer Bodensubstanz (SOM) induziert. Um dies herauszufinden, wurde gehäckselter *Mis* zu zwei C- und N-reichen Düngern verarbeitet. Zum einen wurde Rindergülle (CS) mit *Mis*-Biomasse gemischt (CS-*Mis*), zum anderen wurde *Mis*-Biomasse als Einstreumaterial für Rinder verwendet, um einen Rindermist aus *Mis*-Einstreu (CM-*Mis*) zu erhalten. Beide wurden in Versuchen unter Gewächshaus- und Freilandbedingungen eingesetzt. Zusätzlich wurden eine Mischung aus CS und Weizenstroh (CS-WS) und ein Rindermist aus Weizenstroh-Einstreu (CM-WS) verwendet, um Weizenstroh (WS) als klassische Biomasse zu testen. Der Einfluss auf Boden- (anorganischer N, mikrobieller Boden-C und -N) und Pflanzenparameter (pflanzliche N-Aufnahme, Ertrags- und Qualitätsparameter) wurde mittels Standard-Boden- und -Pflanzenanalysen bestimmt. Die Ergebnisse zeigten, dass die SMB *Mis*-Biomasse als C-Quelle nutzt und mindestens in gleichem Maße zur Bildung von SOM und damit zur C-Sequestrierung beiträgt wie WS. Dadurch wurde ebenfalls eine N-Immobilisierung hervorgerufen, wodurch zum einen die Ertrags- und Qualitätsparameter negativ beeinflusst wurden und zum anderen auf eine Verringerung der Nitratauswaschung geschlossen werden konnte. Zusätzlich wurde der pflanzliche N-Gehalt mit einem Drohnensensor bestimmt und der Einsatz digitaler Elemente im Ackerbau bewertet. Mit zunehmenden Erkenntnissen der C- und N-Flüsse sowie des Metabolismus der SMB besteht ein großes Potenzial, durch deren Management die Nachhaltigkeit im Ackerbau zu erhöhen. *Mis* kann als Instrument für ein nachhaltiges C- und N-Management in Ackerbausystemen eingesetzt werden.

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List of publications

1. Stotter, M.; Wichern, F.; Pude, R.; Hamer, M. Nitrogen Immobilisation and Microbial Biomass Build-Up Induced by *Miscanthus x giganteus* L. Based Fertilisers. Agronomy **2021**, 11(7), 1386. https://doi.org/10.3390/agronomy11071386.

   The published article is reproduced in chapter 2 of the dissertation.

2. Stotter, M.; Wichern, F.; Pude, R.; Hamer, M. Utilisation of *Miscanthus x giganteus* L. Based C-Rich Fertilisers for N Immobilisation and Microbial Biomass Build-Up in a Crop Rotation. Agronomy **2021**, 11(12), 2390. https://doi.org/10.3390/agronomy11122390.

   The published article is reproduced in chapter 3 of the dissertation.

3. Stotter, M.; Hamer, M.; Bohnenkamp, D.; Behmann, J.; Leitenberger, K. und Pude, R., **2019**: Hyperspectral assessment of winter barley, after the fertilisation of different farm manures, based on chopped-*Miscanthus*, at the time of full maturity. Bornimer Agrartechnische Berichte 102, 151-157.

   The published article is reproduced in chapter 4 of the dissertation.

4. Swoboda, P.; Hamer, M.; Stotter, M.; Döring, T.F.; Trimborn, M. Effects of Rock Powder Additions to Cattle Slurry on Ammonia and Greenhouse Gas Emissions. Atmosphere **2021**, 12, 1652. https://doi.org/10.3390/atmos12121652.

5. Stotter, M.; Hamer, M.; Leitenberger, K.; Völkering, G. und Pude, R., **2019**: Effizienter N-Einsatz: N-Dynamik bei Anwendung von *Miscanthus*-Mist in einer zweigliedrigen Fruchtfolge. „Bioökonomie – aber nicht ohne *Miscanthus*“ 10, 77-84.

6. Stotter, M; Hamer, M.; Leitenberger, K.; Völkering, G. und Pude, R., **2019**: Erfassung der N-Dynamik verschiedener Wirtschaftsdünger in Wintergerste und Senf im Dürrejahr 2018. Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften 31, 198-199.

7. Stotter, M.; Hamer, M.; Antonini, S.; Leitenberger, K.; Völkering, G. und Pude, R., **2018:** N-Mineralisierungskinetik von Wirtschaftsdüngern basierend auf *Miscanthus*-Häckselgut. Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften 30, 140-141.

8. Stotter, M.; Hamer, M.; Leitenberger, K., Völkering, G. und Pude, R., **2017**: Nutzungsmöglichkeiten innerbetrieblich anfallender Wirtschaftsdünger mit *Miscanthus*. Bioökonomische Anwendungs- und Forschungsfelder bei *Miscanthus* 9, 33-41.

9. Stotter, M.; Hamer, M.; Völkering, G.; und Pude, R., **2017**: Eigenschaften eines streufähigen Substrates bestehend aus flüssigen Wirtschaftsdüngern und *Miscanthus*. Mitteilungen der Gesellschaft für Pflanzenbauwissenschaften 29, 170-171.

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B	Boron
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
CM-*Mis*	Cattle Manure from *Miscanthus* shredded bedding
CM-WS	Cattle Manure from Wheat Straw bedding
CS	Cattle Slurry
CS-*Mis*	Cattle Slurry with *Miscanthus* addition
CS-WS	Cattle Slurry with Wheat Straw addition
Cu	Copper
CUE	Carbon Use Efficiency
e.g.	exempli gratia – for example
et al.	et alia – and others
EU	European Union
Fe	Ferrum
ha	Hectare
K_2O	Potassium oxide
L	Litre
MB	Microbial Biomass
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
mg	Milligram
Mg	Megagram
Mis	*Miscanthus x giganteus*
N	Nitrogen
ND	Normalized Difference
NH_3	Ammonia

NH_4^+	Ammonium
N_2O	Nitrous oxide
NO_3^-	Nitrate
NoN	No Nitrogen applied
NUE	Nitrogen Use Efficiency
P_2O_5	Phosphorpentoxid
PSRI	Plant senescence reflectance index
SMB	Soil Microbial Biomass
SOC	Soil organic carbon
UAN	Urea Ammonium Nitrate Solution
UN	United Nations
WS	Wheat Straw
Yr	Year

1 Introduction

1.1 Problems of intensive arable farming

Feeding a growing world population, which according to the United Nations (2019) will reach 10.9 billion people by 2100, required a large increase in agricultural food production, which was made possible by the Green Revolution (Evenson and Golling, 2003; United Nations, 2019). The Green Revolution is characterised by the introduction of high-yielding and disease-resistant crops, the development of plant protection products and the synthesis of mineral fertilisers using the Haber-Bosch process (Evenson and Golling, 2003). Other technological developments and economic conditions (agricultural subsidies, world market trade) have reduced agricultural production costs in recent decades, thereby increasing food availability (Tilman et al., 2011; United Nations, 2019). However, the intensification of agricultural production methods, the expansion of agricultural land and the low-cost access to production tools such as N fertilisers and pesticides are leading to imbalances in various ecosystems and also to negative impacts on human health (Tilman et al., 2002; Schrijver et al., 2011; Tilman et al., 2011; Lu and Tian, 2017). In addition to the Green Revolution, industrialisation has led to an increase in global emissions of CO_2, N_2O and CH_4, which has changed the global climate (Seneviratne et al., 2016; Yoro and Daramola, 2020). Extreme weather conditions such as heavy precipitation, storms and droughts will continue to intensify, requiring new adaptation strategies aimed at implementing resilient crop production (Kang et al., 2009; Yoro and Daramola, 2020; Masson-Delmotte et al., 2021).

Soil organic carbon (SOC) is an important source and sink of CO_2 (Lal, 2004; Scharlemann et al., 2014). The type of land use has a major impact on soil C and N dynamics and thus plays an important role in global climate change (Tilman et al., 2002; Lal, 2004; Tilman et al., 2011; Masson-Delmotte et al., 2021). Inadequate soil management in arable farming and conversion of grassland to cropland often lead to SOC degradation, reducing soil fertility, while SOC degradation contributes to CO_2 emissions to the atmosphere (Goidts and van Wesemael, 2007; Steinmann et al., 2016a; Steinmann et al., 2016b). Furthermore, the risk of NH_3 emissions with toxic effects on mammalian and human respiratory system and N_2O emissions, which is a potent greenhouse gas, increases with increased N inputs (Canfield et al., 2010; Hietz et al., 2011; Nacry et al., 2013).

In many cases, inadequate soil management in arable farming leads to soil erosion with negative effects on crop production, which is compensated, for example, by increased fertilisation, often with negative effects on the environment (Naylor, 1996; Tilman et al., 2002). Together with agricultural intensification, increased nitrogen (N) use leads to lower N use efficiency (NUE), N accumulation in soils and nitrate leaching to ground and surface waters, resulting in eutrophication (Galloway et al., 2004; Diaz and Rosenberg, 2008; Bouwman et al., 2013). Particularly at the end of the vegetation period, soil inorganic N can be leached into deeper soil layers with the onset of autumn rains, where it cannot be reached by plant roots. When organic fertilisers are applied, not all of the applied N is available to plants in the year of application. Some organic N remains in the soil and inorganic N is slowly released by microbial mineralisation processes in the months and years following the N application. If this is insufficiently taken into account or underestimated, and if it does not match with the plant N requirement, losses in the form of reactive N compounds to aquatic or terrestrial ecosystems may occur (Daudén et al., 2004; Sørensen, 2004; Sørensen and Thomsen, 2005). In Europe, inappropriate N fertilisation has led to nitrate concentrations in groundwater bodies exceeding the EU limit of 50 mg L^{-1} in some regions (Eurostat, 2012). For the reduction of nitrate concentrations below the critical value, regulations on fertilisation have been tightened in some European countries.

In the context of the negative effects of structural change and intensification in arable farming (SOC degradation, N inputs into ecosystems, loss of biodiversity), as well as the negative effects of increasing climate change (more extreme weather conditions), there is a need for integrated approaches and novel strategies in crop production. These aim to minimise the environmental impact of arable farming and to maintain soil fertility for the protection of ecosystems and sustainable food production based on resilient arable soils. The cultivation of *Miscanthus* (*Mis*) can contribute to manage some of the above challenges by providing numerous ecosystem services (Emmerling and Pude, 2017). Cultivation provides overwintering opportunities for plant beneficial insects, which can provide natural pest control during the vegetation period. By cultivation in stripes along hedge structures, *Mis* can extend and enhance wildlife habitat, act as a stepping stone biotope when planted in the open landscape, and contribute to the restoration of biotope cross-linking (Semere and Slater, 2007; Bellamy et al., 2009). It can be cultivated on marginal sites where cultivation of other crops is not economically viable and can be cultivated as a low-input crop due to low fertiliser requirements and no need for weed control (Emmerling and Pude, 2017). Perennial crops such as *Mis* can protect soil from erosion and contribute to CO_2 storage, accumulating about 1.1 Mg C ha^{-1} yr^{-1}, similar to permanent grassland (Schneckenberger and Kuzyakov, 2007; Felten and Emmerling, 2012; Zang et al., 2018). Harvesting of *Mis* usually takes place in spring, just before new shoots appear. Consequently, *Mis* habitat provides opportunities for rare wildlife to protect themselves from predators and weather conditions during the winter months and increases structural diversity in open agricultural landscapes.

However, these contributions to a more sustainable agriculture will only be effective if *Mis* is actually cultivated. For this purpose, there is a need for utilisation options and thus market opportunities (Pude, 2021). These includes usage as a raw material in anaerobic digestion (Ruf and Emmerling, 2017; Schmidt et al., 2018; Mangold et al., 2019b; Mangold et al., 2019a), as a growing medium in soilless cultivation (Nguyen et al., 2021), cascaded use in livestock farms as bedding material (van Weyenberg et al., 2015; Winkler et al., 2020; Yesufu et al., 2020) or as an additive for the packaging industry or as a construction or building material (Moll et al., 2020; Pude, 2021). However, these potential utilisation pathways are currently rarely requested by industry, which will only invest in specific processing technologies once a reliable supply of *Mis* biomass from agriculture has been established over many years. In turn, the farmer will not invest in establishing a *Mis* crop once he knows that he will be able to purchase it for several years. This lack of coordination between farmers and industry has prevented the establishment of a large market as well. In contrast, the establishment of an on-farm utilisation option for *Mis* biomass provides farmers an independent ability to plan for their own requirements and to be independent of external resources. However, this requires the identification of potential benefits of on-farm utilisation of *Mis*.

1.2 Research design

1.2.1 Research questions

The specific characteristics of *Mis* biomass with a higher C/N ratio compared to cereal straw ((*Miscanthus*: 166/1 to 288/1; wheat straw: 73/1 to 137/1) (Stotter et al., 2021b, 2021a)) indicate the possibility of producing a C-rich fertiliser on the farm, which could contribute to manage some of the above-mentioned challenges (chapter 1.1) such as reducing N losses and increasing soil fertility. Therefore, in this thesis, two different N-containing and C-rich agricultural fertilisers were produced on the basis of chopped *Mis* biomass to test their effects on N- and C-dynamics in arable farming systems. One of the fertilisers was produced by mixing *Mis* biomass with cattle slurry and the other by using *Mis* biomass as a bedding material for cattle, resulting in a *Mis*-based manure.

These two organic fertilisers were then implemented in experiments of this thesis to answer the overall research question whether *Mis* biomass can be used in a similar way to wheat straw for microbial N immobilisation and SOM build-up. In this context, on the one hand, experiments were conducted under greenhouse conditions by cultivation of German ryegrass (*Lolium perenne* L., Valerio) and, on the other hand, under field conditions in a crop rotation of winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris* L.) and winter wheat (*Triticum aestivum* L.). During these experiments, soil parameters (soil inorganic N, soil microbial biomass C and N) and plant parameters (plant N uptake, yield and quality parameter) were continuously determined by standard soil and plant analyses. It was determined whether *Mis* biomass was as effective as wheat straw in reducing N losses in the form of nitrate leaching. In addition, the effect on crop yield and quality parameters was determined. To investigate the effect of the use of *Mis* biomass on SOM build-up, it was tested whether the microbial biomass make use of *Mis* as a C source for biomass build-up (Fig. 1). The dynamics of N mineralisation and N immobilisation of organic fertilisers depend on a large number of influencing factors and are therefore often difficult to estimate. In order to ensure a demand orientated N-supply of crops, a timely determination of the N-supply is required to adjust the N-fertilisation strategy if necessary. Therefore, in this thesis, the drone-based determination of N uptake was investigated and the the implementation of digital farming as an element of sustainable C and N management was evaluated.

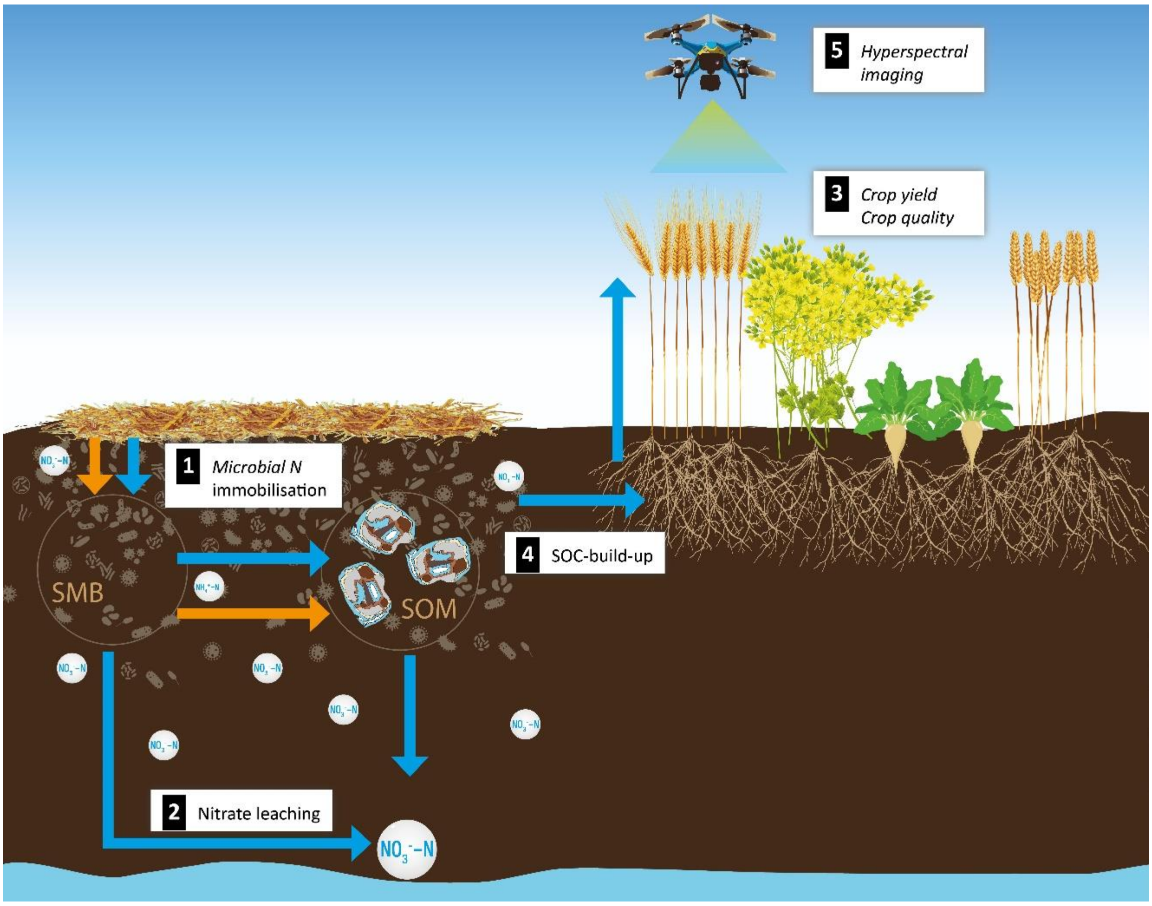

Figure 1: Schematic overview of this PhD thesis, which contains the application of organic fertilisers, based on chopped *Mis* biomass. Coloured arrows identicate the C (orange arrows) and N (blue arrows) fluxes detected and discussed in this thesis. The numbered headwords mark the five hypotheses.

The thesis focuses on the following five hypotheses, which are investigated by three greenhouse experiments and a field experiment:

1) *Miscanthus* is as good as wheat straw in immobilising additional inorganic N from mineralisation of slurry or manure (Paper 1, 2).
2) *Miscanthus* reduces nitrate leaching as effective as wheat straw (Paper 2).
3) *Miscanthus* and wheat straw are identical in affecting yield and quality parameters of crops of a crop rotation (Paper 2).
4) Microbial biomass makes use of *Miscanthus* as a C source for biomass build-up and thus contribute to C sequestration (Paper 1, 2).
5) Drone-based determination of N uptake of winter barley by hyperspectral imaging represents an alternative to destructive measurement (Paper 3).

1.2.2 Outline of the thesis

In order to answer the hypotheses listed in chapter 1.3.1, two main experiments were performed: A pot experiment under controlled conditions in the greenhouse and a 35-month field experiment using a crop rotation typical for the Rhine region. The outline of the thesis is structured as follows:

In **chapter 2**, hypotheses one and four have been worked on (1: *Miscanthus* is as good as wheat straw in immobilising additional inorganic N from mineralisation of slurry or manure; 4: Microbial biomass make use of *Miscanthus* as a C source for biomass build-up and thus contribute to C sequestration). Therefore, two organic farm fertilisers were tested, each containing *Mis* biomass as an amendment. These were a cattle slurry mixed with *Mis* and a cattle manure from *Mis* bedding material. As complementary treatments, cattle slurry mixed with wheat straw and cattle manure from wheat straw bedding were tested. This chapter answers the hypotheses by considering that the experiment was performed in pots under controlled conditions in the greenhouse. The soil used in the experiment was the same as that used in the field experiment. To validate the experimental results, the greenhouse experiment was repeated three times, each time with a different starting time.

In **chapter 3**, hypotheses one to four have been worked on (1: *Miscanthus* is as good as wheat straw in immobilising additional inorganic N from mineralisation of slurry or manure; 2: *Miscanthus* reduces nitrate leaching as effective as wheat straw; 3: *Miscanthus* and wheat straw are identical in affecting yield and quality parameters of crops of a crop rotation 4: Microbial biomass make use of *Miscanthus* as a C source for biomass build-up and thus contribute to C sequestration). Therefore, a field trial with a typical Rhenish crop rotation of winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris* L.) and winter wheat (*Triticum aestivum* L.) was set up, testing the same treatments of the pot experiments. The effects of the fertilisers on SOM, on N dynamics and on yield and quality parameters were determined over a period of 35 months. In order to keep the influence of the existing soil chemical and physical parameters on the measured parameters as identical as possible, an agricultural area with soil characteristics as homogeneous as possible was selected. The soil scanner EM38 was used for the positioning of the experiment.

In **chapter 4**, hypothesis five has been worked on (5: Drone-based determination of N uptake of winter barley by hyperspectral imaging represents an alternative to destructive measurement). Therefore, a DJI Matrice 600 drone with an attached Rikola hyperspectral camera (Senop, Finland) was used in the field experiment at the time of full maturity. The reflectance of winter barley fertilised with different nitrogen fertilisers was mapped. Calculation of the Plant Senescence Reflectance Index (PSRI; Merzlyak et al., 1999) and Normalized Difference 800/680 (ND 800/680; Tucker, 1979) were used for interpretation of destructively detected N uptake by aboveground barley biomass.

The dynamics of N mineralisation and N immobilisation of organic fertilisers are driven by SMB, which is affected by a wide range of factors such as soil chemical and physical properties, climate and fertiliser characteristics. Real-time determination of N supply is required to provide a demand-based N supply to crops in arable farming systems. Therefore, this thesis investigates the drone-based determination of N uptake to validate the implementation of a drone-based sensor as an element of sustainable N management.

1.3 State of knowledge

1.3.1 Management of SOC in arable farming

Global climate change is mainly driven by increasing CO_2 and N_2O emissions in recent decades, which have increased and will continue to increase extreme weather conditions such as heavy precipitation, storms and droughts (Masson-Delmotte et al., 2021). The importance of soil as a sink and source of C compounds and as a source of N_2O is large (Smith et al., 2000; Lal, 2004; Smith et al., 2013). Soils with site-specific levels of SOC are more resilient to extreme weather conditions than soils with lower levels of SOC (Drexler et al., 2020). Therefore, the importance of the development and content of SOC in agricultural used soils has received particular attention in recent years (Jacobs et al., 2018). In many cases, the development of SOC content is correlated with past land use changes (e. g. degradation of organic matter after grassland conversion) (Steinmann et al., 2016b) or by management practices (e. g. application of organic or mineral fertilisers over many years, plant residue management) (Bamminger et al., 2019). A decrease of SOC content due to climate change was not observed in NRW, Germany (Bamminger et al., 2019). In contrast, other studies from other regions show a correlation between SOC degradation and increasing temperatures (Bellamy et al., 2005; Koven et al., 2017; Wiesmeier et al., 2019). However, there is no general agreement on this and the development of SOC content has to be considered and evaluated site-specifically (Scharlemann et al., 2014; Bamminger et al., 2019; Drexler et al., 2020). It is generally acknowledged that increasing of SOC can improve several yield-stabilizing, chemical, physical and biological soil parameters up to a site-specific maximum level of SOC (Gregorich et al., 1994; Johnston et al., 2009; Reeves, 1997; Carter, 2002; La Paz Jimenez et al., 2002; Bot and Benites, 2005). Soils with low organic matter inputs from organic fertilisers or crop residues, tend to have lower SOC contents (Post and Kwon, 2000; Loveland and Webb, 2003; Houghton, 2012; Bamminger et al., 2019) than soils that have been managed for many years to increase SOC (Steinmann et al., 2016b; Bamminger et al., 2019; Drexler et al., 2020).

The potential to increase SOC content is particularly high on soils with low initial SOC content (Bamminger et al., 2019). Appropriate C management can reduce soil C losses (Smith et al., 2013), and increasing organic matter inputs can increase nutrient buffer capacity in the medium term and stimulate soil microbial processes (Blanco-Canqui and Lal, 2009; Šimon et al., 2016; Reichel et al., 2018; Cao et al., 2021; Stotter et al., 2021b, 2021a). The potential for nutrient losses to aquatic and terrestrial ecosystems is thus reduced and consequently nutrient use efficiency may be increased (Chen et al., 2014; Reichel et al., 2018; Cao et al., 2021). However, there is no linear increase in SOC with increased C input (Heitkamp et al., 2012). Nevertheless, there are several approaches to increase the soil fertility, nutrient use efficiency and SMB (Kallenbach and Grandy, 2011) by increasing SOC using biochar (Lehmann et al., 2006; Chan et al., 2007; Haider et al., 2015; Haider et al., 2017), hydrochar (Gajić and Koch, 2012; George et al., 2012; Bargmann et al., 2014a; Bargmann et al., 2014b, 2014c) or sawdust and cereal straw (Reichel et al., 2018; van Duijnen et al., 2018; Dundore-Arias et al., 2019; Wei et al., 2020; Cao et al., 2021; Kamau et al., 2021) as soil amendments.

Due to the higher C/N ratio of *Mis* compared to wheat straw (*Mis*: 166 to 288; wheat straw: 73 to 137) (Stotter et al., 2021b, 2021a), the possibility of on-farm cultivation and the provision of numerous ecosystem services (Emmerling and Pude, 2017; Pude, 2021), this thesis investigates the contribution of *Mis* biomass to SOC sequestration, the stimulation of SMB and its suitability for reducing of nitrate leaching. Other nutrients are essential to maximise the enhancement of soil microorganisms. By adding cattle slurry to chopped *Mis* biomass or by using *Mis* biomass as a bedding material, the microorganisms are provided a number of essential nutrients from the excreta. This can stimulate nutrient uptake into the microbial biomass.

1.3.2 Reduction of N losses using high carbon amendments

An increase in the intensity of agricultural production has mostly resulted in higher mineral and organic N fertiliser applications, with negative environmental impacts (Cameron et al., 1996; Di and Cameron, 2002; Yang et al., 2018). The continuous development of agricultural technology, precision farming, the adaption of N fertilisation to crop-specific N requirements with multiple N fertilisation rates as well as the cultivation of undersown crops and catch crops contribute to the reduction of N excesses and N losses (Cassman et al., 2002; Kanders et al., 2017; Abdalla et al., 2019; Rao et al., 2021). The type of farming also affects soil fertility and nutrient retention capacity (Ogle et al., 2005). Soils characterised by long-term organic matter removal and low organic input often have low SMB, low SOC content and low nutrient retention capacity (Loveland, P., and Webb, J., 2003; Goidts and van Wesemael, 2007;

Bamminger et al., 2019). However, as N mineralisation and N immobilisation processes are driven by SMB, there is great potential to prevent N leaching and contribute to plant N nutrition (Chen et al., 2014). Therefore, strategies to improve soil N retention are being intensively researched. It is well known that incorporation of cereal straw leads to N fixation (Shindo and Nishio, 2005; Chen et al., 2014). The specific addition of high carbon amendments (HCA) such as sawdust, cereals, maize, rice or sugar cane straw has received considerable scientific attention in recent years. First experimental results of the specific application indicate a high potential to increase SMB, SOC and nitrogen use efficiency (NUE) of N-fertilisers as well as to reduce nitrate leaching. By understanding the interactions between soil, microorganisms and plants, it is intended to optimise the usage of the variable N pool of soil microorganisms by using agronomic management tools (Kallenbach and Grandy, 2011; Congreves et al., 2013; Bargmann et al., 2014c, 2014b; Bargmann et al., 2014a; Haider et al., 2017; Reichel et al., 2018; van Duijnen et al., 2018; Dundore-Arias et al., 2019; Wei et al., 2020; Cao et al., 2021; Kamau et al., 2021; Stotter et al., 2021a). During the winter months, soil microorganisms can act as a sink to reduce nitrate leaching. Through soil microbial N mineralisation processes, soil microorganisms can also act as a source to manage plant N nutrition. The addition of easy available organic C compounds can rapidly reduce the soil inorganic N content because SMB can metabolise dissolved organic C compounds for inorganic N incorporation and thus use them for energy generation (Chen et al., 2014). The death of SMB, for example due to microbial predation and microbe-substrate interactions, can allow microbial biomass to remineralise incorporated N compounds (Zelenev et al., 2006). The chemical composition of HCAs affects microbial N immobilisation because N transformation processes depend on C availability (Rummel et al., 2020). A higher holocellulose to lignin ratio results in greater microbial N immobilisation because more available C are available for metabolism by SMB (Burke et al., 2013; Wei et al., 2020). A single addition of lignin did not result in microbial N immobilisation (Reichel et al., 2018). In addition to biotic N immobilisation, abiotic N immobilisation also occurs in soils. For example, NO_3^- can be fixed by the iron wheel mechanism (Davidson et al., 2003; Fitzhugh et al., 2003), NH_4^+ can be adsorbed onto the surface of phyllosilicates, such as clay minerals like montmorillonite or kaolinite and can bound by lignin derivatives (Schmidt-Rohr et al., 2004; Shindo and Nishio, 2005; Olk et al., 2006) or by SOM (Shindo and Nishio, 2005; Olk et al., 2006; Chen et al., 2014).

However, Wei et al. (2020) describe that after HCA addition, consisting of sawdust and wheat straw, biotic N immobilisation dominates and chemical N immobilisation is less important. Reichel et al. (2018) and van Duijen et al. (2018) showed a reduction of soil inorganic N after the addition of HCAs consisting of sawdust and wheat straw in an incubation experiment and in a 2-year field experiment. Higher microbial N immobilisation was observed with wheat straw than with sawdust. HCAs could be used to reduce high soil inorganic N contents after rapeseed and field bean harvest due to decomposing plant residues, which are usually not fully taken up by the following crop such as winter wheat (Sieling and Kage, 2006). The application of HCAs such as sawdust and wheat straw stimulates microbial N immobilisation, which compensates excess N and can partially available to crops in the next vegetation period (Mooshammer et al., 2014; Reichel et al., 2018). However, the stimulation of denitrification can lead to increased N_2O emissions (Yue et al., 2017; Li et al., 2021). Nevertheless, the application of HCAs is effective in maintaining soil fertility and reducing NO_3^- leaching. Considering that the efficiency is determined by the material properties of HCAs and excluding any potential risk of environmental contamination when using HCAs (Li et al., 2021), experiments on site-specific effects on soil, plants and microorganisms are essential before using any plant species for HCA. Due to the possibility of cultivation of *Mis* on the farm and thus obtaining a C-rich biomass, we tested *Mis,* which had not been investigated as an HCA, for its potential for N fixation.

In contrast to many other studies on the utilisation of HCAs for N fixation and stimulation of SMB (Bargmann et al., 2014c; Bargmann et al., 2014a; Bargmann et al., 2014b; Reichel et al., 2018; van Duijnen et al., 2018; Dundore-Arias et al., 2019; Wei et al., 2020; Cao et al., 2021; Kamau et al., 2021), we took two new approaches that, according to our research, have not yet been investigated regarding N fixation. On the one hand, *Mis* was used as a bedding material before application and thus received another agricultural use. On the other hand, *Mis* was mixed with cattle slurry to induce microbial N immobilisation already before application to reduce the NH_4^+ content at the time of application and thus reduce the risk of potential N losses of NH_3 and NO_3^-. It was unclear whether mixing cattle slurry with HCAs such as *Mis* and wheat straw and then applying it as fertiliser could immobilise N and consequently reduce NO_3^- losses. It was also unclear, whether *Mis* was as effective in stimulating SMB.

1.3.3 SOM build-up by soil microbial biomass

Inadequate C management in arable farming, for example due to insufficient input of organic matter or organic compounds caused by insufficient C rhizodeposition as a result of poor soil conditions or insufficient catch crop cultivation, or when organic matter is constantly removed and not sufficiently returned from plant residues or organic fertilisers, lead to a reduction of SOC (Goidts and van Wesemael, 2007; Steinmann et al., 2016b; Bamminger et al., 2019). SOC is directly related to soil fertility and provides an essential function as both a source and a sink for C, thereby mitigating climate change (Richards and Webster, 1999; Lal, 2001; Poissant et al., 2008; Ilumäe et al., 2009). In addition, high SOC contents improve the resilience of soils, making them more resistant to periods of drought, heavy rainfall and strong winds that can lead to soil erosion and yield loss. Changes in SOC content in response to C-inputs are only detectable after several years (Smith, 2004), whereas changes in SMB indicate long-term changes in SOM (Powlson et al., 1987), as SOM is largely composed of cells and cell fragments of dead microorganisms, microbial excreta and cytoplasmic materials of the SMB, the microbial necromass (Amelung et al., 2008; Kallenbach et al., 2016; Khan et al., 2016).

The addition of organic substrates to soil induces their degradation and conversion by soil microorganisms, resulting in microbial residues. The amount of such residues depends on the degradation efficiency of the microorganisms. The chemical heterogeneity of microbial residues follows the structure of the organic input as well as the physiology of the soil microbial community (Bradford et al., 2013; Cotrufo et al., 2013; Kallenbach et al., 2015). The rate of SOM accumulation is not directly related to the chemical composition of an added organic substrate. Rather, the applied organic substrate influences the composition and formation of microbial communities characterised by a specific microbial physiology (Kallenbach et al., 2016). Thus, a higher amount of labile C compounds does not necessarily result in a higher amount of microbially produced SOM, but it does influence the microbial physiology and the formation of an individual microbial carbon use efficiency (CUE), from which the necromass build-up is then determined (Kallenbach et al., 2016). The CUE is “the proportion of C substrate that microbes assimilate into new biosynthetic material relative to the C lost from the system as CO_2” (Kallenbach et al., 2019). The necromass (proteins, lipids and polysaccharides) is protected from further microbial degradation by organic-mineral interactions and compounds and thus forms part of the stable SOM. Plant residues without previous microbial degradation also contribute to SOM build-up, for example if they are physically protected from degradation (Gillabel et al., 2010; Kleber et al., 2015). However, changes in environmental conditions, such as soil tillage or increases of temperature, can lead to degradation of this plant SOM (Lützow et al., 2006; Kleber et al., 2011). SOM build-up does not increase linearly with C input. Rather, microbial CUE determines C accumulation in the soil. Under conventional management conditions, C input to agricultural soils is related to agricultural plant growth, making CUE a key factor in increasing SOM (Kallenbach et al., 2019). An increase in the amount and diversity of organic matter applied to the soil will increase the diversity of SMBs as well as the SMBs themselves, leading to the formation of more interactive microbial networks and thus more efficient ecosystem functioning and potentially improved CUE (Bender et al., 2016; Morriën et al., 2017; Vries and Wallenstein, 2017). CUE is temporally variable and not a static state. Anabolic processes of SMB lead to the incorporation of nutrients into biological structures and catabolic processes lead to the mineralisation of nutrients and organic C to CO_2 (Joergensen and Wichern, 2018b). Changes in resource availability in agroecosystems require SMB to adapt accordingly. When C is limited, SMB enter a dormant state. In order to maintain their own metabolism, part of the C previously incorporated into their own cells is consumed and disappears from the soil system (Kempes et al., 2017; Joergensen and Wichern, 2018b). It is known that dead microbial cells are more likely to stabilise with soil minerals than living cells, but current knowledge of CUE and microbial biomass turnover rates and their interaction is insufficient to determine microbial C accumulation rates (Hagerty et al., 2014; Kallenbach et al., 2019). Accurate knowledge of the management of CUE by agricultural management tools is still lacking. The potential to increase SOM using agricultural management tools to improve the efficiency of microbial C processes has not yet been fully exploited. Following the stoichiometric equilibrium of SMB, microbial CUE is related to microbial cellular NUE. Consequently, the potential for managing the soil microbial N cycle has not yet been fully exploited. Therefore, the possibility of managing the soil microbial N cycle should exist.

Considering that C resources of agricultural farms are usually limited, the biomass of *Mis*, a high C yielding crop, will be tested as an additional C resource for farms in this thesis. It is unknown how *Mis* biomass compared to cereal straw, both as a component of organic fertilisers, affects SMB. It is not yet known if *Mis* biomass can provide an additional C source for SMB build-up and thus C sequestration. It is also unknown whether *Mis* can provide a management element for microbial N dynamics in the cropping system.

1.3.4 Drone-based determination of N supply of crops

Digitalisation in agriculture, Agriculture 4.0, describes the use and interconnection of information and communication technologies such as image processing, sensors, robotics, geographic information and global positioning systems (GIS, GPS), automation, big data management, machine learning and cloud computing, and their integration in all agricultural operations (Aulbur et al., 2019; Trivelli et al., 2019; Saiz-Rubio and Rovira-Más, 2020). The further industrial revolution, Industry 5.0 or Agriculture 5.0, is characterised by the interaction of artificial intelligence, the Internet of Things and humans. This interaction is also referred to as collaborative robots or "cobots". In combination with the implementation of artificial intelligence (AI) -based robotics, the aim is to further improve processes (Mesías-Ruiz et al., 2023; Tziolas et al., 2023). The implementation of digital technologies in agriculture increases productivity and efficiency and can reduce negative environmental impacts (Saiz-Rubio and Rovira-Más, 2020). In crop production, heterogeneities in the crop can be detected early, allowing agronomic tools to respond quickly and precisely (Hrustek, 2020). Digital technologies are used for weed control (Kämpfer and Nordmeyer, 2021), for demand-based plant nutrition (Reckleben, 2014; Argento et al., 2021) and in crop protection for the detection of plant diseases and pests (Bohnenkamp et al., 2019). They allow rapid detection of yield-relevant parameters in the field, enabling precise application of herbicides, pesticides and fertilisers. As a result, they can be used in an economically and ecologically targeted way, reducing negative environmental impacts and thus facilitating resource- and climate-friendly production methods (Piramuthu, 2022).

In plant nutrition, soil physical, soil chemical and soil microbial differences lead to heterogeneous field conditions, resulting in differences, for example, in the dynamics of N mineralisation, in the supply of macro- and micronutrients, in microbial activity, diversity and biomass, and in the usable field moisture capacity within a field (Reckleben, 2014; Kindred et al., 2015; Joergensen and Wichern, 2018a; Praveen et al., 2020). This results in different yield potentials between parts of the field and differences in plant nutrient requirement and uptake. If N availability is above or below plant N requirements, either the yield potential cannot be realised or the risk of negative environmental impacts increases. For agronomic, economic and environmental reasons, site-specific N fertilisation is useful, depending on the current plant N requirement (Zebarth et al.; Jan et al., 2017). Determining site-specific N requirements requires knowledge of a wide range of parameters (soil properties, climate data, management measures) that influence SMB and thus N dynamics (Samborski et al., 2009; Kindred et al., 2015).

Remote sensing, airborne and ground-based sensors are suitable for the detection of heterogeneities in vegetation. In agricultural practice, multispectral sensors are mainly used to detect plant parameters (Reckleben, 2014). They record the reflectance of 5 to 10 bands in the wavelength range from 500 nm to 900 nm, also in the visible, near infrared and partly in the short-wave infrared range. In contrast, hyperspectral imaging is not established in agricultural practice, but are used in science for basic research (Cilia et al., 2014). They have a higher technical sensitivity and capture the spectral information of more than 100 bands of the spectral range. They can cover the wavelength range from 250 nm to 2500 nm, including the ultraviolet to the shortwave infrared, although one camera usually does not cover the entire spectral range (Steiner et al., 2008). They provide spectral information such as colour, gradient and geometry in an additional dimension for each individual pixel. The data complexity and volume is much higher for hyperspectral cameras than for multispectral cameras (Mahlein et al., 2012; Behmann et al., 2015; Mahlein et al., 2018). They are rarely used in agricultural practice due to the large amount of data generated and the high cost.

Spectral vegetation indices are a tool used to detect plant parameters such as plant biomass or plant N uptake. They are calculated from the reflections of specific wavelength ranges, from which specific plant parameters such as chlorophyll or protein content, and thus plant N supply, can be determined (Cilia et al., 2014; Berger et al., 2020; Féret et al., 2021). For this purpose, spectral bands in the red spectral range (around 730 nm) are used, as they are more sensitive to N compounds than the red spectral band (Bean et al., 2018). Specific vegetation indices are also used in algorithms, for example to map the N supply of crops and to adjust crop N fertilisation (FILELLA and PENUELAS, 1994; Gitelson et al., 2003a; Gitelson et al., 2003b; Jongschaap and Booij, 2004; Berntsen et al., 2006; Mutanga and Skidmore, 2007). However, the algorithms that have been developed are not suitable for general use, but usually provide valid results under the specific site and climate conditions for which the algorithm has been developed (Bean et al., 2018). Climatic conditions, farm-specific crop rotation, crop species and the type of soil management all have an impact on SMB, which in turn has a continuous influence on C and N dynamics and thus on the plant nutrient uptake, thus challenging the application of algorithms. Even the soil microbiological processes are not fully understood, so intelligent systems are still reaching their limits in the complex system of soil-SMB-plant interactions. Nevertheless, big data sets help to identify complex relationships using methods such as supervised and unsupervised learning, for example to identify appropriate fertilisation strategies. As an element of sustainable agriculture, where HCAs such as *Mis* are implemented to SOM build-up and manage N dynamics in the arable farming, this thesis summarises the influencing factors of the complex system of SMB, plant, soil and digital farming. The spectral characterisation of crops will be used to evaluate the implementation of drone-based sensors for agricultural practice.

2 Nitrogen Immobilisation and Microbial Biomass Build-Up Induced by *Miscanthus x giganteus* L. Based Fertilisers

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Michael Stotter performed the experiments (100%), MS analysed the data and wrote the paper (100%).

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2.1 Abstract

Cultivation of *Miscanthus* x giganteus L. (*Mis*) with annual harvest of biomass could provide an additional C source for farmers. To test the potential of *Mis*-C for immobilising inorganic N from slurry or manure and as a C source for soil organic matter build-up in comparison to wheat (*Triticum aestivum* L.) straw (WS), a greenhouse experiment was performed. Pot experiments with ryegrass (*Lolium perenne* L.) were set up to investigate the N dynamics of two organic fertilisers based on *Mis* at Campus Klein-Altendorf, Germany. The two fertilisers, a mixture of cattle slurry and *Mis* as well as cattle manure from *Mis*-bedding material resulted in a slightly higher N immobilisation. Especially at the 1st and 2nd harvest, they were partly significantly different compared with the WS treatments. The fertilisers based on *Mis* resulted in a slightly higher microbial biomass C and microbial biomass N and thus can be identified as an additional C source to prevent nitrogen losses and for the build-up of soil organic matter (SOM) in the long-term.

2.2 Introduction

Technological developments, as well as economic conditions (agricultural subsidies, world market trade), have reduced the production costs in agriculture in the last decades. This has changed production methods resulting in nutrient access and pollution of the environment, especially in areas with high livestock density and slurry application and that, ultimately, threaten the long-term stability of agricultural production (Tilman et al., 2002; Bouwman et al., 2013). Inadequate soil management in arable farming can lead to soil degradation with negative effects on crop production being compensated by, for example, increased fertilisation; but more intensive treatments often lead to negative effects on the environment (Naylor, 1996; Cassman, 1999). Along with agricultural intensification, increased nitrogen (N) use resulted in lower N use efficiency (NUE) (Smil, 1999; Cassman, 2002), to an accumulation of N in soil and to nitrate leaching into ground and surface waters, resulting in eutrophication. Furthermore, the risk of NH_3 emissions with toxic effects on the respiratory system of mammals and humans and N_2O emissions, which is a potent greenhouse gas, increased with enhanced N inputs (Galloway et al., 2004; Sandel et al., 2011). In addition, changed production practices, like the replacement of cereals with root crops and fodder crops with a lower C/N ratio (Goidts et al., 2007) and past land use changes by conversion of grassland to cropland (Sleutel et al., 2007; Meersmans et al., 2010; Steinmann et al., 2016a; Steinmann et al., 2016b) all led to a decrease in soil organic carbon (SOC) in many cases. Climate change with rising temperatures increases the decomposition of organic matter further and results in SOC losses (Bellamy et al., 2005). Therefore, it is essential to use organic fertilisers and other C sources in a way that retains N and C in the crop-livestock-soil system and stops further SOC reduction or promotes SOC build-up. Thereby, soil microorganisms have a key function because they regulate essential C and N turnover processes in the soil. Nutrient mobilisation processes are often induced by microbial enzymatic activity, which is like nutrient immobilisation related to the soil microbial biomass (MB) (Geisseler et al., 2010; Geisseler et al., 2014). The MB, which is dominated by fungi and bacteria (Joergensen and Wichern, 2008), fulfils important functions in the soil. Anabolic processes lead to the incorporation of nutrients into biological structures and catabolic processes lead to mineralisation of organic N to NH_4^+ and of organic carbon (C) to CO_2 (Dilly et al., 2003). Thereby, the catabolic turnover rate can result in up to 225 kg N ha^{-1} released as inorganic N per hectare and year (Joergensen and Emmerling, 2006).

When organic fertilisers are applied, not all of the N supplied becomes available to plants in the year of application. Some organic N remains in the soil and only slowly releases inorganic N through microbial mineralisation processes in the months and years after application (Sørensen, 2004; Daudén et al., 2004; Sørensen and Thomsen, 2005). If this is insufficiently taken into account or is underestimated and if it does not occur simultaneously with the N demand of the plants, losses in the form of reactive N compounds to aquatic or terrestrial ecosystems may occur. These tend to increase when the SOC content decreases (Tilman et al., 2002; Bouwman et al., 2013; Cassman et al., 2002; Spiertz, 2009). SOC is directly related to soil fertility and provides an essential function as both, source and sink for C, mitigating climate change (Richards and Webster, 1999; Lal, 2001; Poissant et al., 2008; Ilumäe et al., 2009). Changes in the SOC content, depending on C-input, are detectable not before several years (Smith, 2004), whereas changes in MB indicate long term changes of SOC (Powlson et al., 1987) because SOC consists largely of cells and cell fragments of dead microorganisms, the microbial necromass (Kallenbach et al., 2016; Khan et al., 2016).

Microbial biomass N (MBN) and microbial biomass C (MBC) are closely correlated with each other (Dilly et al., 2003; Joergensen and Emmerling, 2006). In C-limiting agricultural systems with high N inputs, C tends to be the most limiting factor for microbial growth (Joergensen and Mueller, 1996). Chen et al. (2014) identified the incorporation of crop residues as an option to enhance C/N ratios to reduce N leaching, but also describe that the C/N ratio of incorporated substrates does not always predict N dynamics. Rather, soil properties and the biochemical composition of the substrate, especially the holocellulose/lignin ratio, are essential to N dynamics (Chen et al., 2014; Abbasi et al., 2015; Reichel et al., 2018; Wei et al, 2020).

It is well known that keeping and incorporating cereal straw stimulates anabolic processes and consequently reduces N losses, as well as it contributes to SOC content (Nishio and Oka, 2003; Shindo and Nishio, 2005; Simon et al., 2016; García-Ruiz et al., 2019). However, these effects, driven by MBC turnover rates, are limited by the annual C input (Joergensen and Wichern, 2018). A C-export in the form of straw selling and/or high organic N input with low C/N ratio promotes high N mineralisation. If this occurs in the period following harvest of the main crop and if it exceeds the N demand of the following crop, the risk of N losses increases (Schröder, 2014). Considering that microbial N immobilisation is basically related to C input (assuming sufficient supply of N, P, S, etc.) (Chen et al., 2014, Joergensen and Wichern, 2018; Blagodatsky and Richter, 1998; Schulten and Schnitzer, 1997; Lutzow et al., 2006; Olk et al., 2006), especially in agricultural regions with high organic N input and where cereal straw is being limited, N immobilisation should be facilitated by other C sources. Here, *Miscanthus x giganteus* L. can be a solution as multi-purpose crop which performs essential ecosystem services during cultivation. Furthermore, it can be cultivated as a low-input crop because of low fertiliser demand and no weed control (Emmerling and Pude, 2016) and is certified as a greening crop in Germany (a crop subsidised for its ecological value) (European Union. Regulation No. 2017/2393). *Mis* can be used as feedstock in anaerobic digestion (Ruf and Emmerling, 2017; Schmidt et al., 2017), as growing media in soilless cultivation (Nguyen et al., 2021), as an additive for packaging industry or as construction material (Pude, 2021) and can be cascaded to livestock farms in the form of bedding material (Van Weyenberg et al., 2016). Nevertheless, the question is whether *Mis* can be applied as a straw substitute, where cereal straw is lacking (e.g., because cereal straw is exported), for microbial N immobilisation and SOC build-up.

Therefore, in a pot experiment with ryegrass, we tested the effects of two novel N-containing and C-rich organic farm manures on N immobilisation as well as on soil microbial biomass. One was a mixture of cattle slurry with *Mis* and the other was a cattle manure based on *Mis* bedding material.

In this context, our hypotheses were (i) *Miscanthus* is as good as wheat straw in immobilising additional inorganic N from mineralisation of slurry or manure; (ii) Microbial biomass make use of *Miscanthus* as C source for biomass build-up and thus C sequestration.

2.3 Materials and methods

Site Description

In 2018, two pot experiments and in 2019, a third one, were set up in the greenhouse at Campus Klein-Altendorf (University of Bonn, Rheinbach, Germany). The third one was performed with a different composition of slurry and manure than those, used for the first two (see below for details). The set-up was chosen to compare the N dynamics of two organic fertilisers based on *Miscanthus x giganteus* L. (*Mis*) in an arable soil of a conventionally used agricultural site in the Rhine region.

This site from where soil samples were taken, has never received any organic fertilisation before, but was converted from grassland to arable in the year 2013. Soil (Gley-Cambisol) was taken from the Ah horizon (silty clay; up to 30 cm depth) of a field (50°36’3” N, 07°01’37” E; WGS 84) at Campus Klein-Altendorf, sieved to <4 mm and thoroughly homogenised manually. Afterwards, the gravimetric water content and the water holding capacity of the soil were determined (Wilke, 2005). The soil texture was silty loam as determined by particle-size analysis according to DIN ISO 11277:2002-08 (DIN ISO 11277:2002-08). Basic soil properties like pH (VDLUFA A 5.1.1, 2016), P_2O_5, K_2O (VDLUFA A 6.2.1.1, 2012), Mg (VDLUFA A 6.4.1, 1997), B, Cu, Mn, Fe (VDLUFA A 6.4.1, 2002), SOM (DIN ISO 10694:1996-08, 1996) and N_t (DIN ISO 13878: 1998-11, 1998) are given in Table 1.

Table 1. Basic soil properties. Values show means and standard deviation (n = 5; for SOM, SOC, N_t, C/N: n = 6); SOM = soil organic matter; SOC = soil organic carbon.

pH (H_2O)	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Mg (mg kg^{-1})**
6.3 ± 0.06	11.4 ± 2.7	10.4 ± 1.6	14 ± 1.9
B (mg kg^{-1})	**Cu (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Fe (mg kg^{-1})**
0.5 ± 0.04	6.3 ± 0.5	169.4 ± 47.4	196.3 ± 18.6
SOM (%)	**SOC (%)**	**N_t (%)**	**C/N (ratio)**
3.9 ± 0.7	2.3 ± 0.4	0.27 ± 0.02	8.5 ± 1.2
Clay (g kg^{-1})	**Silt (g kg^{-1})**	**Sand (g kg^{-1})**	
229	597	173	

The experiment aimed to stimulate microbial growth by adding an additional agricultural C source to immobilise inorganic N and enhance SOC in the soil. Therefore, the biomass of *Mis* grown on another field was used for two utilisation pathways, mixed with cattle slurry (CS) or used as bedding material creating cattle manure (CM). The *Mis* biomass was harvested in April 2017 (exp. 1, 2) or April 2018 (exp. 3) respectively with a forage harvester (Krone Big X 480) with a set cutting length of 30 mm. The wheat straw (WS; *Triticum aestivum* L.) biomass of the treatment CS-WS was broken up and baled by a Claas Quadrant 3200 FC big baler with a ROTO CUT front chopper and FineCut cutting unit and the WS biomass of the variety CM-WS was not chopped and cut. WS used for exp. 1 and 2 was from August 2018, WS used for exp. 3 was from August 2019.

In one pathway of *Mis* use, CS was mixed with *Mis* (cattle slurry mixed with *Mis* = CS-*Mis*) and, as a complementary treatment, CS was mixed with WS (cattle slurry mixed with wheat straw = CS-WS) and both converted into spreadable substrates. This has the objective to bind odorous compounds of the cattle slurry (CS), to keep nutrients in the topsoil for a longer time against precipitation, to reduce gaseous N emissions and to slow down the nitrification. It also aims to achieve a slower and longer lasting N mineralisation of the mixtures.

For the determination of the best possible mixing ratio of both mixed treatments (CS-*Mis*, CS-WS) concerning maximum absorption of CS to *Mis* and of CS to WS biomass, different amounts of CS (from 1 to 10 kg of CS in steps of 0.5 kg CS) were mixed with 1 kg of *Mis* or WS. As a result of this pre-test, a complete absorption (no excess liquid visible) of the liquid fraction of CS to *Mis* and WS, respectively, over seven days, was achieved at a ratio of 5 kg of CS to 1 kg of *Mis* and at a ratio of 8.5 kg of CS to 1 kg of WS. After mixing, the two mixture treatments were stored for five weeks on a manure slab and covered with a silage film to prevent precipitation intrusion and allow for N immobilisation.

The other option to use *Mis* on a farm was the use of *Mis* as bedding material in livestock. For this purpose, cattle were bedded with *Mis* (cattle manure from *Mis* = CM-*Mis*) and, as a reference, cattle were bedded with WS (cattle manure from wheat straw = CM-WS) according to standard farm practice and mucked out after about six weeks. As a reference treatment for the two mixtures, a pure CS was tested in the experiment. In addition, two further treatments were tested, this was a mineral N-fertilisation (Urea Ammonium Nitrate solution = UAN) as well as a treatment without any N applied (No Nitrogen applied = NoN). All abbreviations of the fertiliser products and fertiliser feedstocks are listed in Table 2.

Table 2. Abbreviation and description of the fertiliser products and fertiliser feedstocks (*Mis*, WS) evaluated in the greenhouse experiments.

Abbreviation	**Fertiliser Description**
CS	**C**attle **S**lurry
CS-*Mis*	**C**attle **S**lurry with ***Miscanthus*** addition (5 kg:1 kg)
CS-WS	**C**attle **S**lurry with **W**heat **S**traw addition (8.5 kg:1 kg)
CM-*Mis*	**C**attle **M**anure from ***Miscanthus*** shredded bedding
CM-WS	**C**attle **M**anure from **W**heat **S**traw bedding
UAN	**U**rea **A**mmonium **N**itrate solution
NoN	**No N**itrogen applied
Mis	***Mis****canthus*-shredding
WS	**W**heat **S**traw-shredding

The application rates of the tested treatments were 120 kg N ha^{-1} (experiment 1) and 170 kg N ha^{-1} (experiments 2 and 3). The nutrient content of the applied fertilisers (Tables 3 and 4) was determined by a certified laboratory following the requirements of the Fertiliser Ordinance 2017 of Germany (Bundesministerium der Justiz und für Verbraucherschutz, 2017).

Table 3. Nutrient contents of the used treatments for experiment 1 (120 kg total N ha^{-1}) and experiment 2 (170 kg total N ha^{-1}), (CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg), CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, *Mis* = *Mis*-shredding, WS = wheat straw-shredding).

Test Parameter	Unit	CS ^{1}	CS-*Mis* ^{2}	CS-WS ^{2}	CM-*Mis* ^{2}	CM-WS ^{2}	UAN ^{1}	*Mis* ^{2}	WS ^{2}
Dry matter	%	9.2	21.6	16.8	32.8	33.2	-	87.8	86.2
Organic matter	%	6.7	19.1	14.2	26.9	22	-	85.2	79.2
Total N	kg m^{-3}/kg t^{-1}	4.0	3.8	4.2	7.4	12.4	358.4	1.7	6.3
NH_4^+-N	kg m^{-3}/kg t^{-1}	1.8	1.2	1.3	0.2	0.1	89.6	<0.1	0.2
NH_4-N in total N	%	45	32	31	3	1	50	5	3
C/N	ratio	10	29	20	21	10	-	288	73

Indication of the nutrient content in: ^{1} kg m^{-3}; ^{2} kg t^{-1}.

Table 4. Nutrient contents of the used treatments for experiment 3 (170 kg total N ha^{-1}), (CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg), CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, *Mis* = *Miscanthus*-shredding, WS = wheat straw-shredding).

Test Parameter	Unit	CS ^{1}	CS-*Mis* ^{2}	CS-WS ^{2}	CM-*Mis* ^{2}	CM-WS ^{2}	UAN ^{1}	*Mis* ^{2}	WS ^{2}
Dry matter	%	8	20.7	16.5	25.4	15.5	-	90.1	90.9
Organic matter	%	5.3	18.0	13.5	22.9	12.4	-	86.9	86.3
Total N	kg m^{-3}/kg t^{-1}	3.5	3.7	3.9	5.0	5.0	358.4	3.0	4.4
NH_4^+	kg m^{-3}/kg t^{-1}	2.1	1.5	1.3	1.4	1.8	89.6	0.2	0.2
NH_4^+-N in total N	%	60	41	33	28	36	50	7	5
C/N	ratio	9	28	20	27	15	-	166	115

Indication of the nutrient content in: ^{1} kg m^{-3}; ^{2} kg t^{-1}.

The tested treatments were mixed with 6.2 kg dry matter soil and with additional plant macro- and micronutrients applied in inorganic form as shown in Table 5. These nutrients were also applied after the second and fourth harvest to avoid nutrient deficiency effects other than N. The soil was then filled into Kick-Brauckmann pots (with closed drainage) and German ryegrass (*Lolium perenne* L., Valerio) was sown at a sowing rate of 0.15 g per pot (sowing rate of 40 kg ha^{-1}). Each of the three experiments was set up as a completely randomised block design with five replicates per treatment (35 pots per experiment, 105 pots for the three experiments). To ensure ideal growth conditions for plants and soil microorganisms, all pots were adjusted to 60 to 70% of the maximum water holding capacity (WHC) by applying distilled water regularly. For this, pots were weighed twice to thrice a week, depending on temperature conditions and then irrigated.

Table 5. Form of supply and amounts of macro- and micronutrients to each pot supplied at the start of the experiment, after the second and after the fourth grass harvest. Nitrogen was only supplied once via the test materials at the start of the experiment.

Nutrient	Nutrient (mg pot^{-1})	Form of Supply
N	188/266 [a]	Organic N, NH_4^+ [b] K_2HPO_4
P	220	K_2HPO_4
K	1800	K_2SO_4
Mg	400	$MgSO_4 \bullet 7H_2O$
B	5	H_3BO_3
Zn	20	$ZnSO_4 \bullet 7H_2O$

[a] For experiment 1, 188 mg pot^{-1} (120 kg ha^{-1}) and for experiment 2 and 3, 266 mg pot^{-1} (170 kg ha^{-1}) were supplied. [b] Tables 4 and 5.

Plant and Soil Analyses

In each experiment, plants were cut six times with scissors to 0.03 m height. The thermal time (cumulative day degrees from 6 to 22 o'clock) and the day after sowing of the respective harvests is shown in Table 6.

Table 6. Thermal time and days after sowing of each harvest of the specific experiment (exp.).

	Harvest Number					
	1	**2**	**3**	**4**	**5**	**6**
exp.	Thermal time [a]/days after sowing					
1	646/55	931/75	1286/102	1808/137	2455/173	3138/209
2	640/47	926/67	1304/96	1880/133	2516/168	3260/204
3	627/42	855/56	1310/84	1935/118	2751/155	3418/188

[a] cumulative day degrees (6 to 22 o'clock) above 5 °C.

The obtained biomass was dried to a constant weight at 60 °C to calculate the dry matter yield. The dried biomass was ground by using a disk mill (TS 250, Siebtechnik GmbH, Mülheim an der Ruhr, Germany) and 6 mg ± 0.2 mg of each ground sample was weighed into tin cartridges. The C and N concentrations of each harvest-biomass was analysed by using an elemental analyser (EA 3000 series, HEKAtech GmbH, Wegberg, Germany). Plant N uptake was calculated by using dry matter yield and N concentration. It was extrapolated to one hectare, assuming a soil bulk density of 1.32 g cm^{-3} (Ah horizon up to 30 cm depth).
At the end of the experiments, a soil aliquot of each pot was used to analyse inorganic N (N_{min} = NH_4^+ + NO_3^-; NO_2^- was not detectable), soil microbial biomass C (MBC) and soil microbial biomass N (MBN). For this, soil samples were sieved at 2 mm and all visible roots were removed. For the analysis of inorganic N, 25 g of the field-fresh soil was weighed into PE bottles, mixed with 100 cm^3 of 1% K_2SO_4 and placed on an overhead shaker at 22 rpm for 60 min. After shaking, all extracts were filtered (VWR 305; particle retention: 2–3 µm). The first 10 cm^3 of the filtrate were discarded to obtain the purest possible extract. The extract was

filled into plastic cuvettes, then stored until further analysis at −18 °C. The inorganic N content was determined with the AutoAnalyzer 3 from Bran + Luebbe GmbH Norderstedt, Germany.

MBC and MBN were analysed by chloroform fumigation-extraction (Brookes et al., 1985; Vance et al., 1987). Therefore, two portions of 10 g of moist soil were weighed into PE bottles. One sample was for fumigation- and the other one for non-fumigation-extraction. The fumigation was carried out in a vacuum desiccator at 25 °C using ethanol-free chloroform ($CHCl_3$) for 24 h in the dark. The fumigated and non-fumigated samples were then extracted with 40 cm^3 of 0.5 M K_2SO_4 and placed on a horizontal shaker at 180 rpm for 30 min. After shaking, all extracts were filtered (VWR 305; particle retention: 2–3 µm) and stored until analysis at −18 °C to avoid microbial transformation processes. Just before starting the analyses, extracts were defrosted rapidly to room temperature. In all extracts, organic C and total N were detected after combustion at 800 °C by using a Multi N/C 2100S (Analytic Jena, Jena, Germany). MBC was calculated as the ratio of extractable C (EC) and k_{EC}. EC is the difference between organic C extracted from fumigated soils and non-fumigated soils, whereas k_{EC} is a coefficient with the value of 0.45 (Wu et al., 1990) and represents the fraction of microbial C released in 24 h of fumigation. MBN gets calculated as the ratio of extractable N (EN) and k_{EN}. EN is the difference in organic N extracted from fumigated soils and non-fumigated soils, where k_{EN} is a coefficient with the value of 0.54 (Joergensen and Mueller, 1996; Brooks et al., 1985) and represents the fraction of microbial N.

Plant N uptake, inorganic N, MBC and MBN were extrapolated to one hectare, assuming a soil bulk density of 1.32 g cm^{-3} (Ah horizon up to 30 cm depth). The amount of N mineralised from applied fertilisers was estimated by subtracting the sum of plant N uptake of NoN treatments from the N uptake of the fertilised ryegrass. The total inorganic N (N_{min}) value at the start and end of the test was included in the calculation by subtracting these differences from the N uptake of each treatment. This calculation does not take into account N losses in the form of ammonia and nitrous oxide. These are assumed to be minimal because the organic fertilisers were incorporated into the soil immediately and the soil moisture was around 60% WHC.

Statistical Analyses

Dry matter yields are shown as arithmetic means (n = 5). Nitrogen uptake was calculated on a pot basis by multiplying the dry matter plant yield by the respective N content of the biomass and by extrapolation to the amount of soil in the upper 30 cm of a hectare. Statistical analyses were performed using IBM SPSS Statistics 27. Normal distribution of data was tested using the Shapiro–Wilk test. Levene's test based on means was used to verify homogeneity of variances. To identify treatment differences between three treatments, a one-way analysis of variance (ANOVA), following by a post hoc Tukey's HSD (honest significance difference) test were used. To identify differences between two treatments, t-test was used. Tukey's HSD and t-test were performed separately for each experiment. When data were not normally distributed or no homogeneity of variance were detected, Welch test and Games–Howell test were used to identify differences for three or more treatments, Mann–Whitney–U test was used to identify differences between two treatments. *p*-values of 0.05 were used as threshold for significant interactions.

2.4 Results

Plant N-uptake

The plant N uptake indicates clear differences in the N availability of the applied N fertilisers. Ryegrass fertilised with mineral N (UAN) showed the highest N uptake at the 1st harvest, but already at the 3rd harvest, no differences in N uptake was detected between the mineral treatment and the treatment without N addition (NoN). When ryegrass was fertilised with cattle slurry (CS), lower amounts of N were plant-available especially at the 1st harvest as compared to mineral fertilisation (Figure 1).

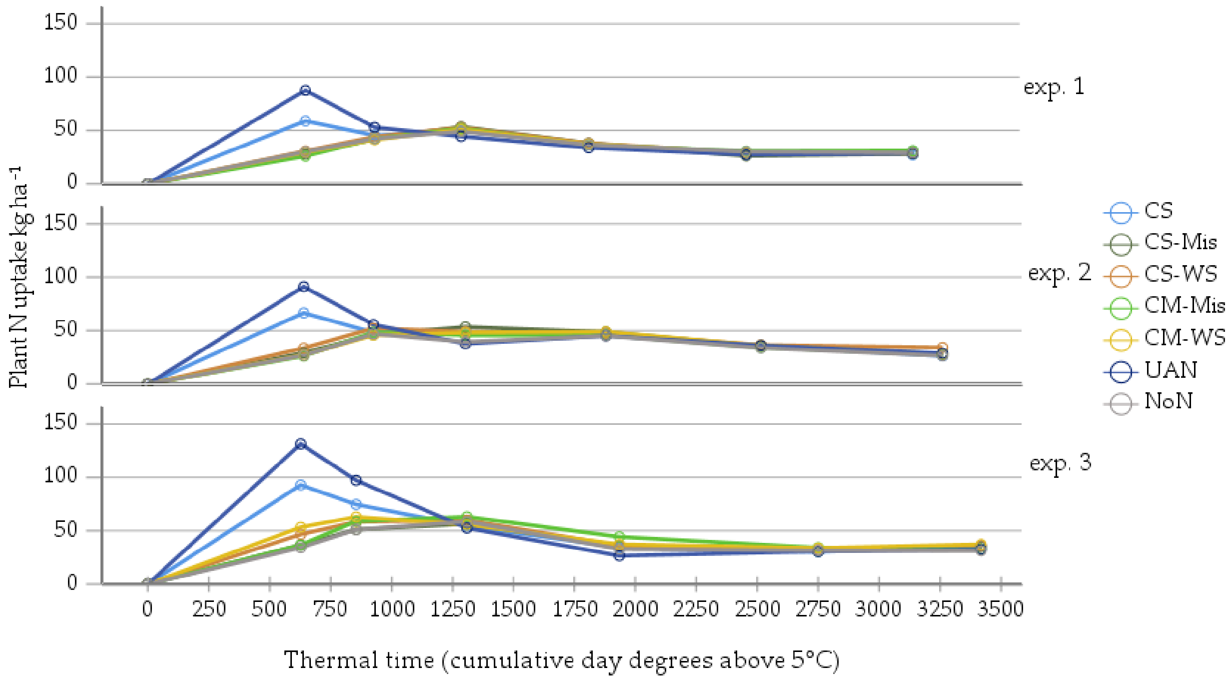

Figure 1. Plant N uptake of ryegrass in relation to harvests (markings) and thermal time (cumulative day degrees above 5 °C) in experiments 1, 2 and 3 for each treatment (CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg), CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied).

The cumulated N uptake of ryegrass was significantly lower (7% to 24%) compared to pure CS when *Mis* or WS were mixed with CS and then applied as C-rich organic N fertiliser (Table 7). Compared to pure CS fertilisation, the addition of WS to CS induced a 7% to 17% reduction in plant N uptake and the addition of *Mis* induced a slightly stronger reduction with plant N uptake being reduced by 12% to 24% (Table 7). Especially in the period after application until the 1st harvest, the addition of *Mis* or WS to CS caused a significant reduction in plant N uptake. The addition of WS to CS significantly reduced plant N uptake by 50% compared to CS fertilisation only. The addition of *Mis* to CS caused an even greater reduction in plant N uptake (53% to 61%), which was statistically significant in exp. 1 and 2, compared to WS addition (48% to 50%) (Table 7). At the 2nd and 3rd harvest, N uptake of ryegrass, fertilised with mixtures of *Mis* or WS and CS slightly increased and the N uptake of ryegrass fertilised with pure CS slightly decreased, compared to the 1st harvest, however, only to a small extent (Figure 1). Therefore, in exp. 1 and 2, at the 2nd harvest, N uptake in ryegrass fertilised with CS-*Mis* is only 4% to 7% lower than that of the ryegrass fertilised with CS only. Moreover, at the 2nd harvest, N uptake of ryegrass fertilised with CS-WS increased so that it was identical (exp. 1) or even higher than plant N uptake after pure CS fertilisation (exp. 2). In exp. 3, at the 2nd harvest, only 70% (CS-*Mis*) to 80% (CS-WS) of the N was taken up by plants compared to fertilisation with pure CS (Table 7). From the 3rd harvest until the end of the experiment (temperature sum of more than 3000 °C), the plant N uptake in ryegrass fertilised with the mixtures (CS-*Mis*, CS-WS) was in most cases higher than that after CS fertilisation only (Figure 1, Table 7). Plant N uptake after CS-*Mis* fertilisation was only slightly lower than that after CS-WS application (Figure 1, Table 7).

Table 7. Percentage of N uptake of the two mixtures (CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg); CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg)) to N uptake of cattle slurry (CS), at the time of each harvest and cumulatively (cum). Listed for experiment (exp.) 1,2 and 3, respectively. Different letters within a column and within each experiment number show significant differences. One-way ANOVA; $p < 0.05$; ns = not significant; n = 5.

		Harvest Number						
		1	**2**	**3**	**4**	**5**	**6**	**cum**
exp.	**treatment**	**N uptake [% of CS]**						
1	CS	100 a	100 a	100 ns	100 ns	100 ns	100 ns	100 a
	CS-*Mis*	47 c	93 b	107 ns	101 ns	95 ns	101 ns	87 b
	CS-WS	52 b	97 ab	105 ns	101 ns	106 ns	107 ns	91 b
2	CS	100 a	100 ab	100 ns	100 ns	100 ns	100 ns	100 a
	CS-*Mis*	44 c	96 b	110 ns	104 ns	100 ns	102 ns	88 b
	CS-WS	51 b	108 a	100 ns	101 ns	103 ns	130 ns	93 b
3	CS	100 a	100 a	100 ns	100 ns	100 ns	100 ns	100 a
	CS-*Mis*	39 b	68 c	104 ns	104 ns	102 ns	99 ns	76 b
	CS-WS	50 b	78 b	110 ns	104 ns	105 ns	101 ns	83 b

When ryegrass was fertilised with the two cattle manure types, with *Mis* or WS, there was no significant difference in the cumulative plant N uptake, but rather in the dynamics of the relative N uptake (Table 8). Until the 1st harvest, the unfertilised ryegrass (NoN) took up the same amount of N as the ryegrass fertilised with CM-WS or CM-*Mis*, respectively (Figure 1). In exp. 1 and 2, at the 2nd harvest, N uptake was slightly higher when CM-*Mis* was applied, but decreased in the further development mainly to a lower N uptake level compared to CM-WS (Table 8). Exp. 3 showed larger differences in the dynamics of plant N uptake between ryegrass fertilised with the two manure types. Here, ryegrass fertilised with CM-*Mis* took up more N (13% to 19%) at the 3rd and especially at the 4th harvests, whereas uptake was lower at the 1st and 2nd harvest (Table 8).

Table 8. Percentage of N uptake of the new type of cattle manure from *Mis* shredded bedding (CM-*Mis*) to N uptake of conventional cattle manure from wheat straw bedding (CM-WS), at the time of each harvest and cumulatively (cum). Listed for experiment (exp.) 1, 2 and 3, respectively. Different letters within a column and within each experiment number show significant differences between the two types of manure ($p < 0.05$, t-test); ns = not significant; n = 5.

		Harvest Number						
		1	**2**	**3**	**4**	**5**	**6**	**cum**
exp.	**treatment**	**N uptake [% of CM-WS]**						
1	CM-*Mis*	88 B	102 ns	102 ns	98 ns	106 ns	109 ns	101 ns
	CM-WS	100 A	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns
2	CM-*Mis*	97 ns	106 ns	95 ns	94 ns	95 ns	98 ns	97 ns
	CM-WS	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns
3	CM-*Mis*	69 B	93 ns	113 ns	119 A	101 ns	88 B	96 ns
	CM-WS	100 A	100 ns	100 ns	100 B	100 ns	100 A	100 ns

Microbial Mineralisation-Immobilisation as Affected by Added *Miscanthus* Straw

The fraction of mineralised N was significantly reduced after adding organic C in the form of *Mis* or WS to cattle slurry in each experiment (Figure 2A). Thereby, the addition of *Mis* resulted in a lower mineralised N fraction compared to the WS addition, in all 3 experiments (Figure 2A). In exp. 2 and 3, the difference was statistically significant. In exp. 1 and 3, *Mis* addition even resulted in no additional N mineralisation compared to unfertilised ryegrass (Figure 2A). In exp. 2, 13% of the fertilised N from CS-*Mis* became plant available as inorganic N. In contrast, more N was mineralised in CS-WS, which was 6% in exp. 1, 20% in exp. 2 and 17% in exp. 3. These differences result mainly from the different N release patterns after application to the soil, as shown by plant N uptake especially at the 1st harvest (Table 7). This reduced N uptake, as a result of *Mis* or WS addition to CS, may indicate lower N release from CS through mineralisation or increased N immobilisation by soil microorganisms facilitated by easily available C added with CS-*Mis* or CS-WS (Table 7).

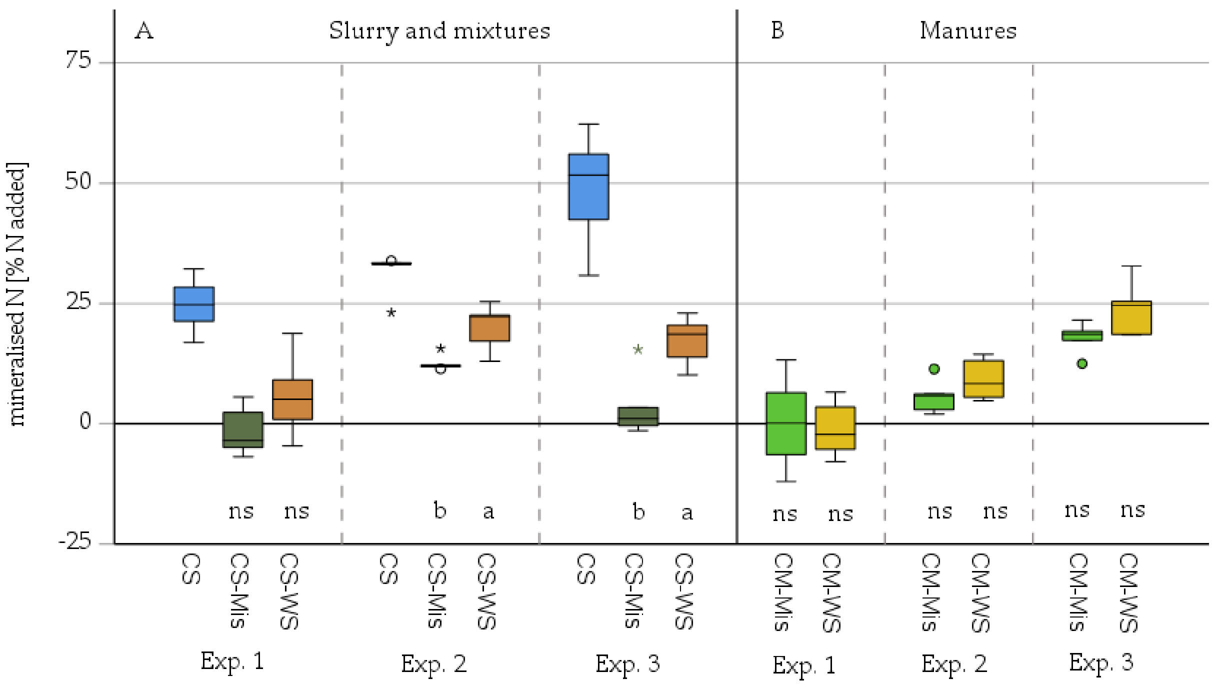

Figure 2. N mineralised expressed as % of N applied for experiment 1 (120 kg total N ha^{-1}) and experiment 2 and 3 (170 kg total N ha^{-1}) cumulated until the end of the experiment for each treatment (**A**: CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg), CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg); **B**: CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding). The dot indicates a "statistical outlier"; the star indicates an "extreme statistical outlier". Different letters within a column and within each experiment number show significant differences between the two types of mixtures (CS-*Mis*, CS-WS) and between the two types of manure (CM-*Mis*, CM-WS) ($p < 0.05$, t-test); ns = not significant; n = 5.

The microbial biomass C (MBC) and N (MBN) were both not significantly affected by adding C to CS either as *Mis* or WS (Figures 3 and 4). However, the mean of MBC was slightly higher in CS-*Mis* compared to CS-WS, moderately in all experiments (Figure 3A). The MBN indicated the same tendency of increasing after addition of CS-*Mis* compared to fertilisation with CS-WS (Figure 4A). Thus, the lower N mineralisation of CS-*Mis* compared to CS-WS (Table 7, Figure 2A) is generally reflected in a slightly higher microbial biomass (Figures 3A and 4A). In CS-*Mis*, MBN was 23 kg ha^{-1} to 60 kg ha^{-1} higher and in CS-WS, MBN was 19 kg ha^{-1} to 51 kg ha^{-1} higher than MBN in the non-fertilised treatment (Table 9). Apparently, when *Mis* was used for mixing with CS, soil microorganisms were able to immobilise more N as compared to WS.

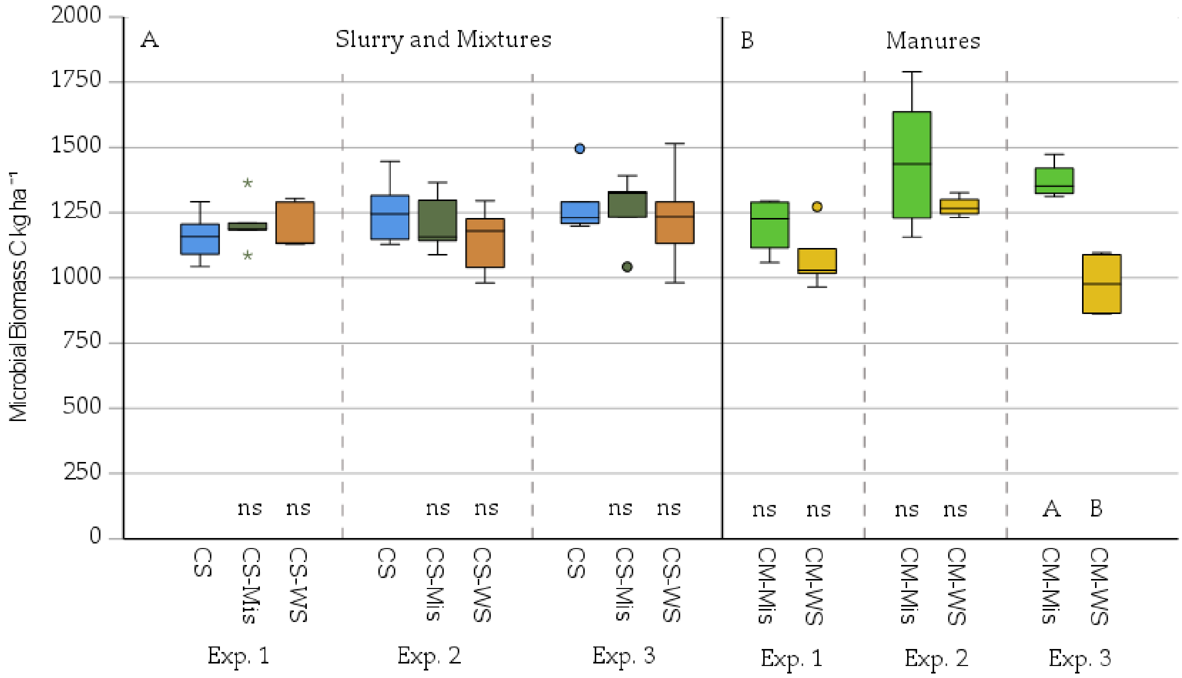

Figure 3. Microbial biomass C kg ha^{-1} of the soils, applied with different organic fertiliser for experiment 1 (120 kg total N ha^{-1}) and exp. 2 and 3 (170 kg total N ha^{-1}) at the end of the experiment for each treatment (**A**: CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg), CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg); **B**: CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding). The dot indicates a “statistical outlier”; the star indicates an “extreme statistical outlier”. Different letters within a column and within each experiment number show significant differences between the two types of mixtures and between the two types of manure ($p < 0.05$, t-test); ns = not significant; n = 5.

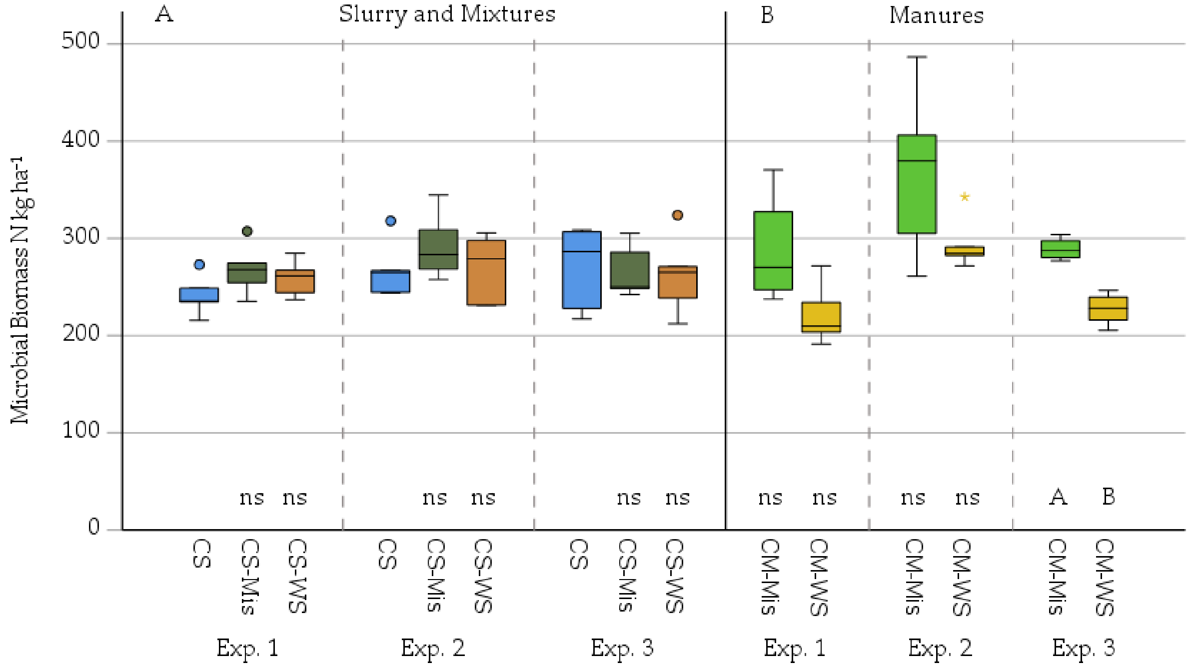

Figure 4. Microbial biomass N kg ha^{-1} of the soils, applied with different organic fertiliser for experiment 1 (120 kg total N ha^{-1}) and exp. 2 and 3 (170 kg total N ha^{-1}) at the end of the experiment for each treatment (**A**: CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg), CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg), **B**: CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding). The dot indicates a "statistical outlier"; the star indicates an "extreme statistical outlier". Different letters within a column and within each experiment number show significant differences between the two types of mixtures and between the two types of manure ($p < 0.05$, t-test); ns = not significant; n = 5.

Table 9. N immobilisation calculated of MBN of fertilised treatments and non-fertilised treatment (CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (5 kg to 1 kg), CS-WS = cattle slurry-wheat straw (8.5 kg to 1 kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate).

Exp.	CS	CS-*Mis*	CS-WS	CM-*Mis*	CM-WS	UAN
	N Immobilisation [kg ha^{-1}]					
1	34	60	51	80	15	21
2	23	48	25	123	50	40
3	26	23	19	46	−16	46

When cattle manure (CM) from *Mis* as well as from WS were used as organic fertiliser, in exp. 2 and 3, a lower fraction, although not statistically significant, of CM-*Mis* was mineralised than of CM-WS (Figure 2B). In exp. 1, the same amount of N was mineralised as became plant available from the soil N pool in the unfertilised ryegrass. Consequently, no additional N was mineralised of both CM-*Mis* and CM-WS (Figure 2B). The tendency for lower N mineralisation after CM-*Mis* fertilisation compared to CM-WS fertilisation was accompanied by a higher MBN in all experiments. In exp. 1 and 2, the difference was slightly lower, in exp. 3 it was obvious and significant for both, MBN and MBC (Figures 3B and 4B). In CM-*Mis*, MBN was higher (46 kg ha^{-1} to 123 kg ha^{-1}) and in CM-WS, MBN was mostly higher compared to the non-fertilised treatment (−16 kg ha^{-1} to 50 kg ha^{-1}) (Table 9).

After application of UAN and in the treatment without any N addition (NoN), the MBC did not differ and the MBN predominantly did not differ statistically significantly from the treatment with organic fertilisation (data not shown). MBC was slightly higher after UAN fertilisation (UAN: exp. 1 = mean 1096 kg ha^{-1} ± SD 104; exp. 2 = 1269 kg ha^{-1} ± 116; exp. 3 = 1254 kg ha^{-1} ± 184) than in the non-fertilised treatment (NoN: exp. 1 = 1024 kg ha^{-1} ± 117; exp. 2 = 1084 kg ha^{-1} ± 128; exp. 3 = 999 kg ha^{-1} ± 131). MBN after UAN fertilisation was 229 kg ha^{-1} ± 29 in exp. 1, 284 kg ha^{-1} ± 40 in exp. 2 and 289 kg ha^{-1} ± 12 in exp. 3, showing a slight increase as a result of UAN addition compared to the treatment without N fertilisation (NoN: exp. 1 = 207 kg ha^{-1} ± 35; exp. 2 = 244 kg ha^{-1} ± 22; exp. 3 = 243 kg ha^{-1} ± 30).

2.5 Discussion

***Miscanthus*-Induced N Immobilisation**

As an organic C source, we tested the utilisation of *Mis* and WS concerning N immobilisation and MB build-up which can yield in C sequestration. We demonstrate that *Mis* is at least as good as WS as a utilisable C source facilitating N immobilisation and microbial growth eventually contributing to the formation of microbial necromass and thus SOC. Nevertheless, the increase in MB is low, which is mainly caused by the large MB background, as caused by grassland conversion to arable land in 2013, overriding the effects of organic fertilisers. Thus, we expect clearer effects in soils with lower SOM.

The process of microbial N mineralisation-immobilisation depends on the biochemical composition of the substrate. In general, these processes are characterised by the NH_4^+ content, the C/N ratio and the holocellulose and lignin contents (Wei et al., 2020; Cabrera et al., 2005; Dittmar and Stubbins et al., 2014; Bhogal et al., 2016). For WS, the holocellulose content is estimated to be 68% to 76% and the lignin content is estimated to be between 8% and 25% (Wei et al., 2020; Rahn et al., 1999; Corbeels et al., 2000; Eiland et al., 2001a; Van Kuijk et al., 2017). For *Mis*, the holocellulose content is given as 70% and the lignin content as between 14% to 19% (Eiland et al., 2001a; Pude et al., 2005), being in the same range as WS. In contrast, the C/N ratio of WS was clearly lower at 73 (exp. 1,2) and 115 (exp. 3) compared to that of *Mis* at 166 (exp. 1,2) and 288 (exp. 3). Additionally, C availability was enhanced by lower mixing ratio of CS-*Mis* (5:1) compared to CS-WS (8.5:1) (Tables 3 and 4), suggesting a higher microbially available C derived from CS in the *Mis*–based fertiliser (CS-*Mis*, CM-*Mis*). In contrast, the NH_4^+ content of both mixtures was almost identical. Thus, the higher microbially available C input in the form of *Mis* appears to have caused greater microbial N immobilisation, especially by the time of the 1st harvest, which is confirmed by a higher MBN in CS-*Mis* treatment (Figure 4A). The addition of *Mis* as bedding material also resulted in a higher C/N ratio of CM-*Mis* compared to CM-WS (Tables 3 and 4) and thus also (like CS-*Mis* already) resulted in lower N uptake at 1st harvest (Figure 1). For CM-*Mis*, we also assume the reason for a stronger N immobilisation being higher easily available C (as already for CS-*Mis*) compared to CM-WS. Another influence on the higher N immobilisation of the *Mis* treatments and for the MBN tending to be higher, might be the smaller particle size of *Mis* or a difference in the surface structure as compared to WS, which accelerates and facilitates microbial degradation processes (Congreves et al., 2013). This, however, needs to be verified in future studies.

Eiland et al. (2001a) and Eiland et al. (2001b) tested the addition of *Mis* to pig manure to produce a growth medium for plants via composting processes. They reported a clear reduction in nitrification at a C/N of 35 compared to a C/N of 11, we observed N immobilisation at a C/N ratio of less than 30 (CS-*Mis*, CM-*Mis*). However, the two experimental settings cannot be compared because, unlike Eiland et al. (2001a) and Eiland et al. (2001b), we incorporated our treatments into the soil and there is a likelihood of NH_4^+ released by microorganisms being fixed at negatively charged sites of clay minerals and SOM. Moreover, soil potassium (K) status, K^+ saturation, moisture conditions and the cation exchange capacity of the soil influence the amount of NH_4^+ that can be fixed and thus reduce its nitrification and plant availability (Nieder et al., 2011). Jensen et al. (2001) and Leth et al. (2001) also conducted composting experiments of *Mis* with pig slurry and other N sources and observed high microbial activities respectively. Like our experiments, these results indicate a high amount of C in *Mis* that can be easily degraded by microorganisms, provided that a sufficient amount of available N is accessible.

Some other studies showed promotion of MB and thus N immobilisation after *Mis* biomass incorporation into the soil or application to the soil surface (Beuch et al., 1998; Mierzwa-Hersztek et al., 2017; O´Toole et al., 2018). In contrast, Schimmelpfennig et al. (2015), detected no initial N immobilisation after addition of *Mis* and slurry, successively, possibly because more N was added than became immobilised. Rex et al., (2015) determined a decrease of fungal biomass, but an increase in bacterial biomass, when *Mis* biomass and pig slurry were applied together compared to pig slurry alone. Especially on agricultural fields with a high potential of pedogenic N-mineralisation, an additional C-input can reduce the risk for N-losses by induced microbial N-immobilisation (Reichel et al., 2018; Wei et al., 2020), causing N to be assimilated into the microbial cells and thus decreasing the inorganic N of the soil (Congreves et al., 2013). Reichel et al., (2018) and Wei et al., (2020) induced N immobilisation by the application of high carbon amendments, such as wheat straw and spruce sawdust and mention the holocellulose/lignin ratio as a future tool to prevent N losses. Our experiments, suggesting a tendency for microbial N immobilisation to be slightly higher when *Mis* is used

compared to WS, each in conjunction with excreta (Figure 1, Table 7), lead us to assume that *Mis* may find suitability for N immobilisation even without the addition of excreta as a high carbon amendment for N immobilisation.
The relative data of N uptake of the two mixtures (CS-*Mis*, CS-WS) to N uptake of CS (Table 7) as well as the percentage of N uptake of the new type of cattle manure from *Mis* shredded bedding (CM-*Mis*) to N uptake of conventional cattle manure from wheat straw bedding (CM-WS) (Table 8) demonstrate to the farmer how these fertilisers can be estimated and applied in comparison to the well-known fertilisers.

Miscanthus **as C Source for Microbial-Derived C Sequestration and SOM Build-Up**

For build-up of SOC, N compounds are essential (Kallenbach et al., 2016; Khan et al., 2016). In many areas, high organic N amounts are already formed by excretions in animal farming. These have a high potential for SOM build-up, which, however, cannot be exploited without sufficient C availability and the SOM build-up can only be insufficiently formed, resulting in a higher risk of N losses instead. In contrast, sufficient C availability with simultaneous N supply, as provided by CS-*Mis* and CM-*Mis*, could contribute to the formation of microbial necromass. Especially necromass has an essential role in the formation, conservation and stability of SOM and is thus a key component of C sequestration in soil (Kallenbach et al., 2016; Khan et al., 2016; Liang et al., 2010; Hobara et al.; 2013). Thus, the increase of SOM as C and N storage improves soil fertility. This would result in a reduction of N losses and in future could increase the NUE of organic N fertilisers. Future studies need to verify the role of *Mis* in microbial necromass formation to better understand its contribution to SOC build-up.
The cultivation of *Mis* enables farmers to develop an additional C source regionally (Pude, 2021). The process of mixing CS with *Mis*, compared to the cascade utilisation as bedding material, does require an additional working step. However, both options contribute an increase in the SOM by promoting MB (Kallenbach et al., 2016; Khan et al., 2016). The additional source of *Mis*-C in areas with high livestock numbers and thus high demand for bedding and fodder material in form of cereal straw may also buffer the demand for cereal-based C in arable regions (Yesufu et al., 2016), leaving more C in arable regions to conserve SOC and thus counteract the continuous SOM losses (Steinmann et al., 2013). Additional C

production through *Mis* cultivation also counteracts dependence on external C sources such as imported organic fertilisers like slurry and farm manure in predominantly arable farming areas. Furthermore, *Mis* can fulfill and compensate the increasing demand for bedding materials in livestock production (Van Weyenberg et al., 2016), which is also a result of societal requests for animal welfare conditions as well as the rising demand for cereal straw as a feed component.

In addition to *Mis* as an accredited crop of ecological compensation conservation areas (European Union. Regulation No. 2017/2393), other greening measures, such as hedges or trees in agroforestry systems also provide an alternative C source that could be used for induced N immobilisation and SOC build-up. Removal of vegetation material from buffer strips, erosion strips and riparian strips as C-source would result in nutrient removal and thus nutrient reduction of the greening part, which benefits biodiversity at these sites. This would be a positive development regarding nature conservation (increase of plant diversity, habitat for insects and other animals). The useful utilisation as a C-source could ensure the removal of mown material, but requires tests concerning the effectiveness on N immobilisation and the effects on the MB.

If organic N-fertilisers containing *Mis* are applied, where N is directly required, yield deficits due to microbial N immobilisation are to be expected, if they are applied exclusively. In this case, the N demand can be supplied by the application of additional ready available N fertilisers and the residual effects can be included in subsequent vegetation periods, as is also common practice with the application of other organic fertilisers (Schröder et al., 2014). An estimation of the amount of accounting for subsequent growing seasons is not possible due to missing data from field trials. Earlier application of organic farm manures in the winter months in order to expect higher N mineralisation in spring is also not recommended due to missing data from field trials and due to the risk of N losses to ecosystems.

2.6 Conclusions

The specific characteristics of *Mis*, such as the higher C/N ratio compared to WS, were reflected in a slightly higher N immobilisation. Especially at the 1st and 2nd harvest, CS-*Mis* and CM-*Mis* were partly significantly different from the comparative treatments CS-WS and CM-WS. The *Mis*-C resulted in a slightly higher MBC and MBN and thus can contribute as an additional C source to prevent N losses and for the maintenance or build-up of SOM on agricultural farms. We assume that high background values of SOM and thus a high starting content of MB, as caused by grassland conversion to arable land, overrode the effects.

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3 Utilisation of *Miscanthus x giganteus* L. Based C-Rich Fertilisers for N Immobilisation and Microbial Biomass Build-Up in a Crop Rotation.

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Michael Stotter performed the experiments (100%), MS analysed the data and wrote the paper (100%).

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3.1 Abstract

Cultivation of perennials such as *Miscanthus x giganteus* (*Mis*) combines the provision of ecosystem services and the generation of additional carbon sources for farming. The potential of *Mis* based fertilisers, regarding immobilisation of inorganic nitrogen (N) and build-up of soil organic matter (SOM) was tested in a field trial. Therefore, a crop rotation of winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris* L.) and winter wheat (*Triticum aestivum* L.) was set up. The tested treatments were a mixture of cattle slurry (CS) and *Mis,* a mixture of CS and wheat straw (CS-WS), cattle manure (CM) from *Mis*-shredded bedding, CM from WS-shredded bedding, a pure CS, urea ammonium nitrate (UAN) and a treatment without any N applied (NoN). When the C-rich fertilisers (both mixtures and manures) were applied to cereals, they lead to a slightly N immobilisation compared to pure CS, whereas differences were mostly not significant. Furthermore, *Mis*-fertilisers were at least as efficient as WS-based organic fertilisers in inducing a contribution of SOM build-up and in reducing inorganic N before winter and thus prevent N losses, whereas differences were mostly not significant.

3.2 Introduction

In the last decades, technological developments, agricultural subsidies and the world market trade have facilitated an increase in animal husbandry and bioenergy production, as well as the access to mineral nitrogen (N) fertilisers (Bouwman et al., 2013; Tilman et al., 2002). This has changed production methods and contributed to specialisation of agricultural production and an intensification of agricultural land use. However, although this has improved the availability of food, access to mineral nitrogen may also threaten the sustainability of agricultural production in the long term (Bouwman et al., 2013; Tilman et al., 2002). Unsustainable soil management in arable farming can lead to soil degradation and consequently to negative effects on crop production. This may often be compensated by an increased fertilisation, but high N input often leads to N losses in form of nitrate leaching into ground and surface waters, resulting in eutrophication (Cordell et al., 2009). Furthermore, due to enhanced N inputs, the risk of NH_3 emissions with toxic effects on the respiratory system of mammals and humans and N_2O emissions, which is a potent greenhouse gas, has increased (Galloway et al., 2004; Good et al., 2004; Hietz et al., 2011; Narcy et al., 2013).

In addition, past land-use changes by conversion of grassland to cropland and changed production practices, like the replacement of cereals with root crops and fodder crops with lower C/N ratio, all led to a decrease in soil organic carbon (SOC) in many cases (Goidts and van Wesemael, 2007; Sleutel et al., 2007; Meersmans et al., 2010; Steinmann et al., 2016a; Steinmann et al., 2016b). Consequently, soils become more vulnerable to extreme weather conditions, including periods of drought, heavy rainfall and strong winds, which can result in soil erosion and yield losses. Compared to annual crops, the cultivation of perennial crops such as *Miscanthus* (*Mis*) has numerous ecological advantages. *Miscanthus x giganteus*, a sterile hybrid of *M. sacchariflorus* and *M. sinensis* (Greef et al., 1997; Hodkinson and Renvoize, 2011) can protect soil against erosion and can cause an accumulation of over one t C ha^{-1} $year^{-1}$ in soil (Felten and Emmerling, 2012). In contrast, soil organic matter (SOM) contents provide a greater level of resilience for plants and soil microbial biomass (MB), contributing to stable yield and quality parameters.

Furthermore, it is essential to use organic fertilisers and other C sources in a way that retains N and C in the crop-livestock-soil system and stops further SOC reduction or that even promotes further SOC storage. Soil microorganisms have a key function because they regulate essential C and N turnover processes in the soil. Nutrient mobilisation processes are induced by enzymatic activity and nutrient immobilisation is caused by microbial uptake of nutrients; both activities are closely related to the size of the soil MB (Geisseler and Horwarth, 2014; Geisseler et al., 2010). The MB, which is dominated by fungi and bacteria Joergensen and Wichern, 2008), fulfils important functions in the soil. Anabolic processes lead to the incorporation of C and nutrients into biological structures and catabolic processes lead to mineralisation of organic N to NH_4^+ and of organic carbon (C) to CO_2 (Dilly et al., 2003). When organic fertilisers are applied, not all of the N supplied gets mineralised in the year of application. A part of the N remains in the soil and some becomes plant available in the following years, through microbial mineralisation processes, depending on the environmental conditions, tillage intensity and the specific characteristics of the applied organics (Daudén et al., 2004; Sørensen, 2004; Sørensen et al., 2005).

If microbial nutrient mineralisation from organic fertilisers does not occur simultaneously with plant N demand and uptake, either N deficiency or residual nitrate leaching can occur. Especially at the end of the vegetation period, inorganic N may be transferred into soil depths with the onset of the autumn rains and cannot be reached by plant roots anymore. Leaching mainly occurs as nitrate (NO_3^-), due to its monovalent negative charged anion. In contrast, NH_4^+ is positively charged and mostly adsorbed at negatively charged colloidal surfaces, avoiding the risk of leaching. Therefore, N fertilisation tailored to crop demand is essential to avoid N surplus and to minimise negative environmental effects. Insufficient N fertilisation fundamentally reduces yield and quality of the crops, thus reducing marketing opportunities and revenue. Therefore, knowledge on the N dynamics of organic fertilisers applied before planting is essential and needs to be taken into account in the farmers´ N fertilisation strategy. In Europe, inappropriate N fertilisation has led to concentrations of nitrate in groundwater bodies in some regions exceeding the EU-limit of 50 mg l^{-1} (Eurostat, 2012). For the reduction of nitrate concentrations below the critical value, regulations on fertiliser application have been tightened in some European countries. The cultivation of catch crops can immobilise inorganic N in plant biomass and, when N is abundantly available, the C rhizodeposition of catch crop stimulates microbial N immobilisation (Thorup-Kristensen et al., 2003; Fritz and Wichern, 2018; Meier et al., 2017).

It is well known that incorporation of C-rich components like cereal straw stimulates anabolic processes in soil microorganisms and consequently reduces N losses, as well as contributing to SOC maintenance (García-Ruiz et al., 2019; Nishio and Oka, 2003; Shindo and Nishio, 2005; Šimon et al., 2016; Reichel et al., 2018; Wei et al., 2020). In greenhouse experiments we already showed that *Mis* biomass provides a suitable C source to induce N immobilisation in soil; part of this N immobilisation is caused by microbial growth which also contributes to C sequestration (Stotter et al., 2021). The cultivation of C-rich crops like annual cereals can be supplemented with perennials such as *Mis*, whereas *Mis* provides important ecosystem services and can create new sales and utilisation opportunities (Pude, 2021). *Mis* is certified as a greening crop in Germany (a crop subsidised for its ecological value) (Publication Office of the European Union. Regulation No. 2017/2093). It can be cultivated on marginal sites where the cultivation of other crops is economically not feasible and can be cultivated as a low-input crop because of low fertiliser demand and no need for weed control (Emmerling and Pude, 2017). Perennial crops like *Mis* can protect soil against erosion. The harvest of *Mis* usually takes place in spring, immediately before new shoots of *Mis* appear. Consequently, the tight *Mis* habitat provides opportunities for rare wildlife to protect themselves from predators and weather conditions during the winter months and increases structural diversity in open agricultural landscapes. The specific cultivation can contribute to the restoration of biotope cross-linking (Bellamy et al., 2009; Semere and Slater, 2007), which has been lost in many cases by the structural change in agriculture in recent decades (Butler et al., 2010; Flade and Schwarz, 2013; Green et al., 2005; Gregory et al., 2005). *Mis* biomass can be used as feedstock in anaerobic digestion (Ruf and Emmerling, 2017; Schmidt et al., 2018), growing media in soilless cultivation (Nguyen et al., 2021), can be cascaded to livestock farms in the form of bedding material (Nowak et al., 2016) or it is used as an additive for the packaging industry or as construction material (Pude, 2021).

However, the question is whether C input in form of *Mis* can be applied as a straw substitute (e.g. because the cultivation of *Mis* has numerous ecological benefits or because cereal straw is exported) for microbial N immobilisation and its effects on nitrate leaching and crop yields. Therefore, in a field trial, a crop rotation with winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris* L.) and winter wheat (*Triticum* *aestivum* L.) was set-up to test the effects of two novel N-containing and C-rich organic farm fertilisers based on *Mis*.

In this context, our hypotheses were (i) *Mis* is as effective as wheat straw in immobilising additional inorganic N from mineralisation of slurry or manure and thus reduces nitrate leaching as effective as wheat straw; (ii) *Mis* and wheat straw are identical in affecting yield and quality parameters of crops of a crop rotation (iii) Microbial biomass make use of *Mis* as C source for biomass build-up and thus contribute to C sequestration.

3.3 Materials and Methods

Site Description

A field trial was set-up at Campus Klein-Altendorf (University of Bonn, Rheinbach, Germany) from September 2017 to August 2020. A typical crop rotation for the Rhine region was chosen, this consisted of winter barley (*Hordeum vulgare* L.), mustard (*Sinapis alba* L.) as catch crop, sugar beet (*Beta vulgaris*) and winter wheat (*Triticum aestivum* L.). The set-up was chosen to compare the N dynamics of two organic fertilisers based on *Miscanthus x giganteus* L. (*Mis*) in an arable soil of a conventionally farmed agricultural site. Average annual precipitation for 2018 and 2019 (420 and 490 mm) was lower than the long term average (2007 to 2016) of 633 mm. Average annual temperature for 2018 and 2019 (11.0 and 11.5°C) was greater than the long term average (2007 to 2016) of 10.2 °C.

The experiment was carried out on a Gley-Cambisol. The previous, unfertilised grassland was converted to arable in 2013. As determined by particle-size analysis according to DIN ISO 11277:2002-08 (ISO and DIN.), the soil texture is a silty loam (Table 1) and the location of the field is 50°36’3” N, 07°01’37” E; WGS 84. The basic soil properties like pH (VDLUFA A 5.1.1, 2016), P_2O_5, K_2O (VDLUFA A 6.2.1.1, 2012, Mg (VDLUFA A 6.2.4.1, 1997), B, Cu, Mn, Fe (VDLUFA A 6.4.1, 2002, SOM (DIN ISO 10694:1996-08, 1996) and N_t (DIN ISO 13878: 1998-11, 1998) are given in Table 1.

Table 1. Contents and amounts of basic soil properties of the experimental site for the top 30 cm. Values show means and standard deviation (n = 5; for SOM, SOC, N_t, C/N: n = 6).

pH (H_2O)	**P (mg kg^{-1})**	**K (mg kg^{-1})**	**Mg (mg kg^{-1})**
6.3 ± 0.06	11.4 ± 2.7	10.4 ± 1.6	14 ± 1.9
B (mg kg^{-1})	**Cu (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Fe (mg kg^{-1})**
0.5 ± 0.04	6.3 ± 0.5	169.4 ± 47.4	196.3 ± 18.6
SOM (%)	**SOC (%)**	**N_t (%)**	**C/N (ratio)**
3.9 ± 0.7	2.3 ± 0.4	0.27 ± 0.02	8.5 ± 1.2
Clay (g kg^{-1})	**Silt (g kg^{-1})**	**Sand (g kg^{-1})**	
229	597	173	

SOM = soil organic matter; SOC = soil organic carbon; N_t = total N

The biomass of *Mis* grown on another field was used for two utilisation pathways; first mixed with cattle slurry (CS) to create a CS-*Mis* mixture and second used as bedding material creating cattle manure based on *Mis* (CM-*Mis*). For comparison with *Mis*, wheat straw (WS; *Triticum aestivum* L.) biomass was used to create a CS-WS mixture and cattle manure based on WS (CM-WS). The *Mis* biomass was harvested in April 2017 (used for organic N fertilisation of winter barley), in April 2018 (used for organic N fertilisation of mustard and sugar beet) and in April 2019 (used for organic N fertilisation of winter wheat), respectively with a forage harvester (Krone Big X 480) with a set cutting length of 30 mm. WS biomass, used for mixing with CS, was broken up and baled by a Claas Quadrant 3200 FC big baler with a ROTO CUT front chopper and FineCut cutting unit. WS biomass, used for comparison to *Mis* as bedding, was not chopped and cut. WS biomass was harvested in August 2017 (used for organic N fertilisation of winter barley and mustard), in August 2018 (used for organic N fertilisation of sugar beet) and in August 2019 (used for organic N fertilisation of winter wheat). As a reference treatment for the two mixtures, a pure CS was tested. For the determination of the best possible mixing ratio of both mixed treatments (CS-*Mis*, CS-WS) concerning maximum absorption of CS to *Mis* and of CS to WS biomass, different amounts of CS (from one to ten kg of CS in steps of 0.5 kg CS) were mixed with one kg of *Mis* or WS. A complete absorption was achieved by soaking the biomass for seven days. The final mixing mass ratios were five to one for CS to *Mis* and 8.5 to one for CS to WS. After mixing, the two mixture treatments were stored for five weeks on a manure slab and covered with a silage film to prevent precipitation intrusion and allow for N immobilisation. The other option to use *Mis* on a farm was the use of *Mis* as bedding material in livestock. For this purpose, cattle were bedded with *Mis* (cattle manure from *Mis* = CM-*Mis*) and, as a reference, cattle were bedded with WS (cattle manure from wheat straw = CM-WS) according to standard farm practice and mucked out after about six weeks. In addition, two further treatments were tested, this was a mineral N-fertilisation (urea ammonium nitrate solution = UAN) as well as a treatment without any N applied (no nitrogen applied = NoN).

The application dates and rates of the N fertilisers used in the field trial are listed in Figure 1. The nutrient content of the applied fertilisers was determined by a certified laboratory and is listed in Table 2. Based on the nutrient analyses of each fertiliser, the amount of applied fertilisers was calculated, to ensure that the same amount of N across all treatments could applied, except for NoN. The solid fertilisers were applied by manure forks and the liquid fertiliser by watering cans. The crops were grown conventionally and the straw of winter barley and mustard was removed. Before the start of the field experiment, winter barley (2016/2017) and winter wheat (2015/2016) were cultivated and straw was removed for both. The trial was set up as a randomised block design with four replicates per treatment. Each plot had a size of 6 m x 16 m, with a sampled area of 3 x 8 m (to ensure uniformity of incorporation of the organic fertilisers).

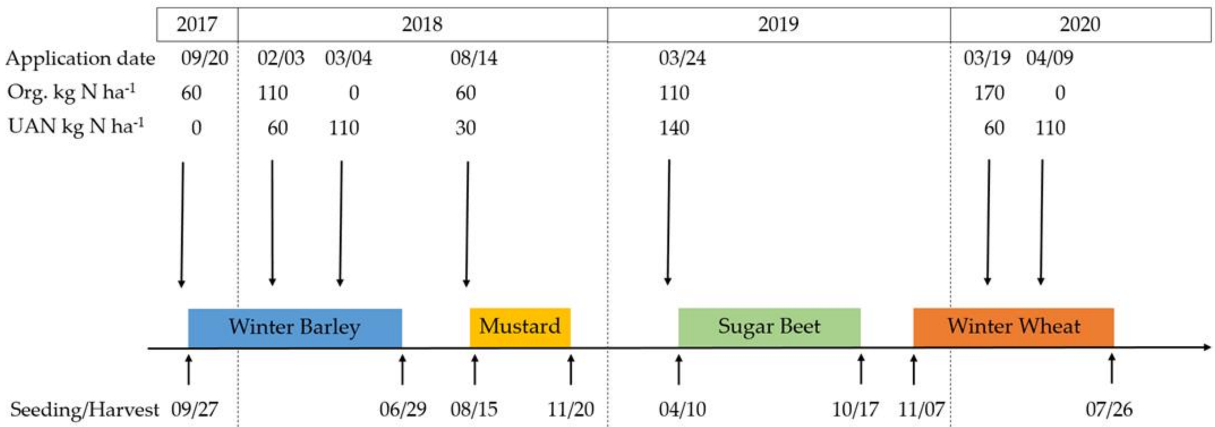

Figure 1. Dates of sowing, harvesting and N apply in kg total N ha^{-1} (Org. = Organic N apply in form of cattle slurry, cattle slurry-*Mis* (five kg to one kg), cattle slurry-wheat straw (8.5 kg to one kg), cattle manure from *Mis* shredded bedding, cattle manure from wheat straw shredded bedding; UAN = urea ammonium nitrate) during crop rotation.

Table 2. Nutrient contents of the used treatments, respectively for each N apply.

	Test Parameter	Unit	CS [1]	CS-*Mis* [2]	CS-WS [2]	CM-*Mis* [2]	CM-WS [2]	UAN [1]	*Mis* [2]	WS [2]
Autumn 2017	Dry matter	%	9.7	20.9	16.4	32.7	25.4	-	87.8	86.2
	Organic matter	%	7.2	18.3	13.4	29.2	17.3	-	85.2	79.2
	Total N	kg m^{-3}/kg t^{-1}	4.6	4.2	5.1	5.6	8.3	-	1.7	6.3
	NH_4^+-N	kg m^{-3}/kg t^{-1}	2.3	1.4	1.1	0.5	0.2	-	<0.1	0.2
	NH_4^+-N in total N	%	50	33	22	9	2	-	5	3
	pH	value	7.3	8.4	8.2	8.3	8.2	-	6	6.8
	C/N	ratio	9.0	26.0	15.0	30.0	12.0	-	288	73
Spring 2018	Dry matter	%	9.2	20.1	16.0	32.5	26.1	-	87.8	86.2
	Organic matter	%	6.7	17.6	13.0	29.8	22.6	-	85.2	79.2
	Total N	kg m^{-3}/kg t^{-1}	4.0	3.9	4.7	6.5	6.4	358	1.7	6.3
	NH_4^+-N	kg m^{-3}/kg t^{-1}	1.8	1.0	1.0	0.7	1.3	90	<0.1	0.2
	NH_4^+-N in total N	%	45	26	21	11	20	25	5	3
	pH	value	7.7	8.1	7.8	8.2	8.3	-	6	6.3
	C/N	ratio	9.8	27.0	16.0	27.0	20.0	-	288	73
Autumn 2018	Dry matter	%	9.9	23.7	18.6	24.1	39.6	-	90.1	90.9
	Organic matter	%	7.3	21.0	15.6	21.8	22.1	-	86.9	86.3
	Total N	kg m^{-3}/kg t^{-1}	4.7	4.3	5.2	5.2	11.4	358	3.0	4.4
	NH_4^+-N	kg m^{-3}/kg t^{-1}	2.2	1.1	1.4	1.4	2.1	90	0.2	0.2
	NH_4^+-N in total N	%	47	26	27	27	18	25	7	5
	pH	value	-	-	-	-	-	-	-	-
	C/N	ratio	8.9	28.2	17.5	24.5	11.2	-	166	114.8
Spring 2019	Dry matter	%	7.0	20.9	15.5	22.8	17.5	-	90.1	90.9
	Organic matter	%	5.2	17.4	12.5	20.1	14.1	-	86.9	86.3
	Total N	kg m^{-3}/kg t^{-1}	3.8	2.9	3.8	4.6	5.2	358	3.0	4.4
	NH_4^+-N	kg m^{-3}/kg t^{-1}	2.1	0.4	0.4	0.8	1.6	90	0.2	0.2
	NH_4^+-N in total N	%	55	14	11	17	31	25	7	5
	pH	value	7.8	8.3	8.1	7.1	7.4	-	6.1	6.3
	C/N	ratio	7.9	35.0	19.2	25.3	15.7	-	166	114.8

Spring 2020	Dry matter	%	8.7	21.7	16.5	32.6	17.9	-	89.8	92.1
	Organic matter	%	6.7	18.3	13.3	28.4	12.6	-	86.8	87.8
	Total N	kg m^{-3}/kg t^{-1}	4.6	3.8	4.3	6.5	4.9	358	1.9	3.7
	NH_4^+-N	kg m^{-3}/kg t^{-1}	2.1	0.6	0.8	0.7	1.3	90	0.1	0.2
	NH_4^+-N in total N	%	46	16	19	11	27	25	5	5
	pH	value	7.3	8.4	8.3	8.3	8.1	-	6	6.4
	C/N	ratio	8.4	27.6	17.9	25.5	14.8	-	262.3	136.8

CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, *Mis* = *Miscanthus*-shredding, WS = wheat straw-shredding. Indication of the nutrient content in: [1] kg m^{-3}; [2] kg t^{-1}.

Soil Analyses

Per sampling and plot, six soil samples were taken for analysis. The analysis was carried out by forming a soil aliquot of each plot and determining inorganic N, soil microbial biomass C (MBC) and soil microbial biomass N (MBN). The N analysis quantified inorganic N as NH_4^+ plus NO_3^-, since NO_2^- was not detectable. For the preparation of analysis, soil samples were sieved at 2 mm and visible roots were removed. For the analysis of inorganic N, field-fresh soil was weighed into polyethylene bottles, filled with 100 cm^3 of 1% K_2SO_4 and then placed on an overhead shaker. All extracts were filtered (VWR 305; particle retention: 2–3 µm) and then stored until further analysis at −18 °C to avoid microbial transformation processes. Just before starting the analyses, extracts were defrosted rapidly to room temperature. Then the content of inorganic N was determined with the AutoAnalyzer 3 from Bran + Luebbe GmbH Norderstedt, Germany.

For the analysis of MBC and MBN, chloroform fumigation-extraction (Vance et al., 1987; Brookes et al., 1985) was used. For this, field-fresh soil was used for fumigation- and for direct extraction. The fumigation was carried out in a vacuum desiccator at 25 °C using ethanol-free chloroform ($CHCl_3$) for 24 h in the dark. The fumigated and non-fumigated samples were then extracted with 40 cm^3 of 0.5 M K_2SO_4 and placed on a horizontal shaker. All extracts were filtered (VWR 305; particle retention: 2–3 µm) and stored until analysis at −18 °C. Just before starting the analyses, extracts were defrosted rapidly to room temperature. In all extracts, organic C and total N were detected after combustion at 800 °C by using a Multi N/C 2100S (Analytic Jena, Jena, Germany). MBC was calculated as the ratio of extractable C (EC) and k_{EC}. EC is the difference between organic C extracted from fumigated soils and non-fumigated soils, whereas kEC is a correction factor with the value of 0.45 (Wu et al., 1990) and represents the fraction of microbial C released in 24 h of fumigation. MBN gets calculated as the ratio of extractable N (EN) and k_{EN}. EN is the difference in organic N extracted from fumigated soils and non-fumigated soils, where kEN is a correction factor with the value of 0.54 (Brooks et al., 1985; Joergensen and Mueller, 1996) and represents the fraction of microbial N. Inorganic N, MBC and MBN were extrapolated to one hectare by assuming a soil bulk density of 1.32 g cm^{-3}.

Plant Analyses

In each crop, during the vegetation period and at harvest time, plants were cut and used for determination of N uptake. Plant biomass were cut during vegetation period. Five times when winter barley, one time when mustard, nine times when sugar beet and six times when winter wheat was cultivated. For winter barley and winter wheat, 1.2 m^2 and for sugar beet, 0.9 m^2 were taken for each sampling of each plot. At harvest date, 12 m^2 was sampled of each crop and of each plot by a plot combine (winter barley, winter wheat), by plot beet lifters (sugar beet) and by hand (mustard). Of the 1.2 m^2 and 0.9 m^2 plant samples, the whole plant biomass and of the 12 m^2 samples only a plant aliquot was placed in a drying oven at 50 °C until constant weight to calculate dry matter yield. Whole dried plant material was shredded and pulverised by using a cutting mill (Retsch, Haan, Germany) at 3,000 rpm and by using a sieve of 0.25 mm. The C and N concentrations of each harvest-biomass were analysed by combustion and gas chromatographic analysis, using an elemental analyser (EA 3000 series, HEKAtech GmbH, Wegberg, Germany). Plant N uptake was calculated by using dry matter yield and N concentration of each sampling and then extrapolated to one hectare, without considering N uptake of the root biomass.

The amount of N released from applied fertilisers was estimated by subtracting the sum of plant N uptake of NoN treatments from the N uptake of the fertilised treatments at harvest. The total inorganic N value just before seeding of each crop and just after each harvest was included in the calculation by subtracting these differences from the N uptake of each treatment. This calculation does not take into account N losses in the form of ammonia and nitrous oxide and nitrate leaching.

The yields of each crop were calculated by extrapolating corn yield, sugar beet yield and total biomass of each 12 m² harvested plot to one hectare. The protein content of winter barley and winter wheat and the sugar content of sugar beet were analysed, after cleaning the grain and beet, using near-infrared spectroscopy.

Statistical Analyses

Statistical analyses and visualisations were performed using IBM SPSS Statistics 27 (IBM Ehningen, Germany). Normal distribution of data was tested using the Shapiro–Wilk test. Levene's test based on means was used to verify the homogeneity of variances. To identify treatment differences between three treatments, a one-way analysis of variance (ANOVA), following by a post hoc Tukey's HSD (honest significance difference) test were used. To identify differences between two treatments, a t-test was used. Tukey's HSD and t-test were performed separately for each experiment. When data were not normally distributed or no homogeneity of variance was detected, Welch test and Games–Howell test were used to identify differences for three or more treatments, Mann–Whitney–U test was used to identify differences between two treatments. A p-value of 0.05 was used as threshold for significant interactions.

3.4 Results

Plant N-Uptake

The plant N uptake indicates differences in the N availability of the applied N fertilisers. The main crops (winter barley, sugar beet, winter wheat), fertilised with mineral N (UAN) showed the highest N uptake during the whole vegetation period of all main crops throughout the crop rotation, respectively from the first sampling until harvesting (Figure 2). Only non-significant differences in N uptake from mineral or organic fertiliser were detected in the mustard catch crop. When crops were fertilised with cattle slurry (CS), lower amounts of N were plant-available throughout the crop rotation as compared to mineral fertilisation (UAN). When cereals were cultivated (winter barley, winter wheat), tendency of greater amounts of N (non-significant) were taken up after CS fertilisation, compared to the other organic fertilisers (CS-*Mis*, CS-WS, CM-*Mis*, CM-WS).

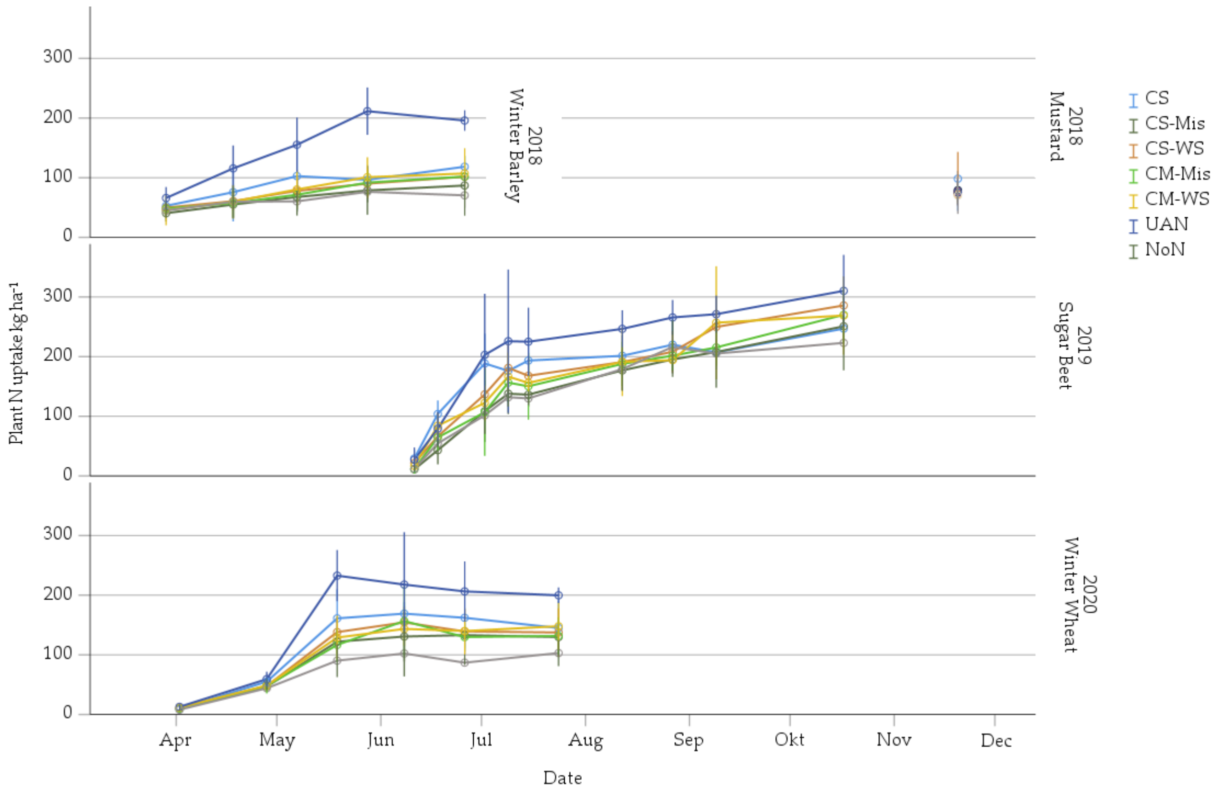

Figure 2. Plant N uptake during vegetation period (markings) from seeding to harvest of winter barley, mustard, sugar beet and winter wheat for each treatment (CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied). Error bars show Standard Deviations.

When *Mis* or WS were each mixed with CS and then applied as C-rich organic fertilisers to cereals and mustard, the N uptake at harvest was slightly lower (non-significant) compared to pure CS (Figure 2, Table 3). Thereby, the addition of *Mis* to CS induced a greater reduction of N uptake compared to the addition of WS to CS, respectively in winter barley and winter wheat (Figure 2, Table 3). At the first samplings, the N uptake was stronger reduced than at the last samplings, respectively for CS-*Mis* and CS-WS for both, winter barley and winter wheat (Figure 2, Table 3).

In contrast, when both mixtures were applied to sugar beet, at harvest the N uptake was slightly greater (CS-*Mis* = 2%, CS-WS = 16%), compared to pure CS (Figure 2, Table 3). However, when CS-*Mis* was applied, in four of the first five samplings, it led to a statistically significant initial N immobilisation (*sampling one* = $F(2, 9) = 6.98$, $p = 0.015$; *sampling two* = $F(2, 9) = 25.39$, $p < 0.001$; *sampling three* = $F(2, 9) = 6.95$, $p = 0.015$; *sampling four* = $F(2, 9) = 3.06$, $p = 0.097$; *sampling five* = $F(2, 9) = 8.58$, $p = 0.008$), indicated a greater N immobilisation compared to CS-WS (only in one of the first five samplings) (Table 3). Although the reductions of N uptakes, induced by added *Mis* and WS to CS, are mostly not significant, they are apparent, indicated by the means (Figure 2, Table 3).

When the two manure types were applied as fertiliser, no statistically significant differences between both manures in N uptake were detectable. In most samplings during vegetation period, N uptake was slightly lower after fertilisation of main crops with CM-*Mis* (Table 4).

Table 3. Percentage of N uptake of the two mixtures (CS-*Mis* = cattle slurry-*Mis* (five kg to one kg); CS-WS = cattle slurry-wheat straw (8.5 kg to one kg)) to N uptake of cattle slurry (CS), to different samplings during vegetation period and at time of harvest. Listed for crop rotation, which consists of winter barley, mustard (catch crop), sugar beet and winter wheat, respectively. Different letters within a column and within each cultivar show significant differences. One-way ANOVA; $p < 0.05$; ns = not significant; n = 4.

		Sampling								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Harvest***
Cultivar	**Treat-ment**	**N uptake [% of CS]**								
Winter Barley	CS	100 ns	100 ns	100 ns	100 ns					100 ns
	CS-*Mis*	77 ns	73 ns	66 ns	82 ns					73 ns
	CS-WS	94 ns	80 ns	76 ns	93 ns					86 ns
Mustard	CS									100 a
	CS-*Mis*									88 b
	CS-WS									93 b
Sugar Beet	CS	100 a	100 a	100 a	100 ns	100 a	100 ns	100 ns	100 b	100 b
	CS-*Mis*	38 b	42 b	58 b	78 ns	70 b	88 ns	89 ns	100 b	102 b
	CS-WS	74 ab	64 b	73 ab	103 ns	87 ab	95 ns	95 ns	121 a	116 a
Winter Wheat	CS	100 ns	100 ns	100 ns	100 a	100 ns				100 ns
	CS-*Mis*	101 ns	85 ns	76 ns	77 b	82 ns				90 ns
	CS-WS	118 ns	87 ns	86 ns	91 ab	86 ns				95 ns
Cumu-lated	CS									100 ns
	CS-*Mis*									90 ns
	CS-WS									99 ns

*final Sampling

Table 4. Percentage of N uptake of the new type of cattle manure from *Mis* shredded bedding (CM-*Mis*) to N uptake of conventional cattle manure from wheat straw bedding (CM-WS) to different samplings during the vegetation period and at time of harvest. Listed for crop rotation, which consists of winter barley, mustard (catch crop), sugar beet and winter wheat, respectively. Different letters within a column and within each cultivar show significant differences. One-way ANOVA; $p < 0.05$; ns = not significant; $n = 4$.

		Sampling								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Harvest***
Cultivar	**Treat-ment**	**N uptake [% of CM-WS]**								
Winter Barley	CM-*Mis*	106 ns	96 ns	89 ns	91 ns					95 ns
	CM-WS	100 ns	100 ns	100 ns	100 ns					100 ns
Mustard	CM-*Mis*									109 ns
	CM-WS									100 ns
Sugar Beet	CM-*Mis*	72 ns	77 ns	87 ns	94 ns	96 ns	98 ns	104 ns	84 ns	100 ns
	CM-WS	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns
Winter Wheat	CM-*Mis*	81 ns	99 ns	90 ns	109 ns	93 ns				89 ns
	CM-WS	100 ns	100 ns	100 ns	100 ns	100 ns				100 ns
Cumu-lated	CM-*Mis*									98 ns
	CM-WS									100 ns

*final Sampling

Microbial Mineralisation-Immobilisation as Affected by Added *Miscanthus* Straw

After mixing *Mis* or WS as a source of organic C with CS and applying it as fertiliser to cereals and mustard (catch crop), the fraction of mineralised N was in tendency slightly reduced, but not significant, compared to pure CS (*winter barley: $F(2, 9) = 1.03$, $p = 0.396$; mustard: $F(2, 9) = 0.94$, $p = 0.448$; winter wheat: $F(2, 9) = 2.37$, $p = 0.149$*). Thereby, for winter barley and winter wheat, the addition of *Mis* resulted in a slightly lower mineralised N fraction compared to WS addition, though the difference was not significant (*winter barley: $T(6) = -0.88$, $p = 0.428$, winter wheat: $T(6) = -0.90$, $p = 0.405$*) (Figure 3A).

After the application of CS-*Mis* and CS-WS as fertilisers to sugar beet, the fraction of mineralised N was in tendency slightly increased, though the difference was not significant, compared to pure CS (*$F(2, 9) = 0.90$, $p = 0.442$*). This increased N uptake of sugar beet after fertilisation with the mixtures (CS-*Mis*, CS-WS) may indicate an increased N release through mineralisation after initial N immobilisation by soil microorganisms.

When cattle manure (CM) from *Mis* as well as from WS were used as organic fertilisers, about the same fraction of both manures were mineralised, respectively for each crop. When they were applied to sugar beet, the fraction of N mineralised was greater than when they were applied to the cereals. When they were applied to the catch crop, the same amount of N was mineralised as became plant available from the soil N pool in the unfertilised mustard. Consequently, no additional N was mineralised compared to the plots without any N supplied (Figure 3B).

Cumulated for the total crop rotation, a slightly lower N fraction of the two mixtures was mineralised (CS-*Mis* = mean 12% ± SD = 12, CS-WS = 23% ± 12), although not statistically significant, from CS (CS = 29% ± 12) (*$F(2, 9) = 1.49$, $p = 0.277$*). Between both manure types, no statistically significant difference in the fraction of mineralised N was detected (CM-*Mis* = 20% ± 7, CM-WS = 23% ± 12) (*$T(6) = -0.27$, $p = 0.793$*) (Figure 3A, 3B).

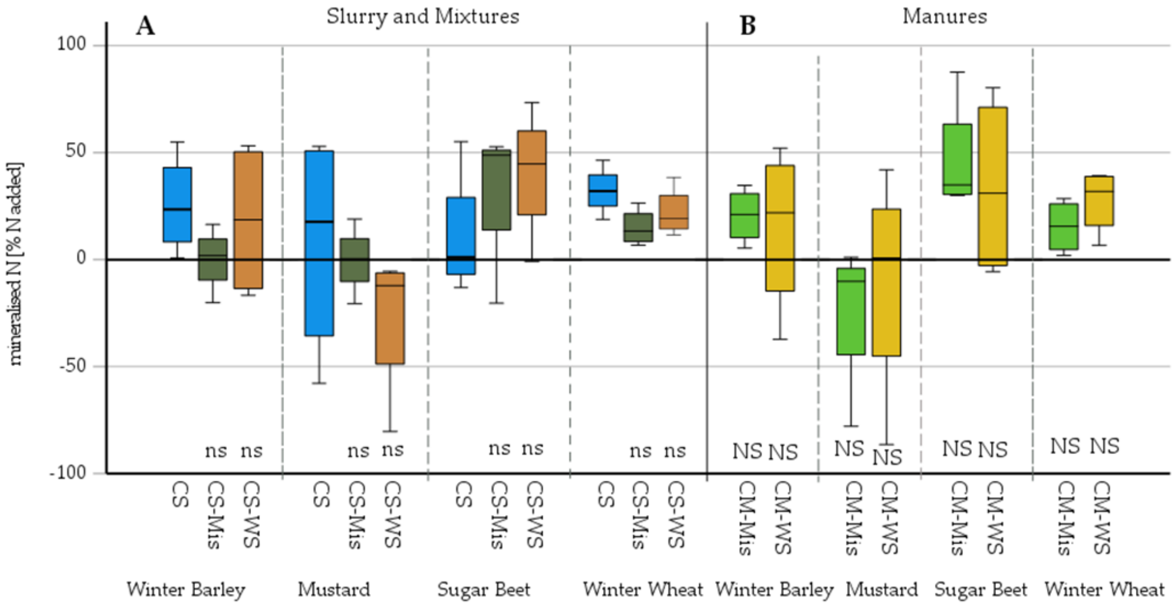

Figure 3. N mineralised expressed as % of N applied for winter barley, mustard (catch crop), sugar beet and winter wheat, calculated using the amount of N uptake and N apply of each cultivar. **(A)**: CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg); **(B)**: CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding). The horizontal bars indicate the median and the whiskers indicate the 1.5 x IQR (interquartile range). No significant differences were indicated between the two types of mixtures (CS-*Mis*, CS-WS) and between the two types of manures (CM-*Mis*, CM-WS); $p < 0.05$, t-test; not significant; $n = 4$.

At the end of the field experiment, no significant differences in soil microbial biomass C (MBC) and N (MBN) were analysed. Neither between both mixtures (*MBC:* $T(6) = 0.18$, $p = 0.862$; *MBN:* $T(6) = 0.27$, $p = 0.800$), nor between both manures (*MBC:* $T(6) = 0.67$, $p = 0.529$; *MBN:* $T(6) = -0.43$, $p = 0.683$) (Figure 4A and 4B).

After the addition of C to CS, as *Mis* or WS and applied as organic fertilisers during crop rotation, the MBC and MBN were both slightly greater (Figures 4A and 4B), compared to CS only, although not statistically significant (*MBC:* $F(2, 9) = 0.47$, $p = 0.641$; *MBN:* $F(2, 9) = 1.15$, $p = 0.361$). Thus, the slightly lower N mineralisation of the mixtures compared to pure CS (Figure 2 and 3) is generally reflected by a slightly greater MB (Figures 4A and 4B). After the crop rotation and after the fertilisation with CS-*Mis*, MBC was of 12% (156 kg ha^{-1}) and CS-WS was of 9% (123 kg ha^{-1}) higher than the non-fertilised plots (Figure 4A) and MBN was of 26%

(60 kg ha^{-1}, CS-*Mis*) and of 23% (54 kg ha^{-1}, CS-WS) higher than the non-fertilised plots (Figure 4B). Apparently, when *Mis* and WS were used for mixing with CS, soil microorganisms were able to assimilate slightly more N and C as compared to CS, when they were used as fertilisers during a crop rotation.

When the manure types (CM-*Mis*, CM-WS) were applied as fertilisers, both, the MBC and MBN were greater compared to the mixtures (CS-*Mis*, CS-WS), although not statistically significant (Figure 4A and 4B) (*MBC:* $F(3, 12) = 0.84$, $p = 0.499$; *MBN:* $F(3, 6.17) = 1.67$, $p = 0.270$)). Although there was no difference between the cumulated fractions of mineralised N after fertilisation with manures and mixtures (Figure 3 and description in text above), the MBC and MBN of manures were non-significantly, but slightly greater, compared to mixtures (Figure 4A and 4B). This indicates an increased uptake of applied manure-N by the MB, compared to applied CS-mixtures.

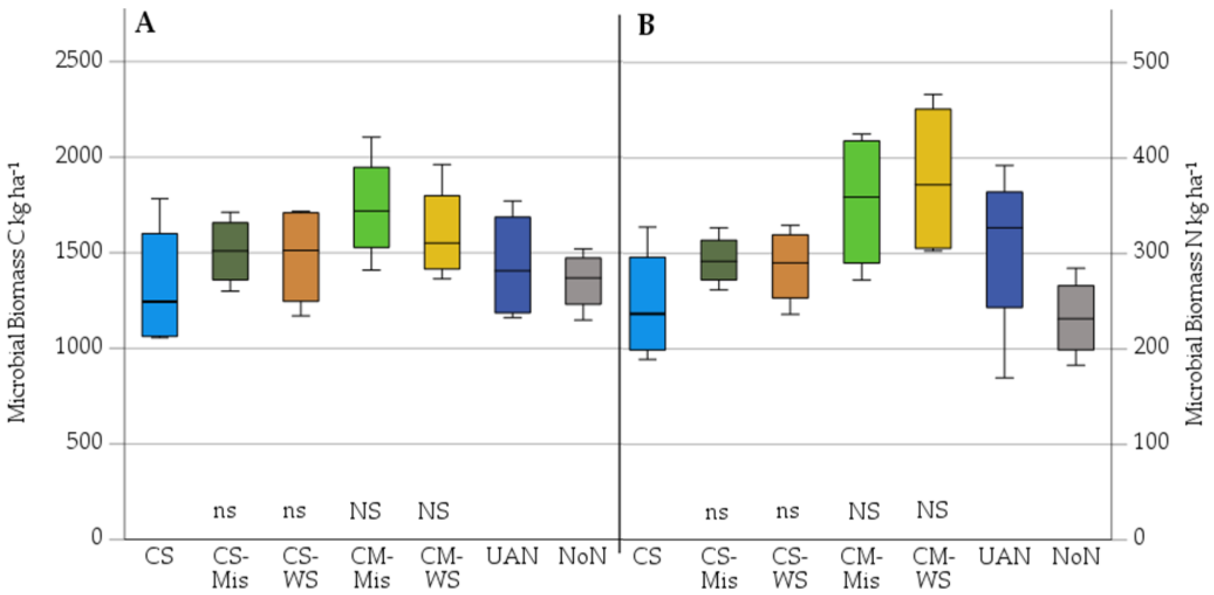

Figure 4. (A): Microbial biomass C kg ha^{-1} and **(B)** Microbial biomass N kg ha^{-1} of the soils, following the application of different treatments (CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied). The horizontal bars indicate the median and the whiskers indicate the 1.5 x IQR (interquartile range). T-test, $p < 0.05$, ns = not significant; n = 4.

Inorganic N as affected by added *Miscanthus* straw

The soil inorganic N indicates the amount of plant-available N during the crop rotation before, during and after the application of the different types of N fertilisers and in the plots without any N applied (Figure 5). After adding organic C in the form of *Mis* or WS to CS and then applied as fertilisers just before seeding of winter barley in September 2017, in the middle of December 2017, the inorganic N of the soils (soil layer 0 – 90 cm) was slightly reduced to an amount of 26 kg ha^{-1} and 23 kg ha^{-1} (December 2017 in Figure 5) ($F(2, 4.213) = 4.92, p = 0.079$). Apparently, winter barley was not able to take up the entire amount of mineralised N from the CS, before the end of the vegetation period in 2017. The greater amount of inorganic N in autumn 2017, after CS-fertilisation, was not detectable anymore at the end of winter (February 2018), so that N loss in the form of nitrate-leaching after CS-fertilisation might have been at least 20 kg N ha^{-1} greater compared to all other organic fertilisers. Accordingly, the addition of C in the form of *Mis* and WS, was effective in reducing N loss over the winter months (February 2018 in Figure 5).

In April 2018, when N demand of winter barley was high, N mineralisation of the mixtures was reduced, indicated by a reduced N uptake (Figure 2, Table 3) and by a reduced amount of inorganic N after fertilisation with CS-*Mis* and CS-WS, compared to pure CS (April 2018 in Figure 5) ($F(2, 5.27) = 4.10, p = 0.084$). After fertilisation with manures (CM-*Mis*, CM-WS) and after no fertilisation, the amounts of inorganic N were almost identical, compared to the CS-mixtures. When both mixtures (CS-*Mis*, CS-WS) were applied just before seeding of the catch crop mustard, both caused a slightly lower amount of inorganic N compared to pure CS fertilisation ($F(2, 5.11) = 2.13, p = 0.212$), whereby the application of CS-*Mis* caused a slightly greater N immobilisation and thus lower amount of inorganic N, compared to CS-WS application ($T(6) = -1.70, p = 0.140$) (September 2018 in Figure 5). Although at the end of the vegetation period of 2018, mustard N uptake was slightly greater after CS fertilisation compared to the other treatments (Figure 2), the inorganic N was also slightly greater, compared to the amount of inorganic N of mixtures and of manures ($F(4, 7.11) = 0.43, p = 0.783$) (November 2018 in Figure 5). The amount of inorganic N after UAN fertilisation was even greater, but not significant ($F(5, 8.04) = 3.37, p = 0.062$) (November 2018 in Figure 5). This indicates again that the addition of *Mis* to CS was as effective as WS addition to CS in reducing the amount of potential N leaching, compared to pure CS. Neither the application of the mixtures nor the application of the manures to mustard resulted in a greater amount of inorganic N, compared to the mustard without any N applied (November 2018 in Figure 5).

In February of 2019, in the CS-plots, the amount of inorganic N was 94 kg ha^{-1}, compared to 66 kg ha^{-1} in the CS-*Mis*- and to 84 kg ha^{-1} in the CS-WS-plots. This indicates an earlier start of N mineralisation of CS, compared to the mixtures, which were applied in 2018. Obviously, *Mis* and WS addition to CS also resulted in a lower N mineralisation in the year following application, whereas *Mis* addition caused a slightly slower N mineralisation compared to WS addition ($T(6) = -1.48, p = 0.190$).

In spring 2019 (sampling date May 27th), after the N fertilisers were applied to sugar beet, the added *Mis* and WS to CS caused N immobilisation. Thereby the added *Mis* to CS caused a significant greater N immobilisation compared to added WS to CS, indicated by the amount of inorganic N of 69 kg ha^{-1} (CS-*Mis)*, compared to the amount of inorganic N of 99 kg ha^{-1} (CS-WS) ($T(6) = -2.87, p = 0.028$) (May 2019 in Figure 5). Until the harvest of sugar beet, nearly all available N was taken up by, except after UAN application (October 2019 in Figure 5). After harvest of winter wheat, CS-*Mis* fertilisation caused a slightly lower amount of inorganic N compared to CS-WS fertilisation ($T(6) = -1.43, p = 0.201$) (August 2020 in Figure 5).

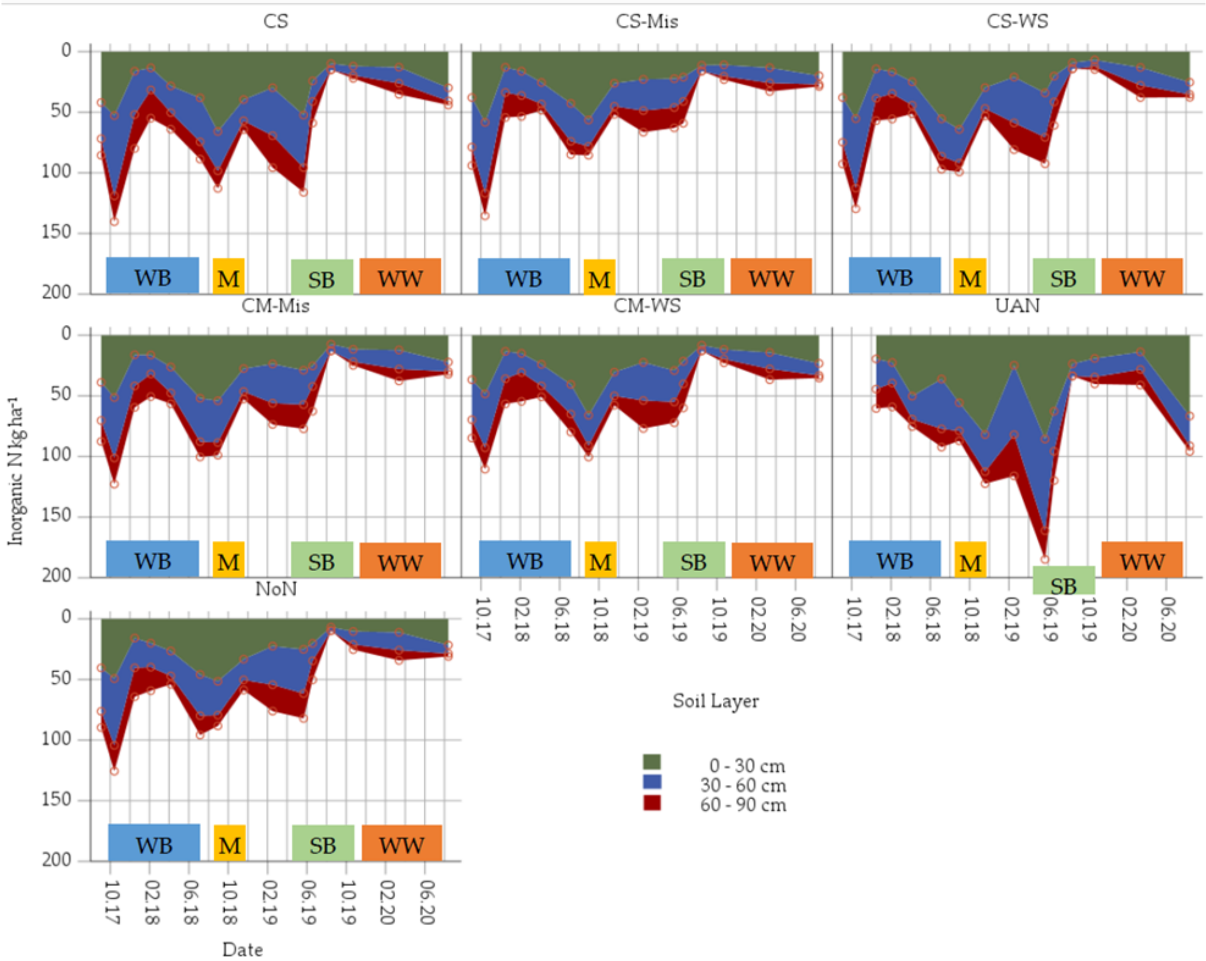

Figure 5. Inorganic N (NH_4^+, NO_3^-) in soil layers of 0 – 30 cm, 30 – 60 cm and 60 – 90 cm during crop rotation, consisting of winter barley (WB), mustard (M), sugar beet (SB) and winter wheat (WW) for each treatment (CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = No nitrogen applied). Labeling on the x-axis indicates the month and the year; data points represent the mean of n = 4.

Yield and quality parameters as affected by added *Miscanthus* Straw

The yield and quality parameters of the main crops were differently affected by the application of the types of N fertilisers (Table 5). When CS-*Mis* or CS-WS were applied as fertiliser to winter barley, both caused a slightly reduction in protein content (*F(2, 9) = 2.02, p = 0.188)*, compared to pure CS fertilisation. In contrast, the application of CS-*Mis* and CS-WS to sugar beet caused a significant greater beet yield of around 15 Mg ha^{-1}, compared to pure CS fertilisation (*F(2, 9) = 5.87, p = 0.023)*. However, N availability to later vegetation stages after CS fertilisation must have been greater, indicated by a slightly greater amino-N content in the beets, compared to the amino-N content after fertilisation with mixtures (*F(2, 9) = 2.16, p = 0.17)*. Thereby, the amino-N content after CS-*Mis* application was slightly, but non-significantly, lower compared to the CS-WS application (*T(6) = -2,383, p = 0.055)*. When the mixtures were applied to winter wheat, both did not affect the grain yield nor the protein content, compared to pure CS fertilisation, *F(2, 9) = 1.82, p = 0.217* (Table 5).

When both manure types were applied to winter barley, no difference were detected in grain yield *(T(6) = -0.165, p = 0.875)* and protein content *(T(6) = -1.852, p = 0.114)*. Beet yields were not significantly different between the two manure treatments *(F(1, 6) = 5.68, p = 0.055)*, yields were around 94 Mg ha^{-1}, with a corresponding sugar yield around 12 Mg ha^{-1}. Amino-N after CM-*Mis* application was slightly lower, compared to Amino-N after CM-WS application (*T(6) = -2.149, p = 0.075)*. Apparently, the N fertilisers with a greater organic C content (Table 2) resulted in greater beet and sugar yields (Table 5). The N mineralisation in the plots without any N supply resulted in the same beet and sugar yield, compared to CS fertilisation (Table 5). In contrast, the beet yield was negatively affected by UAN application, with an amount of around 80 Mg ha^{-1}, apparently due to an excessively high N input (Table 5). This also led to an excessively high amino-N content of 37 Mmol kg^{-1}, making sugar extraction more difficult. Thereby, the sugar yield only reached an amount of about 10 Mg ha^{-1} (Table 5). When manure was applied to winter wheat, no difference in grain yield, but a slightly lower protein content after CM-*Mis* fertilisation was detected *(T(6) = -1.879, p = 0.109)*, indicating a slightly lower N availability of CM-*Mis*, compared to CM-WS (Table 5).

No statistical evidence for negative effects in yields and quality parameters are provided, after application of each of the organic fertilisers compared to UAN fertilisation. Nevertheless, slightly differences in the means of yields and quality parameters of winter barley and winter wheat are shown (Table 5).

Table 5. Yield and quality parameter of each of the cultivars of the crop rotation (winter barley, mustard, sugar beet, winter wheat) and for each treatment. The value represents the mean and standard deviation of n = 4. One-way ANOVA for CS, CS-*Mis* and CS-WS; t-test for CM-*Mis* and CM-WS, $p < 0.05$; Different letters within a column show significant differences; ns = not significant.

Treat-ment	Winter Barley		Mustard	Sugar Beet			Winter Wheat	
	Corn Yield	Protein	Biomass yield	Beet Yield	Sugar Yield	AmN	Corn Yield	Protein
	Mg ha^{-1}	%	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	Mmol kg^{-1}	Mg ha^{-1}	%
CS	5.0 ± 1.2 ns	11.0 ± 0.7 ns	2.3 ± 0.9 ns	84.3 ± 2.9 b	11.2 ± 4.0 b	21.2 ± 4.4 ns	8.9 ± 0.6 ns	10.0 ± 0.4 ns
CS-*Mis*	4.7 ± 1.1 ns	10.6 ± 0.1 ns	1.8 ± 0.3 ns	98.0 ± 4.4 a	12.1 ± 4.5 ab	14.3 ± 2.7 ns	8.4 ± 0.2 ns	9.4 ± 0.4 ns
CS-WS	4.9 ± 0.5 ns	10.4 ± 0.1 ns	1.6 ± 0.3 ns	99.3 ± 10.7 a	12.2 ± 4.7 b	16.9 ± 3.6 ns	8.9 ± 0.6 ns	9.5 ± 0.4 ns
CM-*Mis*	4.8 ± 0.7 NS	10.3 ± 0.3 NS	1.8 ± 0.6 NS	94.5 ± 12.2 NS	12.0 ± 4.5 NS	15.3 ± 3.0 NS	8.8 ± 0.5 NS	9.4 ± 0.4 NS
CM-WS	4.9 ± 1.0 NS	10.6 ± 0.1 NS	1.7 ± 0.3 NS	92.9 ± 9.4 NS	12.0 ± 4.4 NS	17.7 ± 3.8 NS	8.8 ± 0.7 NS	9.9 ± 0.3 NS
UAN	6.9 ± 1.7	14.1 ± 0.5	1.2 ± 0.3	79.7 ± 4.7	9.9 ± 3.8	37.3 ± 4.9	9.1 ± 0.2	12.0 ± 0.3
NoN	3.9 ± 0.5	10.2 ± 0.3	1.5 ± 0.2	85.7 ± 13.0	11.3 ± 4.4	16.6 ± 3.4	7.2 ± 0.4	9.1 ± 0.6

CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied.

3.5 Discussion

As an additional C source in arable farming, we tested the use of *Mis* concerning N immobilisation-mineralisation, soil inorganic N, effects on yield and quality parameters of cultivated crops and microbial biomass build-up. We demonstrate that *Mis* as C source caused at least as effective as WS an N immobilisation, with a tendency of a more pronounced impact on N immobilisation. This also resulted in a lower amount of soil inorganic N in the plots fertilised with the mixtures compared to the CS-treatment, resulting in lower N losses over winter. We also demonstrate that the impact on N immobilisation-mineralisation was depending on the duration of the N uptake of the respective crop.

***Miscanthus* as C source for microbial N Immobilisation**

The fertilisers with *Mis* biomass (CS-*Mis*, CM-*Mis*) caused at least as effective as fertilisers with WS biomass (CS-WS, CM-WS) an N immobilisation, when they were applied to winter wheat and winter barley. This field trial showed a tendency of greater N immobilisation and lower inorganic N contents after application of *Mis* based fertilisers, whereas differences were not significant. In pot experiments, the effectiveness of *Mis* and WS biomass amended fertilisers for N immobilisation was already detected (Stotter et al., 2021). There, a tendency of a greater N immobilisation after application of fertilisers with *Mis* biomass (CS-*Mis*, CM-*Mis*) compared to WS amended fertilisers were explained by the microbial processes due to the biochemical differences in the C/N ratio of the added substrates (Wei et al., 2020; Aiken, 2014; Bhogal et al., 2016; Cabrera et al, 2005). In our field experiment, the differences of N immobilisation between *Mis* amended and WS amended fertilisers were less obvious compared to those of Stotter et al. (2021). The *Mis* based fertilisers were characterised by a greater C/N ratio of between 25 to 35 for CS-*Mis* and CM-*Mis*, compared to a C/N ratio of 11 to 19 for CS-WS and CM-WS (Table 2). The differences in the C/N ratio between the *Mis*- and WS-based fertilisers are due to greater C/N ratio of the *Mis* raw biomass material of 166 to 288, compared to the C/N ratio of 73 to 137 of the WS biomass feedstock (Table 2). Additionally, the C amount of the CS-*Mis*-mixture was enhanced by a lower mixing ratio of CS-*Mis* of 5:1, compared to CS-WS of 8.5:1, which could suggest a greater microbially available C from *Mis* biomass. The lower mixing ratio of CS-*Mis* also resulted in a lower content of total N of the CS-*Mis*-fertiliser and therefore, in greater application rates to the crops to achieve the same total N application rate. Consequently, greater amounts of C due to CS-Mis compared to CS-WS and due to CM-Mis compared to CM-WS were applied to the soil. Nevertheless, no significant difference in N immobilisation was analysed.

Furthermore, contents of holocellulose and lignin of *Mis* and WS (*Mis*: holocellulose 70%, lignin = 14% to 19%; WS: holocellulose = 68% to 76%, lignin = 8% to 25%) and as well NH_4^+ content, a biochemical factor that strongly influences N availability (Wei et al., 2020; Eiland et al., 2001; Corbeels et al., 2000; Rahn et al., 1999; Van Kuijk et al., 2017), were almost identical for the *Mis*- and WS-based fertilisers (Table 2). Therefore, we suggest that available C input in the form of *Mis* may in principle appear to have greater effects on microbial N immobilisation than C input in form of WS. In this field trial, this potential effect was apparently overridden by other factors, like the high SOM content of the previous grassland area.

When the organic fertilisers were applied to sugar beet, a greater amount of N uptake after fertilisation with mixtures and manures compared to pure CS was observed (Figure 2 and Figure 3), which was not expected and apparently was attributed to a combination of influences. Missing precipitation for weeks with radiation intensity above the average from June to September of 2019, as well as amounts of precipitation below the average in the previous year, have led to drought and thus to extremely dry conditions also in the subsoil. Incorporated mixtures and manures consisted of a greater fraction of organic matter, compared to pure CS, which could have resulted in greater soil moisture, resulting in better N mineralisation, improved growth conditions and yield formation with a greater amount of N uptake in these plots in August and September of 2019 as organic matter increases water holding capacity in the soil. Furthermore, technical complications, which hindered the immediate incorporation of the applied CS, may have resulted in greater N losses from the CS in the form of ammonia than usual and thus resulting in a lower N uptake than expected. However, the C input of *Mis* in the form of mixtures and manures shows that *Mis* is at least as effective as WS in providing inorganic N over the winter months.

Obviously, the period of one crop rotation was too short for analysing clear differences between both C-rich amendments. The extension of the experiment over a longer period of time with further C input could increase SOM and potentially result in measurable differences between *Mis* and WS addition.

Miscanthus as C source for microbial-derived C sequestration

The greater microbially available C input in form of *Mis* compared to WS, respectively for mixtures and manures, was not reflected in a greater MB, both for MBC and MBN (Figure 4A and 4B). In contrast, the greater C-input of *Mis*-manure (CM-*Mis*) compared to CS-*Mis*-mixture and the greater C-input of WS-manure (CM-WS) compared to CS-WS-mixture each resulted in a slightly greater MBC, though not significant. This indicates that the amount of C input could not have been the only factor influencing the slightly greater MBC in the plots, fertilised with manures compared to the MBC of the plots fertilised with mixtures, but rather the characteristics of the manures were responsible for stronger growth of MB compared to the mixtures (Figure 4B). Other potential factors influencing the MB such as the pH value of the mixtures and manures (pH-values: CS-*Mis* = 8.3, CS-WS = 8.1, CM-*Mis* = 8.0, CM-WS = 8.0 (Table 2)) and natural site factors (soil type, soil temperature, soil moisture) (Dilly et al., 2004) can be excluded as potential factors influencing MB, based on the fact that they were comparable of each fertiliser and field plot. Possibly, the period during the manure accumulated in the animal barn could have led to a stimulation of the manure-microorganisms, which had a positive effect on the soil MB.

Nevertheless, both, the mixture and manure from *Mis* each promote, just like the mixture and manure from WS, the soil MB, though not significant. The C and N supply by the *Mis*-based fertilisers caused a promotion of soil MB, which contributes to the formation of microbial necromass (Kallenbach et al., 2016). Microbial necromass has an essential role in the formation and stability of SOM and is thus a key component of C sequestration (Miltner et al., 2011; Hobara et al., 2014; Khan et al., 2016; Liang et al., 2010). Therefore, the soil fertility can be improved, the SOM can contribute to a reduction of N losses and the promotion of MB contributes to ecological soil functioning for the resilience of arable soils (Bengtsson, 2002). Especially, regions with high organic N occurrences, for example, already formed by excretions in animal farming, have the potential for SOM build-up, because N is an essential N compound for the build-up of SOC. However, SOM build-up cannot be exploited without C availability, thus *Mis* biomass can provide an additionally essential C source for many agricultural regions. Farmers can cultivate it on semi-productive arable land to develop a C source regionally (Pude, 2021) and can be cultivated as a subsidised crop (Emmerling and Pude, 2017). The utilisation as bedding materials for animals is more economical due to the cascading than to mix the cattle slurry with *Mis* by an additional working step.

The cultivation of *Mis* in areas with high livestock farming and thus with a high demand of bedding or biomass for bioenergy-production can buffer the demand for cereal-C in arable regions (Yesufu et al., 2020) and thus counteract the continuous SOM losses (Steinmann et al., 2016). In arable regions, *Mis* cultivation on low-yield potential sites and thus C-production counteracts the dependence on external C sources such as imported organic fertilisers like slurry or manures of predominantly farming areas. Furthermore, *Mis* can contribute to fulfilling the expected increase in demand for bedding materials (Van Weyenberg et al., 2016), as a result of the increasing transformation of animal farming from slatted floors to bedding with straw.

In soils with high available N contents, *Mis* could find suitability for N immobilisation as high C amendment without any addition of excreta. Wheat straw and spruce sawdust were already designated and applied as high C amendments to prevent N losses (Reichel et al., 2018; Wei et al., 2020). Especially in soils with a high potential of N mineralisation, C application could be a functional step in arable farming to contribute to reducing N losses.

However, the induced N immobilisation after the addition of C-rich plant material is not a new knowledge at all (Eiland et al., 2001a; Eiland et al., 2001b), but can be increasingly implemented in the future as a tool to counteract SOM degradation as well as nitrate loss on susceptible arable land. The special cultivation of C-rich plants in the form of perennial crops such as *Mis* can be used as a component of future arable farming systems to ensure the yield capacity of soils. In addition to *Mis*, other greening measures and non-used fields of grassland can be transferred into a usage that aims C-production, which either provides bedding material or directly applied to the cropland for SOM build-up. Therefore, unused grasslands are suitable, as they usually have to be cut once per year.

3.6 Conclusions

Integration of *Miscanthus x giganteus* (*Mis*) in arable farming for provision of ecosystem services and utilisation as additional C source of *Mis*-amended fertilisers is resulting in different N dynamics, depending on the crop. The application of fertilisers amended with *Mis* was at least as effective as fertilisers amended with wheat straw (WS) biomass in the immobilisation of inorganic N from cattle slurry (CS) and cattle manure (CM), when they were applied to cereals. Furthermore, both *Mis*-amended fertilisers (CS-*Mis* and CM-*Mis*), led to better growth conditions, compared to CS application, under the given dry weather conditions during parts of the crop rotation, indicated by the N uptake and yield of sugar beet. Application of *Mis*-fertilisers during a three-year crop rotation caused a temporary significant reduction of inorganic soil N and thus reduced N loss compared to CS. Compared to WS addition to CS, added *Mis* to CS caused an identical reduction of inorganic N during winter. We suggest that application of C-rich fertilisers over a longer period of time may have greater influences on SOM and on the reduction of inorganic N over winter months than has been analysed during this rotation period.

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4 Hyperspectral assessment of winter barley, after the fertilisation of different farm manures, based on chopped *Miscanthus*, at the time of full maturity

Hyperspektrale Erfassung eines mit unterschiedlichen Wirtschaftsdüngern aus *Miscanthus*-Häckselgut versorgten Wintergerstenbestandes zum Zeitpunkt der Vollreife

This work has been published as:

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Michael Stotter performed the experiments (90%), MS analysed the data and wrote the paper (90%).

Text was not modified from the published version.

4.1 Abstract

Knowledge of the dynamics of nitrogen mineralisation of organic fertilisers is essential with regard to the regionally high nitrogen inputs and the further strictness of the new fertiliser regulations. Especially when new organic nitrogen fertilisers are being introduced, their environmental effects, especially those of reactive nitrogen compounds, are of great importance. To replace expensive and time-consuming destructive measurements by non-invasive methods, the implementation of sensors has to be tested and validated. As part of the INTERREG project "food pro.tec.ts", different agricultural fertilisers, in particular those based on chopped *Miscanthus* (*Mis*), were tested in field experiments and the N uptake of winter barley was determined. Hyperspectral imaging was used to detect the plant conditions, once at the time of full maturity. The spectral range of the sensor was between 500 nm and

900 nm. Just before harvesting, differences between several treatments are indicated and their nitrogen supply can be determined by using two spectral indices.

Zusammenfassung: Vor dem Hintergrund der regional hohen N-Einträge aus der Landwirtschaft und der anstehenden weiteren Verschärfung der Düngeverordnung ist die Kenntnis der N-Mineralisierungsdynamik verschiedener Wirtschaftsdünger unerlässlich. Insbesondere bei der Etablierung neuartiger organischer N-Dünger ist deren Umweltwirkung, insbesondere die der reaktiven N-Verbindungen, von hoher Relevanz. Um zukünftig kosten- und zeitintensive destruktive Bonituren durch nicht-invasive Bonituren ersetzen zu können, ist die Erprobung und Validierung der sensorbasierten Methodik erforderlich. Dazu werden im Rahmen des INTERREG-Projektes „food pro.tec.ts“ in einem Feldversuch mit Wintergerste verschiedene Wirtschaftsdünger, insbesondere Miscanthus-Häcksel, ausgebracht und die N-Aufnahmemengen erfasst. Eine einmalige hyperspektrale Messung im Wellenlängenbereich von 500 nm bis 900 nm mittels Drohne zum Zeitpunkt der Vollreife dokumentiert den Zustand des Bestandes. Mit Hilfe zweier Indizes können kurz vor der Ernte noch Unterschiede durch die Applikation verschiedener N-Dünger erfasst und auf den N-Versorgungszustand geschlossen werden.

4.2 Introduction

As part of the reform process of the Common Agricultural Policy (CAP) in 2017, *Mis* was recognised as a crop eligible for greening. As a specific regulation, the utilisation of biomass for material and energy purposes is allowed (LWK NRW, 2018). The cultivation of *Mis* is expected to expand, especially with regard to on-farm use as animal bedding. The resulting product, a cattle manure of *Mis* bedding, has not yet been characterised, nor is the dynamics of nitrogen mineralisation after application to arable crops known. There is a lack of data on N uptake to determine fertiliser requirements and on the effect on soil N dynamics. The current use of this new type of fertiliser is therefore taking place without knowledge of its environmental impact and cannot therefore be used in accordance with good professional practice. The expected tightening of the fertiliser regulation of 2017, to prevent massive financial sanctions by the EU due to inadequate implementation of programmes to protect water resources from nitrates pollution (EuGH, 2018), illustrates the importance of the issue and the knowledge required (BMEL, 2019).

In this field experiment, organic fertilisers with added chopped *Mis* are tested for nitrogen mineralisation by cultivation of winter barley using destructive methods. At the time of full maturity, the spectral characteristics of winter barley were determined using a drone and a hyperspectral camera to estimate the N supplied to the crop. To validate the hyperspectral data, destructive measurements of yield-relevant plant parameters were taken at the same time. On the one hand, the aim is to evaluate the hyperspectral data and the conventionally measured plant parameters as a factor of N uptake. In this way, the potential and practical suitability of hyperspectral sensors at the time of full maturity of winter barley will be determined. On the other hand, the applicability of the non-destructive method to detect differences between fertiliser treatments at harvest time will be tested.

4.3 Material and Methods

As part of the EU INTERREG project "Food Pro.tec.ts", a field trial was performed on the Campus Klein-Altendorf of the University of Bonn. The experiment was conducted on a Gley-Cambisol. The soil texture was a silty loam. Before planting winter barley, 60 kg N ha^{-1} of different organic fertilisers were applied in four replications.

The biomass of *Mis*, grown on another field, was used in two ways; first mixed with cattle slurry (CS) to produce a CS-*Mis* mixture and secondly used as bedding material to produce cattle manure based on *Mis* (CM-*Mis*). For comparison with *Mis*, wheat straw (WS; *Triticum aestivum* L.) biomass was used to create a CS-WS mixture and cattle manure, based on WS (CM-WS). For the determination of the best possible mixing ratio of both mixed treatments (CS-*Mis*, CS-WS) concerning maximum absorption of CS to *Mis* and of CS to WS biomass, different amounts of CS (from one to ten kg of CS in steps of 0.5 kg CS) were mixed with one kg of *Mis* or WS. A complete absorption was achieved by soaking the biomass for seven days. The final mixing mass ratios were five to one for CS to *Mis* and 8.5 to one for CS to WS. A pure CS was tested as a reference treatment for the two mixtures.

At the start of the vegetation in the following spring, a further 110 kg N ha^{-1} was applied using the above treatments. The urea ammonium nitrate (UAN) fertilised treatment received no N application in the autumn, but three applications from the spring onwards, amounting to a total of 170 kg N ha^{-1}. Another treatment was tested with no N application (NoN). The nutrient content of the applied fertilisers was determined by a certified laboratory and is shown in Table 1.

Table 1: Nutrient contents of the applied fertilisers.

	CS [kg m^{-3}]	CS-*Mis* [kg t^{-1}]	CS-WS [kg t^{-1}]	CM-*Mis* [kg t^{-1}]	CM-WS [kg t^{-1}]	UAN [kg/m^{-3}]
Total N	4,6	4,2	5,1	5,6	8,3	358
NH_4^+-N	2,3	1,4	1,1	0,5	0,2	90
C/N ratio	9,0	26,0	15,0	30,0	12,0	-

From sowing to harvest, several measurements of plant biomass were taken (plant biomass, number of ears, plant height, plant chlorophyll content using SPAD-502, CNS content of the whole plant). At the time of full maturity, a DJI Matrice 600 drone with a Rikola hyperspectral camera (Senop, Finland) was used, providing a wavelength range of 500 nm to 900 nm. Each spectrum was averaged based on reflectance characteristics. The Plant Senescence Reflectance Index (PSRI; Merzlyak et al., 1999) and the Normalised Difference 800/680 (ND 800/680; Tucker, 1979) were used for the interpretation of the amount of aboveground barley biomass N uptake, measured destructively. The indices are defined by

$$\mathrm{PSRI} = (R_{678} - R_{500}) / R_{750})$$

$$\mathrm{ND800/680} = (R_{800} - R_{680}) / (R_{800} + R_{680}).$$

4.4 Results

The application of organic and mineral fertilisers was generally performed as expected. The amount of N applied with UAN was taken up by the winter barley. This was followed, at a much lower level, by N uptake after CS fertilisation. The N uptake after fertilisation with mixtures and manures (CS-*Mis*, CS-WS, CM-*Mis*, CM-WS) was less low and did not differ from each other. When no N was applied, the N uptake of winter barley was 70 kg ha^{-1} (Figure 1).

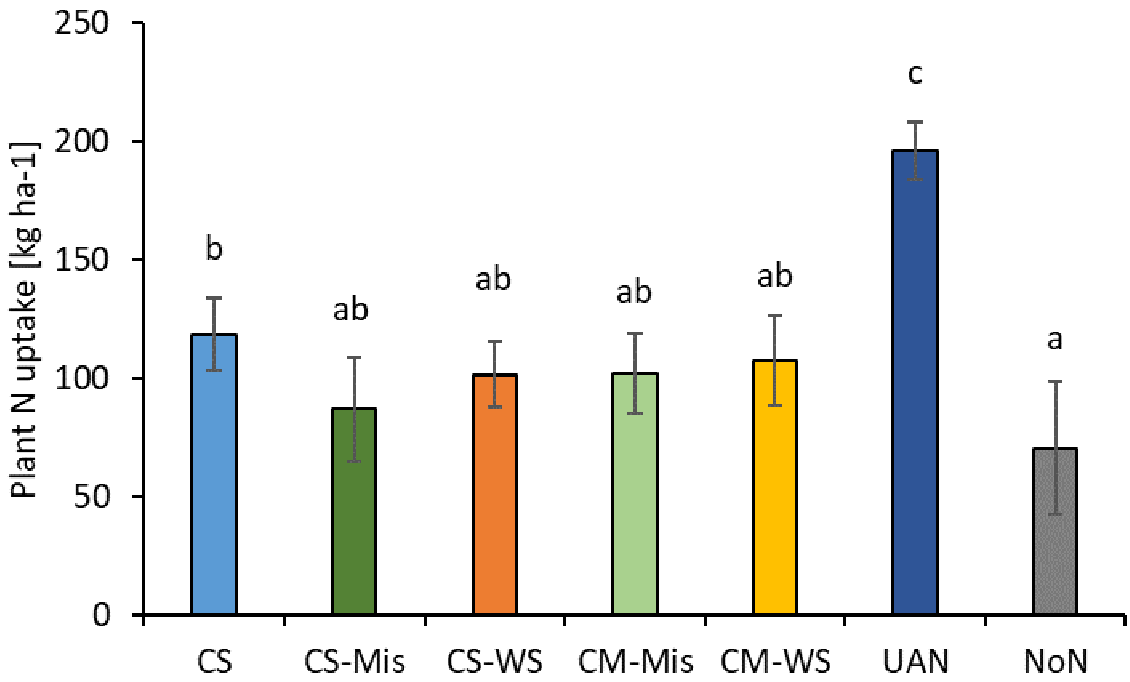

Figure 1: Plant N uptake of winter barley at harvest time for each treatment ((CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied). Error bars show standard deviations; $p < 0.05$; $n = 4$.

The "Plant senescence reflectance index (PSRI)", recorded at the same time, indicated a difference in reflectance between UAN fertilisation and fertilisation with the organic fertilisers (CS, CS-*Mis*, CS-WS, CM-*Mis*, CM-WS) and when no N was applied (NoN) (Figure 2). When the plant N uptake was higher, the reflectance was lower (UAN). When the plant N uptake was reduced (CS, CS-*Mis*, CS-WS, CM-*Mis*, CM-WS, NoN), the reflectance was higher, which was expressed by the PSRI (Figure 2). After Normalised Difference 800/680 was adjusted, the differences in reflectance were less sensitive than after using PSRI. No differences were observed when the organic fertilisers were applied. Only after UAN fertilisation, the reflectance was significantly higher compared to all other treatments.

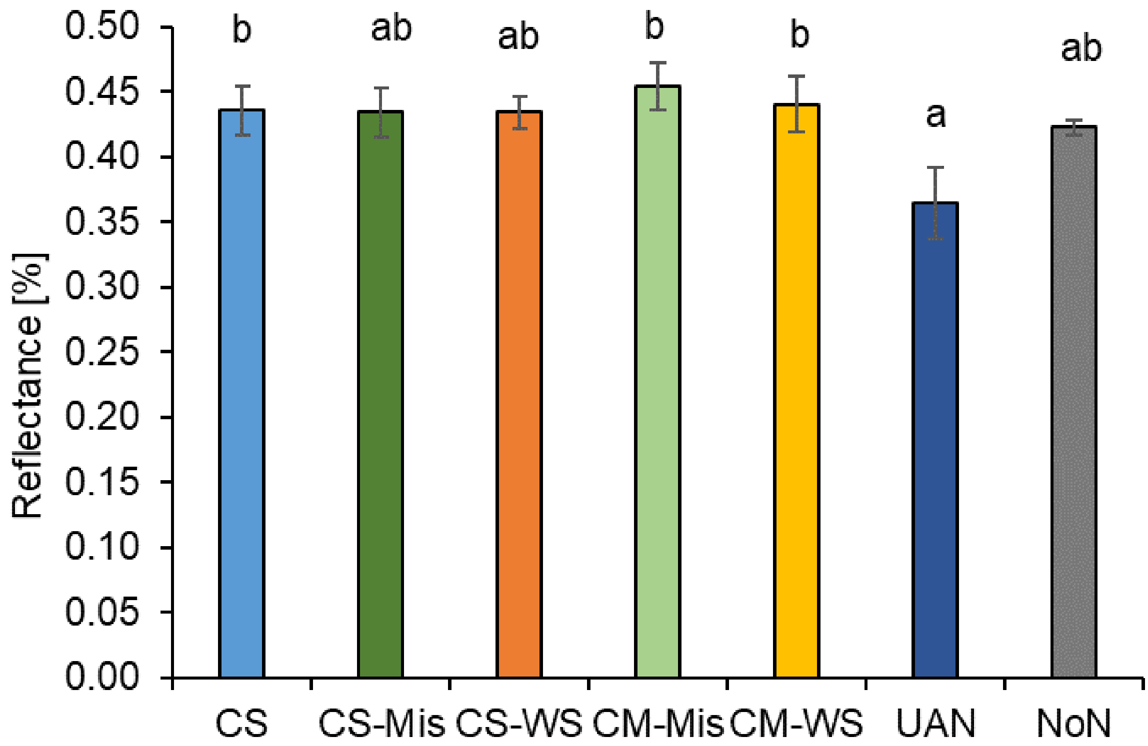

Figure 2: Average reflectance according to the plant senescence reflectance index (PSRI) for each treatment ((CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied). Error bars show standard deviations; $p < 0.05$; $n = 4$.

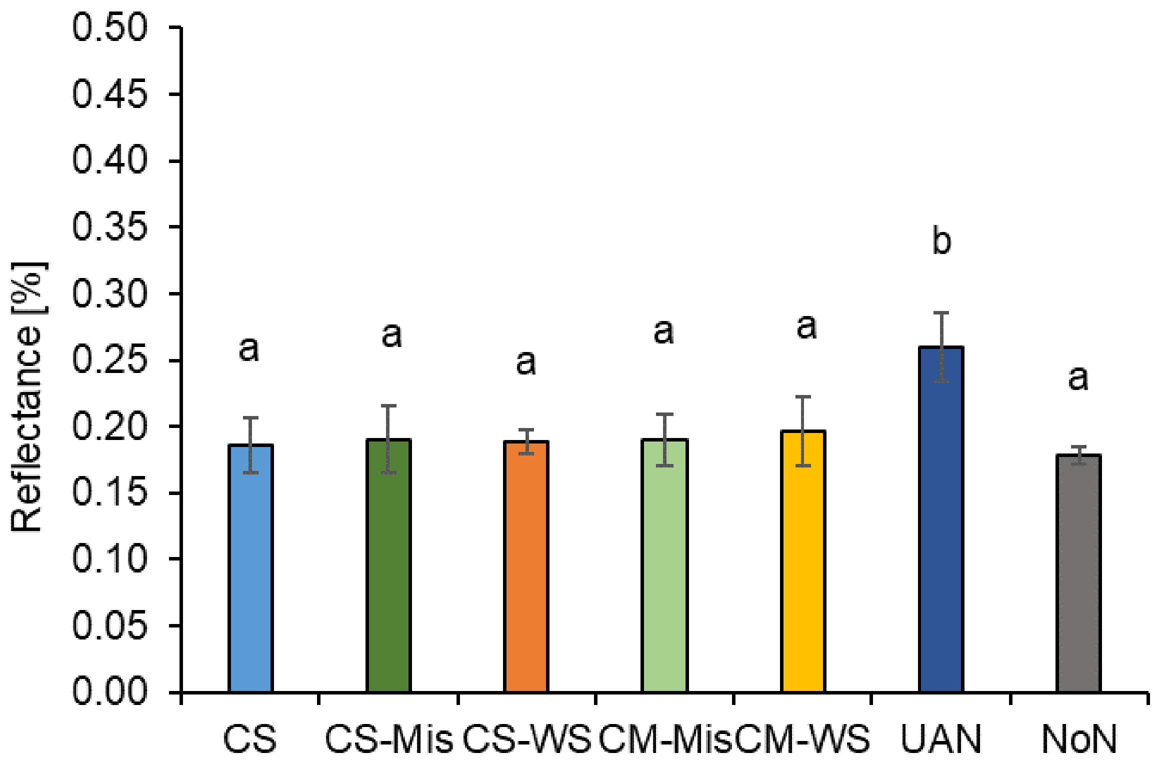

Figure 3: Average reflectance according to the Normalized Difference 800/680 (ND 800/680) for each treatment ((CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied). Error bars show standard deviations; $p < 0.05$; $n = 4$.

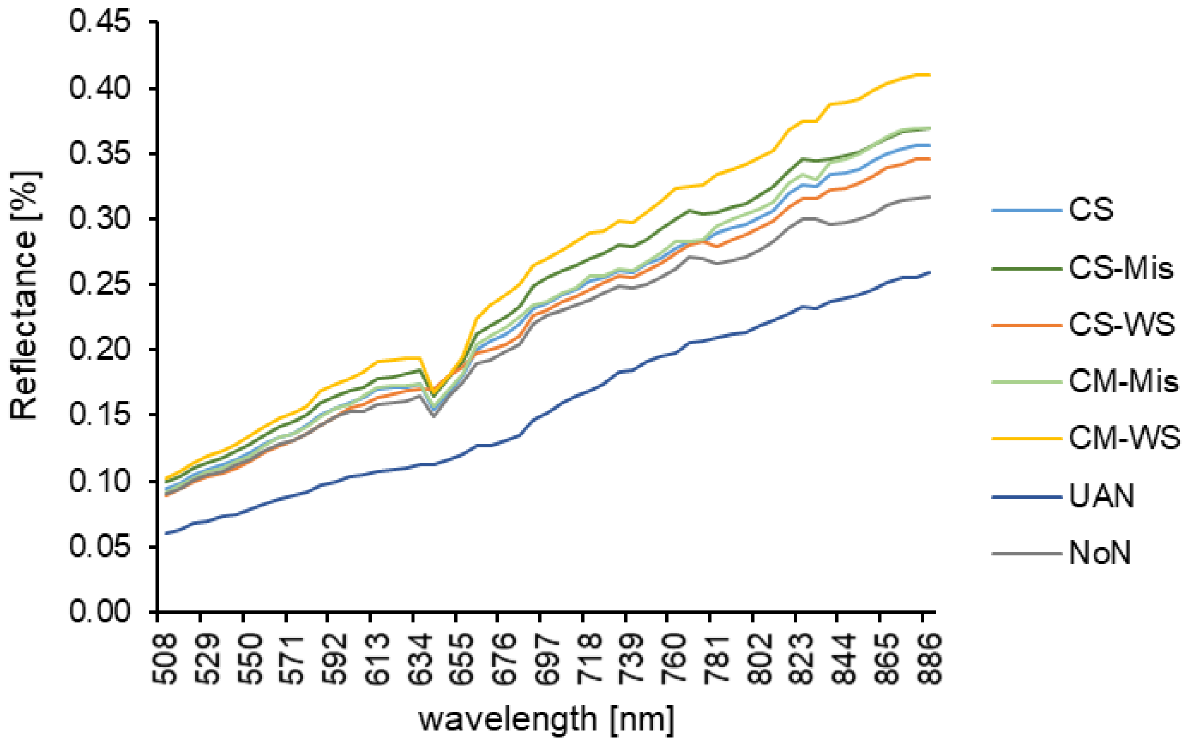

Figure 4: Spectrum of winter barley at time of full maturity for each treatment ((CS = cattle slurry, CS-*Mis* = cattle slurry-*Mis* (five kg to one kg), CS-WS = cattle slurry-wheat straw (8.5 kg to one kg), CM-*Mis* = cattle manure from *Mis* shredded bedding, CM-WS = cattle manure from wheat straw shredded bedding, UAN = urea ammonium nitrate, NoN = no nitrogen applied). Error bars show standard deviations; $p < 0.05$; $n = 4$.

The spectrum of winter barley at time of full maturity does not show the typical characteristic of a vital leaf, indicated by a peak around 550 nm and a rise around 690 nm (Figure 4). Rather, after UAN fertilisation, the spectrum increases continuously with increasing wavelengths and is characterised by a lower reflectance compared to the other fertilisers. The reflectance of winter barley after application of organic fertilisers and winter barley without N application were higher than that after UAN fertilisation. Of these, NoN has the lowest reflectance. This is most similar to UAN. After CM-WS application, the spectrum is indicated by the highest reflectance over the whole wavelength. In contrast to the UAN fertilisation, there is a decrease in the spectrum around 645 nm after the application of organic fertilisers and after no nitrogen application.

4.5 Discussion

The differences in N uptake of winter wheat after application of different organic fertilisers and one mineral fertiliser are due to the different compounds of N in the different fertilisers. UAN only contains mineral nitrogen compounds, which are generally available to plants in the year of application. In contrast, organic fertilisers contain a large part of the N in organically bound form and only part of the N is available to plants in the year of application. The N availability in the year of application is about twice as high for slurry as for manure, which explains the slightly higher N uptake of slurry compared to manure.

An equivalence between the time-consuming destructive determination of the total N uptake and the hyperspectral detections could not be confirmed at the time of full maturity. The absolute N uptake determined destructively could be analysed using the hyperspectral imaging so that differences between the applied fertilisers could be identified using indices. The calculation of the PSRI shows differences in the senescence stage of the crop, which was applied with different fertilisers. Merzlyak et al. (1999) used the PSRI to describe the decrease in reflectance of a leaf with decreasing chlorophyll content. It is also known that the chlorophyll content in the leaf decreases with increasing senescence and that a higher N uptake leads to a later onset of senescence. Therefore, the lower reflectance after UAN application can be explained by a higher N uptake. There is no significant relationship between senescence progression and N uptake after the other fertilisers (CS, CS-*Mis*, CS-WS, CM-*Mis*-CM-WS) were applied.

Using the Normalised Vegetation 800/680, and similar to the PSRI, a difference can be identified between UAN fertilisation and organic fertilisation as well as NoN. The main difference is the lower reflectance of the winter barley with higher N uptake compared to lower N uptake. This is explained by the higher reflectance of the crops with higher N uptake in the near infrared region (from 780 nm), which is used by the ND800/680. The small differences between the organic fertilisers that could be detected with the destructive N measurement are probably due to the fact that the winter barley has reached an advanced stage of maturity. They may be at the same stage of senescence and the spectral analysis should have been done earlier to distinguish between them. This is also suggested by the spectra of all fertiliser treatments (Figure 4). They do not show the typical spectrum of a vital leaf, characterised by a peak at 550 nm and a steep rise around 690 nm. Due to advanced senescence, the amount of chlorophyll in the leaf would have been insignificant for detection and extrapolation of nitrogen uptake. Instead, the spectrum is similar to a soil signal. Its intensity is influenced by the different development of the plant biomass and can be particularly strong in crops with an LAI < 1.5 (Lilienthal, 2013).

In addition, the organic fertilisers were applied to the soil surface without being incorporated. There was no complete decomposition until harvest and this must have had a significant effect on the spectral signatures. This is confirmed by the spectrum of the NoN, characterised by the lowest plant biomass, which is closer to the spectrum of UAN, characterised by the highest plant biomass, than the spectrums after organic fertilisation, characterised by more plant biomass than in NoN. After the application of CM-WS, the reflectance and the organic matter applied were the highest. Probably, the organic matter of CM-WS could have had the greatest effect on the reflectivity of the soil signal. Finally, a hyperspectral detection of the different organic fertilisers and the soil signal was not performed. It is therefore recommended to include the soil signal and the applied organic fertilisers in a future hyperspectral imaging of crops.

5 Final Discussion

The type of arable farming has a substantial impact on soil C and N dynamics of soils and thus plays an important role in global climate change and for aquatic and terrestrial ecosystems (Tilman et al., 2002; Lal, 2004; Tilman et al., 2011; Masson-Delmotte et al., 2021, Chen et al., 2014). Therefore, it is important to manage the agricultural strategy in a way that minimises negative and maximises positive impacts. This work aims to improve the productivity, resilience and environmental sustainability of agriculture through specific C and N management, while protecting and restoring biodiversity, as intended by the United Nations (2015) and the European Commission (2019). Global climate change has increased the demands on agriculture. New management strategies in cropping systems, through adjustments in C and N management, could lead to a reduction in climate change and to an increase in the resilience of cropping systems to extreme weather conditions.

It is well known that the resilience of arable soils can be increased by SOM build-up. The maintenance and SOM build-up is essential for the mitigation of global climate change (Tilman et al., 2001). SOM build-up requires organic matter and nutrients that are stabilised in clay-humus complexes over time. They are not available to plants during the period of fixation. Plant available nutrients are required for crop production. Their application must be adapted to the plant N requirements in order to minimise and prevent nutrient losses to the environment. Soil C and nutrient dynamics are largely controlled by SMB. Depending on the understanding of their metabolic processes, the microbial metabolism can be specifically influenced by agronomic measures (soil tillage, crop rotation, residue management etcetera) in order to optimise N fertilisation according to plant N requirements, to reduce N losses and to improve SOM conversion and build-up.

The experiments were performed to test whether the application of organic C biomass as chopped *Mis* contributes to SOM build-up, promotes N immobilisation of inorganic N from the mineralisation of slurry or manure, reduce nitrate leaching during winter, while determining yield and quality parameters. As an organic C biomass, chopped *Mis* was chosen to test this novel and additional C source for agriculture, as *Mis* cultivation is already established and in line with the objectives of the Green Deal (Emmerling and Pude 2017, European Commission, 2019). Furthermore, the specific characteristics of chopped Mis biomass with a higher C/N ratio compared to cereal straw indicate a higher potential affecting C and N dynamics, as is known from cereal straw (Corbeels et al., 2000; Shindo and Nishio, 2005; Reichel et al., 2018). Therefore, two possible on-farm utilisation pathways for Mis biomass were considered. One was the utilisation of Mis biomass as bedding and the other was the mixing of Mis biomass with cattle slurry. Both were then implemented in cropping system experiments. Mis as a bedding material represents a possible agricultural utilisation, while Mis mixed with cattle slurry is not practical due to an additional processing step, but represents an alternative for comparison with WS.

The first main objective (hypotheses one, two and three) of this thesis was the identification of the potential of fertilisers with Mis biomass to immobilise inorganic N. Under controlled growth conditions in the greenhouse, the N uptake of german ryegrass indicated a higher tendency for N immobilisation after application of fertilisers containing *Mis* biomass (CS-*Mis*, CM-*Mis*) compared to fertilisers containing WS (CS-WS, CM-WS). In the field experiment, N immobilisation was almost identical between fertilisers containing *Mis* and those containing WS.

The higher N immobilisation of inorganic N from slurry and manure in the greenhouse can be explained by the biochemical characteristics of the *Mis* biomass. The higher C/N ratio of the fertilisers with *Mis* (CS-*Mis* and CM-*Mis* = 25 - 35) compared to the fertilisers with WS (CS-WS and CM-WS = 11 - 19) and the lower mixing ratio of the CS-*Mis* (5:1) compared to the CS-WS (8.5:1) led to a higher microbial C availability provided by the *Mis* biomass and thus to a higher microbial N immobilisation. Also in the field experiment, the applied amount of C was higher in the *Mis*-based fertilisers, but N immobilisation was not higher than in the WS fertilisers, but was at the same level. It is possible that agricultural management practices, such as tillage, and the effects of weather may have led to greater remineralisation of N. It is also possible that the greater complexity of factors under field conditions overlay the marginal differences detected under controlled conditions. Based on the results that both, *Mis* and WS, induced immobilisation of inorganic N, the first hypothesis can be confirmed (1: *Miscanthus* is as good as wheat straw in immobilising additional inorganic N from mineralisation of slurry or manure). Holocellulose and lignin content also influence the microbial metabolism of C and N compounds (Wei et al., 2020; Eiland et al., 2001; Corbeels et al., 2000; Rahn et al., 1999; Van Kuijk et al., 2017). However, these were not investigated in the present thesis. In the literature, similar holocellulose and lignin contents are reported for both biomasses, but with a large range, especially for WS (*Mis*: Holocellulose = 70%, Lignin = 14% to 19%; WS: Holocellulose = 68% to 76%, Lignin = 8% to 25%) (Rahn et al., 1999; Corbeels et al., 2000; Eiland et al., 2001a; Cabrera et al., 2005; Dittmar and Stubbins et al., 2014; Bhogal et al., 2016; Van Kuijk et al., 2017; Wei et al., 2020). Considering the variability, it is possible that there were stoichiometric differences that may have affected microbial C availability. A higher holocellulose-lignin ratio increases microbial metabolism and thus N immobilisation and N remineralisation. Therefore, it is recommended to include the analysis of holocellulose and lignin content in future experiments. Induced N-immobilisation can have different objectives and can have both agronomic advantages and disadvantages. The benefits are SOM build-up and the reduction of reactive N losses. Fixing part of the N input to SOM build-up is an investment in soil fertility and a contribution to global climate change mitigation through C sequestration. However, N immobilisation also reduces plant N uptake, which can have a negative impact on yield and direct monetary returns. Reducing N losses through N immobilisation reduces the potential monetary loss of N and reduces negative environmental impacts. The most critical period for N leaching is the winter months when no plant N uptake takes place. Especially the main crops rapeseed and field bean and, depending on the previous N fertilisation, also maize remain with high soil inorganic N contents after harvest (Sieling and Kage, 2006). This N cannot be completely taken up by the following winter wheat, which only requires about 30 kg ha^{-1} N in the autumn. The risk of nitrate leaching increases if microbially available C compounds are insufficient for microbial N immobilisation or if abiotic N immobilisation is insufficient. Therefore, reduction of inorganic N in soil by plant N uptake or (microbial) N immobilisation is essential to minimise N losses, especially until vegetation dormancy.

Although the influence of fertilisers with added *Mis* was small, in each year of the field experiment, at the beginning of the vegetation period, there was a general reduction in soil inorganic N after the application of fertilisers with added *Mis* and WS (CS-*Mis*, CS-WS) compared to the application of pure CS. The application of manures (CM-*Mis*, CM-WS) also did not result in a higher amount of soil inorganic N compared to the mixtures (CS-*Mis*, CS-WS). This indicates that the addition of *Mis* to CS was as effective as the addition of WS in reducing the amount of potential N leaching, compared to pure CS. Thus, the 2. Hypothesis can be confirmed (2: *Miscanthus* reduces nitrate leaching as effective as wheat straw (Paper 2)).

It is not known whether the inorganic N that was moved to soil layers deeper than 90 cm was actually leached out. It can be assumed that due to the rooting depth of winter cereals and sugar beet (around 120 cm), some of the N moved to deeper soil layers could still have been taken up by the plants. The results of this thesis cannot be directly transferred to the practice because, for reasons of organic fertiliser comparability, no mineral N fertiliser was applied as a supplement, deviated from determination of N requirements in arable faming practise. In addition, the application of *Mis* biomass was mainly aimed at SOM build-up. Yield optimisation was not an objective of the thesis.

The N-immobilisation induced by *Mis* and WS biomass had a slightly negative but non-significant effect on the yield and protein content of winter barley, mustard and winter wheat compared to pure CS fertilisation. In contrast, all solid fertilisers (CS-*Mis*, CS-WS, CM-*Mis*, CM-WS) resulted in higher yields in the year of cultivation of sugar beet compared to the pure CS and UAN fertilisers. Inorganic N was not the limiting factor, because the amino N content of the sugar beet was higher after the CS application, but the crop yields were lower than after the application of the *Mis* and WS biomass fertilisers. Due to the drought conditions of this year, it is possible that the addition of organic matter may have already had a positive effect on soil fertility (water holding capacity, nutrient storage, SMB) of both the *Mis* and WS added fertilisers, resulting in better growth conditions. No differences in yield and quality parameters were detected between *Mis* and WS added fertilisers. Thus, hypothesis 3 can be confirmed (*Miscanthus* and wheat straw are identical in affecting yield and quality parameters of crops of a crop rotation (Paper 2)). It remains to be investigated whether the long-term application of *Mis* and WS added fertilisers can increase SMB and labile SOM to a level where (especially the first) N application to cereals and rapeseed can be reduced by remineralisation of previously immobilised N. This would have advantages for arable farming, especially in years with high soil moisture and limited soil access, as the application of organic fertilisers to frozen soils, even if they defrost during the day, is prohibited by law in Germany (DüV, 2020). If the application of organic N fertiliser is delayed due to wet soil conditions to such an extent that it cannot contribute to the initial N requirement of the crop, the NUE of the organic fertiliser is not fully exploited. If, the initial plant N requirement is replaced by additional mineral N fertilisation, which can technically be applied even under wet soil conditions, this is contrary to the requirements of sustainable agriculture. Future experiments could investigate whether, depending on the desired time of application, SMB and thus C and N dynamics can be affected by adjusting the C/N ratio of the applied fertilisers. The C/N ratios of the fertiliser applied with *Mis* and WS were around 20 - 30 and thus around the limit of 25 described in the literature, which indicates the mineralisation and immobilisation equilibrium, taking into account of other biochemical properties (Holocellulose/Lignin ratio) (Verberne et al., 1990). The stoichiometry could be modified by adjusting the mixing ratio (CS-*Mis*, CS-WS) or by the application of mineral N fertilisers. Depending on the date of application and the intended aim of the fertilisation (in autumn for N-immobilisation and SOM build-up or in spring to mainly provide plant N requirements), the application could be used for specific management of C and N dynamics by SMB. Increased N-mineralisation at the beginning of vegetation could be achieved by using shallow tillage techniques that promote microbial metabolic processes. Mechanical weed control using harrows and hoeing techniques could provide a synergetic effect to increase N mineralisation. The field experiment did not investigate the incorporation of fertilisers applied in spring, to winter barley and winter wheat and its effect on N dynamics. Probably one or even more till- and weed control treatments could have reduced N immobilisation and increased N mineralisation, or vice versa. It is also expected that microbial processes will have different effects under wet soil conditions. The precipitation intensity throughout the experiment was well below the long-term average, so that the results presented here are only valid for extremely dry soil conditions.

The second main objective (hypothesis four) of this thesis was to determine how the utilisation of *Mis* biomass affects SOM build-up and whether SMB make use of *Mis*-C. For this purpose, MBC and MBN were determined, considering that microbial necromass is accumulated by microbial metabolism of *Mis* and WS biomass, which is the main C source for SOC and thus contributes to C sequestration (Kallenbach et al., 2016). The higher microbially available C input in the form of *Mis* compared to WS, both in mixtures (CS-*Mis* and CS-WS) and manures (CM-*Mis* and CM-WS), was generally not reflected in higher SMB in either MBC or MBN. However, in the field experiment, the higher C input of manure from the *Mis* bedding (CM-*Mis*) compared to the mixture of CS and *Mis* (CS-*Mis*) and the higher C input of manure from the WS bedding (CM-WS) compared to the mixture of CS and WS resulted to a slightly higher

MBC, although not significantly. This indicates that the amount of C input alone does not affect the metabolism of SMB. Other factors, such as the stoichiometric characteristics of the fertiliser or the microorganisms already present in the manure and their microbial residues, such as amino sugars as readily available organic compounds, could also have stimulated microbial anabolism. Nevertheless, the experiments indicate that *Mis* is assimilated as a C source by SMB and thus contributes to C sequestration, as reported for WS. This confirms the fourth hypothesis (4: Microbial biomass make use of *Mis* as a C source for biomass build-up and thus contributes to C sequestration (Paper 1, 2).

The cultivation of *Mis* provides a new source of C that can be integrated into agricultural management (e.g. as bedding) and arable farming. The cultivation of *Mis* increases plant diversity, the harvested material has a high C/N ratio and the C-rich biomass provides a source for microbial metabolism. Due to the similar material characteristics of *Mis* and cereal straw, it is assumed that *Mis* stimulates microbial processes that promote the accumulation of microbial C-necromass and contribute to C sequestration. The part of the microbial C- and N necromass that is emitted to the atmosphere as CO_2 or N_2O by further transformation processes, or the NO_3^- that is lost by leaching, does not contribute to C sequestration. To determine the amount of C sequestration a more extensive experimental study is required to quantify microbial necromass formation, turnover rates, efficiency and stabilisation processes (Zhou et al., 2023; Wang et al., 2022). In addition to the C input provided by *Mis* or other C-rich biomass such as maize and cereal straw, microbial metabolism is also influenced by the type of soil tillage, the cultivation of catch crops, undersown crops and the biochemical characteristics of the fertiliser. These can contribute to both the accumulation and degradation of C compounds. For example, mineral N fertilisers stimulate microbial metabolism resulting in C-degrading enzymes that consume SOC, some of which is respired and released to the atmosphere as CO_2. In the field experiment, the application of UAN caused a slight increase in MBN. It may have increased and possibly enhanced the microbial metabolism processes, resulting in no more than 65% of the N applied by UAN being taken up by crops in the whole crop rotation (in the greenhouse experiment between 45% and 77% of applied N was mineralised). Thus, up to 35% of the N applied by UAN could have been metabolised by microbial anabolism and catabolism into N compounds that are not plant available. In addition to gaseous N_2O emissions, N could also be physically or chemically immobilised in soil aggregates and later remineralised. The amount of mineralised N of N applied was also low for the other applied fertilisers. Cumulated for the whole crop rotation, the amount of mineralised N was 12% (CS-*Mis*) and 23% (CS-WS) for the two mixtures, 20% (CM-*Mis*) and 23% (CM-WS) for the two manure types and 29% of the pure CS fertilisation. The amount of N that was not plant available remained in the initial organic compounds of each organic fertiliser or was immobilised by SMB or in the microbial necromass. The high initial SOM content of 3.9% and the soil texture (clay content: 229 g kg^{-1}, silt content: 597 g kg^{-1}) indicate a high potential for abiotic N immobilisation in soil aggregates or by chemical fixation to clay minerals and iron oxides and hydroxides (Angst et al., 2021; Wang et al., 2022). Different effects on C and N dynamics within a crop rotation are expected on soils with higher sand content or lower SOM content. However, it is unclear whether C sequestration would be increased. On soils with limited nutrient availability, usually characterised by a low SOM content, SMB may respond with reduced growth due to increased energy requirements to compensate for nutrient limitation (Clayton et al., 2002).

Fungal microorganisms were not studied separately in this thesis. Considering that biomass with a high C/N ratio is known to stimulate fungal biomass growth (Rousk & Bååth, 2007), a large part of the increase in MBN and MBC could be explained by an increase in fungal biomass, which in turn could be induced by *Mis* biomass. Reduced tillage would have increased SMB, but was not practised in the field experiment, suggesting that the potential for increased SMB was not fully exploited. Potentially, the combination of reduced tillage and the application of high C/N biomass represents a more effective management strategy for fungal SMB build-up and thus C sequestration. This needs to be tested in future experiments. In addition, weather and climate change, soil pH and other chemical and physical soil parameters, as well as crop management practices, affect the accumulation of microbial necromass. The effects are still poorly understood. Further research is therefore needed to improve arable farming practices to increase microbial necromass accumulation. Site-specific farming management could increase microbial necromass build-up and thus C sequestration in agricultural soils to counteract climate change and improve the resilience of arable soils. This would contribute to preserve and increase the productivity of arable soils and thus contribute to global food resources. In arable farming, C and N dynamics are often driven by residue management.

Due to the high pre-winter N requirement of rapeseed (about 80 kg ha^{-1}), the removal of straw is intended to reduce N immobilisation and thus contribute to N uptake by the crop. Other possibilities to manage C and N dynamics are the cultivation of catch crops and the reduction of tillage. Catch crops are thought to have great potential for influencing C and N dynamics. In the context of this thesis, the following options, which are currently not common in arable farming practice and require research on their effects on C and N dynamics, have been designed.

In the future, due to climate change, rapeseed and faba beans will be harvested earlier, while winter wheat will be sown later. This will extend the period of establishment of catch crops that promote SMB and take up any excess soil inorganic N. When winter wheat is sown using no-till systems, microbial turnover processes are not stimulated before winter and the remineralisation of organic N compounds is kept at a low level. N losses through leaching are reduced compared to tillage. No-tillage preserves fungal hyphae, which can lead to an increase in fungal necromass and thus C sequestration (Yang et al., 2022). In addition, catch crops with a rich diversity of plant species result in greater soil cover and a diverse input of root exudates, leading to SMB build-up and probably to additional microbial necromass (Baumhardt & Blanco-Canqui, 2014).

It could also be tested to combine the sowing of a catch crop and winter wheat. It should be tested whether the growth of winter wheat is inhibited by the presence of a fast growing catch crop. Growth inhibition of winter wheat by the catch crop could allow early sowing of the catch crop without inducing an excessive pre-winter development of winter wheat. Catch crops could reduce the soil inorganic N content and thus nitrate leaching after the harvest of rapeseed and faba bean and also contribute to SMB build-up.

Further research is required on the management of C and N dynamics in arable farming, focusing on the potential to maintain the growth of the catch crop in spring for as long as possible until just before the summer crop is planted. Catch crops from the previous year with overwintering species start to grow again at the beginning of vegetation. If the growth of the catch crop is allowed to continue until just before the summer crop is planted and is not interrupted by early tillage or herbicide treatment, a previously unused green cover potential is developed. Root exudation and the above and below ground biomass of the catch crop contribute to SMB build-up and thus to SOC sequestration. After the winter months, soil temperature rises and microbial processes begin. Mineralised N compounds can be taken up by the catch crop at the beginning of the vegetation period, so that soil inorganic N is neither biotically nor abiotically immobilised. Part of the N taken up by the catch crop in spring could already be plant available to the summer crop due to the low C/N ratio of the catch crop, indicating a rapid remineralisation of a part of its biomass. As a result, more mineralised N could potentially be mineralised to the summer crop than would be the case without the catch crop in spring. However, this effect on C and N dynamics should be determined in further experiments. These research approaches and the experiments of this thesis demonstrate the potential of C and N management in arable farming by controlling SMB through C inputs, tillage or catch crops.

The third main objective (hypothesis five) of this thesis was to determine the potential of hyperspectral sensors for the detection of plant N content at the time of full maturity of a cereal crop and then to evaluate this potential using the destructive method. In the context of this thesis, the utilisation of drone-based sensors as a part of digital technologies in agriculture was also used to integrate and evaluate the potential of these technologies regarding the management of C and N dynamics.

In contrast to the destructive method, the spectral detection at the time of full maturity of winter barley, which was designed to detect differences in N uptake after the application of different fertilisers, was not useful. The plants were already well into senescence and the chlorophyll content in the leaves was already too low to accurately detect differences. Due to the advanced senescence, the spectrum probably showed a mixture of plant and soil signal. In addition, the soil signal may not have been identical after application of the different fertilisers, which created different structures on the soil surface that may have strongly influenced the spectral signatures. There was no separate detection of the soil signal and the organic fertiliser inputs. Therefore, hyperspectral imaging was not an alternative to destructive N measurement at that time. At an earlier stage, before senescence, hyperspectral imaging would have been an alternative to destructive N measurement. Hypothesis 5 is therefore rejected (5: Drone-based determination of N uptake of winter barley using hyperspectral imaging is an alternative to destructive measurement (Paper 3).

In arable farming, hyperspectral imaging is not widely used due to the large data sets it produces and the lack of advantages compared to multispectral imaging (Cilia et al., 2014).

The spectral detection of plant N supply by multispectral imaging has been implemented in practice since 2001 and is increasingly used in arable farming (Drücker, 2016). Using the offline method, plant parameters are collected by drones and satellites before N application. These are evaluated using biomass and soil maps, manually adapted if necessary based on the farmer's experience, and used for georeferenced N application. Using the online method, multi-spectral sensors installed on the tractors detect the spectral characteristics of the crop while driving.

The close distance between the sensors and the crop provides more detailed detection. This makes them suitable for homogeneous and more productive soils. They are less affected by weather conditions. Data is processed in real time, allowing decisions to be made in real time. (Guerrero, 2022). Both methods can contribute to nutrient management according to the crop's requirements and thus to the reduction of excess N. However, they only detect the current plant status. Sufficient development of the plant biomass is required for correct detection of the spectral plant characteristics. For example, they are not suitable for the first application of N in cereals because of the limited plant biomass. The amount of the first N application is mainly determined by the amount of soil inorganic N in spring, which can vary significantly within a field. The results of the field experiment show the effect of the biomass characteristics of the applied N fertiliser on the soil inorganic N content at the end of winter. For example, the soil inorganic N content at the end of February 2019 was 66 kg ha^{-1} after CS-*Mis*- and 119 kg ha^{-1} after UAN fertilisation. The soil inorganic N content at the beginning of the vegetation period is the calculation basis for the following N fertilisation (DüV, 2020).

As microbial activity increases in spring, microbial metabolism processes, which are affected by soil characteristics and previous C and N management, also increase and thus effect soil N content. As there are usually several days between the sampling for soil inorganic N and the first N application, the soil inorganic N content can already differ from the previously measured value. The calculation of the first N application, which is influenced by the amount of soil inorganic N, can affect both the SMB and the N uptake of the crop. For example, if the first N application to cereals is applied with a higher N input than required, due to an incorrect estimation of the soil inorganic N content, the risk of lodged grain increases, unproductive side shoots are induced and, due to the increased plant density, plant diseases tend to increase, resulting in increased number of fungicide applications, which is contrary to the reduction targets for pesticides. The type of soil management, such as mechanical weed control and changes in soil moisture and temperature due to weather conditions, intensify the dynamics of microbial metabolism and lead to variability in the calculation of N fertilisation. In maize cultivation, daily determination of soil inorganic N content and estimation of continuous N mineralisation would be a tool to optimise N fertilisation, especially for the last N application in maize. Due to agro-technical limitations, the last N application is already applied at the closing of the plant row (around the 10th leaf). If N mineralisation of organic matter (SMB, SOM, N fertiliser) is estimated to be lower or higher than actually occurs, the risk of N losses or yield reduction increases. Therefore, possible digital technology approaches that combine SOM build-up and soil conditions for more sustainable C and N management are presented.

For the daily determination of soil inorganic N, in-field soil sensors could be implemented for direct detection of daily soil inorganic N within a field or part of a field. The daily data set could be continuously accessed by the farmer through an application. Management practices, such as N fertilisation, could be continuously adjusted to the soil N content. In addition, soil moisture and soil temperature could be detected by in-field soil sensors and transferred to an application. The data set could be continuously processed in predictive algorithms and supplemented with manually added site conditions and management practices for both, estimation of daily soil inorganic N content and continuous N mineralisation, provided to the farmer. Modelling requires data sets of C and N dynamics from experiments under well-defined site conditions and management practices. Regression and dynamic models could be a suitable tool for modelling of N mineralisation under different site and management conditions.

The input of important parameters into an application, such as site conditions, continuous management activities, spectral reflections detected by on- or offline methods, soil maps and continuous and automatic detection of soil moisture and soil temperature, could be provided for artificially intelligence systems. Machine learning and deep learning could be used to improve the identification and mapping of interactions and processes such as C and N dynamics. The data sets that are relevant to farmers could be made available, thus contributing to plant N fertilisation that is adapted to the crop's requirements and reduces N losses.

Digital tools can also be implemented for site-specific prioritisation of SOM build-up. Biomass and soil maps can be used to identify high and low yielding parts of the field, as well as zones at high risk of erosion and N leaching. A manual prioritisation of the objectives to be achieved by SOM build-up (climate change mitigation, erosion mitigation, N leaching mitigation) could be visualised in a tool for farmers. On these parts of the field, SOM build-up could then be targeted and primarily induced, either by cultivation of *Mis* itself or by applying C-rich fertiliser, e.g. by fertiliser with added chopped *Mis*.

6 Final Conclusion

Mis can be cultivated as a low-input crop. It can protect the soil from erosion and contribute to CO_2 storage in the soil, similar to permanent grassland. *Mis* increases the structural diversity of the open agricultural landscape. The current focus of material and energy use of *Mis* biomass has been on the development of products, for example as an additive for the packaging industry, as a construction or building material, as a growing material or as a feedstock for anaerobic digestion. The development of additional C-rich biomass through the cultivation of *Mis* for specific use as an organic fertiliser for SOM build-up in arable farming has created a new utilisation option that was not the focus of utilisation pathways for *Mis* biomass until now. Particularly in areas with low livestock concentrations, which often result in high biomass exports as crop products, plant biomass with a high C content, such as cereal straw, is often limited and can be supplemented by *Mis*.

The experiments in this thesis showed that *Mis* biomass is used as a C source by SMB and contributes at least as much as WS to SOM build-up and thus C sequestration. *Mis* is as good as WS in immobilising additional inorganic N from mineralisation of slurry or manure, which had a negative effect on yield and quality parameters, and also indicated a reduction in nitrate leaching.

These results indicate that soil C and N fluxes can be controlled and influenced by SMB. The specific management of SMB, e.g. through the application of C-rich organic fertilisers based on *Mis* biomass, has great potential to reduce the negative and increase the positive environmental impacts of arable farming. An improved knowledge of C and N fluxes and metabolism of SMB and the implementation of soil sensors could contribute to a more environmentally sustainable C and N management in arable farming. The results presented in this thesis provide the basic knowledge for the specific implementation of chopped *Mis* as a C-rich biomass for SOM build-up and for the reduction of N losses in arable farming systems.

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