Reduction of agricultural soil N losses during the winter season by straw amendments, nitrification inhibitor and by intercropping

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Abstract

Nitrogen (N) is a fundamental element for plant and crop growth, but soil N losses may cause low crop N use efficiency and reduced biomass. Moreover, soil N losses such as nitrous oxide (N_2O) emissions and nitrate leaching are causing environmental problems. In order to reduce N losses soil amendment with straw and the application of nitrification inhibitors are used in agriculture. Straw amendment stimulates the microbial uptake of N for stoichiometric reasons, thus reducing N losses form soil. However, significant parts of N may still remain sequestered in soil microbial biomass and cause N shortage of crops if N is not released in time, studies on long-term effects of straw incorporation and its effects on the performance subsequent crops are quite rare. Besides of soil amendment, a good crop management (i.e. intercropping and crop rotation) can also reduce soil N losses and improve crop N use efficiency. Different from intercropping, the effects of crop rotation are mediated through indirect feedback interactions known as plant-soil feedback (PSF) effects, the preceding plant affects the soil abiotic and biotic components that influence the succeeding plant generation. However, little is known about the ecological interactions and how the underlying mechanisms can also be utilized to enhance cropping systems productivity. The research of combining spatio-temporal diversity (intercropping together with crop rotation) in cropping systems is needed. Therefore, this thesis aimed to evaluate the effects of crop management (i.e. intercropping) and soil amendments application (i.e. application of straw and nitrification inhibitor) on crop growth, and the effects of soil amendments application on soil N losses under temperature variation (i.e. cooling-warming and freezing-thawing cycling).

A mesocosm experiment was designed in chapter 1, to reproduce the dynamics of soil N cycling before and after crop growth, and simulate winter temperature fluctuations under three different N fertilisation rates, incorporate with straw and nitrification inhibitor (NI). Straw addition promoted microbial fixation of N, thereby reducing soil N leaching and total N losses. NI was effective in reducing soil N_2O emissions and mitigate N_2O emissions caused by straw application. Soil cooling-warming enhanced ammonification and straw induced microbial fixation of N was released at the end of the experiment. But re-mineralization in no-N and low-N rates was insufficient at critical times of early barley growth, concurrently, barley biomass was decreased at both no-N and low-N fertilization rates. Therefore, adequate N fertilisation, combined with straw application, is essential to regulate the timely re-mineralisation of N for succeeding crops.

A mesocosm experiment that simulated the seasonal temperature variation was conducted in chapter 2, to investigate the effects of wheat straw amendment NI and temperature variation (both freezing-thawing and cooling-warming) on N losses from soil as N_2O and leachate with and without N fertilization. We observed straw addition reduced N leaching and total N losses, but increased the N_2O emission by denitrification in high N fertilizer levels. NI effectively reduced N_2O emission after fertilization. Cooling-warming strongly induced N mineralization and caused N_2O emission peak even in the absence of freezing-thawing, while freezing-thawing increased the N_2O emission furtherly.

In chapter 3, we set up a field trial consisting of an intercropping phase followed by a rotation phase. In the intercropping phase, maize, faba beans and lupins were grown as monocultures or mixtures of the two as intercrops. In the subsequent rotation phase, barley monocultures were grown to test the plant-soil feedback (PSF) effects of the intercropping phase. The results were both good intercropping species combinations (maize and broad bean) and inefficient intercropping combinations (lupin with other crops). In addition, the intercropping phase altered soil mineral N content, but the feedback effect of intercropping had no impact on barley biomass. Although intercropping had no significant PSF effects in this experiment, PSF effects could be important for crops on soils with low fertiliser application or poor fertility.

In conclusion, straw addition significantly reduced soil N losses, NI addition counterbalanced the increased N_2O emissions due to straw amendment at high N fertilization, a great challenge of straw application is the timing of N-release for the following crop. Positive effects of intercropping on above-ground biomass are species specific, but soil legacies from the intercropping phase had no effect on soil microbial parameters and barley above-ground biomass in the rotation phase. In order to maximise crop yield and benefit the environment, management practices like intercropping, crop rotation, and the use of soil amendments like NI, straw should be carefully chosen for adoption based on the unique characteristics of the arable land as well as the costs and labour inputs.

Introduction

N fertilizer increased crop yield but induced environmental problems i.e. N_2O emission and N leaching

The use of chemical N fertilisers in agricultural production is essential to feed the growing global population (Sutton et al., 2011). However, more than half of the N fertiliser is lost into the environment, but not taken up by crops in agricultural fields (Lassaletta et al., 2014). Urea and anhydrous ammonia, as a common form of N fertiliser, are rapidly converted to nitrate (NO_3^-) by the microbial nitrification process when applied to soil (Huber et al., 1977). NO_3^- is highly mobile in soil and is therefore susceptible to a number of environmental problems, including water pollution and greenhouse gas emissions. The application of N in leachable forms (e.g. NO_3^-) can lead to the N losses, especially in intensive agriculture (Nixon and Agency, 2003), endangering human health through eutrophication of surface waters or contamination of drinking water (Di and Cameron, 2002). Moreover, autumn and winter are the main periods of N leaching in temperate agricultural systems, when crop residues decompose, and plant N uptake and evapotranspiration is low (Di and Cameron, 2002), preventing N leaching at critical times is crucial.

In addition to N leaching, N can also be lost to the atmosphere as N_2O through the nitrification and denitrification processes, making it the third largest greenhouse gas after carbon dioxide (CO_2) and methane. N_2O has an atmospheric lifetime of 116 ± 9 years and therefore depletes atmospheric ozone over a long timescale (Prather et al., 2015), with a single-molecule warming potential 298 times that of CO_2 (Montzka et al., 2011). Soil is the major source of N_2O emissions, while agricultural production accounts for half of the anthropogenic N_2O emissions (Tian et al., 2020). Atmospheric concentrations of N_2O have increased by 20% over the past two hundred years and are still rising at a rate of 2% per decade (Tian et al., 2020). N_2O emissions are expected to increase in the future as the population and demand for food keep growing (Godfray et al., 2010). Furthermore, the recent growth in N_2O emissions exceeds some of the highest projected emission scenarios (Davidson, 2012), emphasizing the importance to mitigate N_2O emissions.

Temperature variation (cooling-warming cycle) and freezing-thawing affect the N transformation

In order to predict N losses from agriculture and devise mitigation strategies, theoretical knowledge must be applied to the unique environmental circumstances of a region or ecosystem. In this regard, temperature must be taken into account as one of the most important factors influencing the soil N transformation. During the fallow period of variable soil moisture and temperature, such as periods of cooling, warming, freezing and thawing, large amounts of N are lost before it can be captured by the subsequent crop generation (Cookson et al., 2002; Sieling and Kage, 2006). Previous studies investigated the effects of temperature on soil N content (Tan et al., 2021), ammonia volatilization (Siman et al., 2020), N_2O emission (Smith et al., 1998), temperature sensitivity (Q10) of soil N transformation rate (Guntiñas et al., 2012; Liu et al., 2016). However, these studies either investigated the soil N at one-time point in the natural environment ((Tan et al., 2021), or soils incubated under constant temperature (Li et al., 2017; Tan et al., 2021). The temperature is unstable during late winter and early spring, with temperature fluctuations of more than 20 °C within days in Europe (https://www.visualcrossing.com/weather-history), especially at times of winter warming or spring frost. Winter warming combined with late spring frost can significantly decrease crop yield (Li et al., 2015). Despite seasonal temperature variations can greatly influence soil N fixation processes (Li et al., 2021), experiments on seasonal N cycling in agricultural soils are rare (Sieling and Kage, 2006).

Variation of temperatures below and above 0 °C causes freezing-thawing of soils, which is a common phenomenon in temperate environments and 55 % of the northern hemisphere are subject to seasonal soil freezing-thawing (Kreyling et al., 2008). This leads to more N losses as leachate and N_2O emission due to the increased release of inorganic N from soil (Gao et al., 2018). Soil freezing and thawing cause physical disruption of aggregates and cleavage of microbial cells, resulting in the release of nutrients. The released nutrients combined with the creation of anoxic microsites promote emissions of N_2O by up to one-fold. A burst of N_2O emissions from thawing soils is often observed (Matzner and Borken, 2008), and more than half of the annual N_2O emissions may result from thaw-related fluxes (Wagner-Riddle et al., 2017). The complete understanding of biological mechanisms explaining N loss during soil freezing and thawing, i.e. involved functional microbial genes and enzymes still needs further research (Hu et al., 2015).

Nitrification inhibitor application reduce N losses

To combat the problem of soil N losses, soil amendments are the most commonly used methods, among which nitrification inhibitors (NIs) are widely used to improve crops NUE and reduce N losses (Wu et al., 2017). NIs are compounds that inhibit the oxidation of soil ammonium (NH_4^+) to nitrite (NO_2^-) by ammonia-oxidizing bacteria, such as the genus Nitrosomonas (Zerulla et al., 2001). Ammonia monooxygenase (AMO) is responsible for catalysing the first step of nitrification and most NIs can inhibit AMO activity (Subbarao et al., 2006). Furthermore, NI may indirectly affect N_2O emissions from denitrification (Hatch et al., 2005; Ruser and Schulz, 2015) Although NI are known to reduce N_2O emissions from arable soils, their effectiveness varies widely (Menéndez et al., 2012). The longevity of NI can greatly limit its effectiveness, which is determined by soil temperature, moisture and fertiliser levels (Norton and Ouyang, 2019; Thapa et al., 2016). The effects of NIs on crop yields are affected by soil factors including soil pH, which influence NH_3 volatilization, and soil texture, which influence N leaching (Zaman et al., 2008). The management factors including irrigation, N fertilizer rate, crop varieties and NI species also influence the effect of NIs on yield (Abalos et al., 2014), thus, NIs can have positive (Zaman et al., 2013) or no effects on crops (Linquist et al., 2013). It is known that the inhibitory capacity of NIs decreased with increasing temperature (Irigoyen et al., 2003; McGeough et al., 2016). Menéndez et al (2012) reported that the effect of soil temperature on the efficiency of NI was dependent on soil water content: the efficiency of NI decreased with temperature under 80% WFPS, but the mitigating effect of DMPP increases with temperature at 40% WFPS. Therefore, it is still no clear how soil temperature and moisture modify the efficiency of NI on soil N_2O emissions.

Among the NIs, pyrazole derivatives (PD) are a class of effective NI. Piadin (SKW, Piesteritc, Germany) is a commercial formulation incorporating two active PD compounds: 1H-1,2,4-triazole and 3-methylpyrazole at inclusion rates of approximately 3.1% and 1.6%, respectively, which commonly used as livestock slurry amendment (Barneze et al., 2015). PIADIN® retards the transformation of NH_4^+ into NO_3^- for about two months and weeks immediately after application to the soil (https://www.piadin.de/en/#home). However, up to now only few publications on the use of this product exist (Federolf et al., 2016; Misselbrook et al., 2014), and few investigations studied the effect of Piadin on soil N leaching.

Straw amendment improve the N uptake of crops and reduce N losses

In addition to NI application, straw as a high carbon additive, is one of the most commonly used soil amendments to control soil N loss (Diacono and Montemurro, 2011; Norton and Ouyang, 2019; Xia et al., 2018). Straw is a carbon (C) rich biomass resource that can act as a source of organic fertiliser (Liu et al., 2021). Compared with burning, returning straw to the field decreases air pollution, improves soil aggregation, enhances biological activity and reduces soil water evaporation (Yang et al., 2018). Soil C content is one of the main limiting factors for microbial growth and the addition of higher carbon straw promotes microbial N uptake due to stoichiometry (Chen et al., 2023). To fix excess mineral N in the soil by soil microorganisms, farmers apply straw from the previous season's crop to the field before sowing (van Duijnen et al., 2018). Although microorganisms compete with plants for soil N in the short term, because microbial cycles continually remobilize microbial biomass N (MB-N) in the rhizosphere, which is subsequently taken up by the root system or mycorrhizae (Bonkowski, 2004; Koller et al., 2013a; Koller et al., 2013b; Koller et al., 2013c; Rozmoš et al., 2021), the plant may win out in the long term (Hodge et al., 2000; Kuzyakov and Xu, 2013; Sieling and Kage, 2006).

However, intensive farming can hinder these processes of N released from microbial fixation (Roy et al., 2017). If N is not released in a timely manner, large amounts of N can be retained in the soil microbial biomass, leading to N deficiency in the crop (Jingguo and Bakken, 1997), requiring more N fertiliser for the next crop growth. Nevertheless, the long-term effects of straw addition on crop growth remain unclear (Congreves et al., 2013). A more accurate administration affecting the timing of N fixation and remobilization after straw addition can significantly mitigate global agricultural N losses and greenhouse gas emissions.

By immobilizing N in soil, both the NO_3^- and nitrite concentrations in surface water and in percolating water was reduced after straw application (Yang et al., 2018a). Besides that, straw application could enhance soil aggregation and water penetration by the extension of fungal hyphae (Peng et al., 2013). An increased soil water holding capacity due to a reduced soil bulk density and increased soil porosity will also reduce soil NO_3^-–N leaching (Yang et al., 2018a). While the effects of straw application on N leaching are influenced by e.g. soil texture, particle size of straw, time of percolation and type of straw amendment (Hansen et al., 2015; Nicholson et al., 1997; Zheng et al., 2019), this has led to widely varying results and understanding the causes is essential for efficient straw application (Gong et al., 2022; Hansen et al., 2010; Hansen et al., 2015; Yang et al., 2015).

Moreover, although straw is often used to mitigate soil N_2O emissions (Li et al., 2014; Ma et al., 2009), some doubts were expressed about potential N_2O emissions under straw incorporation (Huang et al., 2017; Yang et al., 2015a; Zhang et al., 2019). Because the effects of straw addition on soil N_2O emissions appear inconsistent, ranging from positive (Li et al., 2013; Xia et al., 2018) to neutral (John et al., 2020; Malhi and Lemke, 2007), and negative (Shan and Yan, 2013; Yao et al., 2017). The divergent N_2O responses to straw addition have been attributed to differences in soil physicochemical properties, fertilization practices, C/N ratio of straw, soil moisture and so on (Chen et al., 2013; Ma et al., 2009; Yu et al., 2019), indicating that the fundamental mechanisms controlling the impact of straw on N_2O

emissions are complicated and still little understood (Wu et al., 2020). Positive effect of straw amendment on leachate due to increased N immobilization can be counterbalanced by acceleration of N_2O emission via denitrification. Critical role in respect to N losses in this case play the dynamics of soil water content and oxygen availability. NI could suppress the provision of NO_3^- for denitrification and therefore, its combination with straw could potentially lead to an overall positive effect on soil N contents. But this hypothetical interaction needs to be proven under controlled conditions. It was reported that the efficiency of NI in reducing N_2O emissions was negatively correlated with soil organic matter content (Lin and Hernandez-Ramirez, 2020; McGeough et al., 2016), while Wu et al (2017) reported that NIs mitigate N_2O emissions more effectively with straw incorporation in soil. At present it is even unclear whether NIs and straw can be used in combination to effectively reduce N loss from the soil, especially under cooling-warming and freezing-thawing.

Intercropping improve crop growth and reduce N losses

Besides of soil amendment, a good crop diversification can improve the crop NUE (Gurr et al., 2016). Intercropping increases crop diversification in cropping systems by planting different varieties or crops concurrently in the same area (Kumar et al., 2021). Compared with monoculture, intercropping can ensure crop yields through crop diversification with less nutrient supply and pesticide use (Chen et al., 2019). Intercropping increases yields by raising the crop's efficiency of use of nutrients, light and water, it also suppresses pathogens, weeds and pests and promotes the growth of beneficial microorganisms (Döring et al., 2012; Hauggaard-Nielsen and Jensen, 2005). Meanwhile, intercropping also reduces soil erosion, and combining with legumes can enhance crop N uptake (Hauggaard-Nielsen and Jensen, 2005; Trenbath, 1993; Zougmore et al., 2000). Two prime advantages of intercropping, which make the practice particularly beneficial for developing country farmers, are diet diversification and risk reduction (Heywood et al., 2013). Intercropping can increase farmers' resilience to risk; when one crop is damaged by climate or insect pests, the companion crop may remain unaffected through physiological responses or by attracting different pests (Whitmore and Schröder, 2007).

Increasing crop diversification including intercropping was shown to have positive effects on plant productivity (Dong et al., 2018; Zhang et al., 2017) and reduced N_2O emission (Chen et al., 2019), but negative effects have also been observed (Bukowski et al., 2018; Polley et al., 2003). It is a huge task to develop a sustainable cropping system based on agro-climatic conditions and available resources (Maitra et al., 2019). Few reports on successful development project initiatives that have led to widespread adoption of intercropping (Panel, 2013). The complexity of intercropping combinations contributes to this limitation, as inappropriate intercropping combinations promote increased pests, spatial competition and allelopathic effects of intercropping partners (Karpenstein-Machan and Stuelpnagel, 2000). The promotion of intercropping is limited by various factors, including the lack of necessary inputs, such as herbicides, and the fact that adjusting crop spacing impedes field traffic and discourages mechanisation and weeding (Chauhan et al., 2012; Rusinamhodzi et al., 2012).

In addition to intercropping, crop rotation is another way of diversifying crops, reducing pests and pathogens, improving nutrient use and utilising complementary ecological niches to increase yields (Dias et al., 2015). Crop rotation has similar benefits to intercropping, including benefit on crop yield, improved water use efficiency, the control of diseases, weeds and reduced N losses (Hegewald et al., 2018). While the effects are affected by climate condition, soil texture, soil C, N contents, fertilizer, previously cultivated crops e.g. (Zhao et al., 2020). Different from intercropping, the effects of crop rotation are achieved via indirect feedback interactions, known as PSF effects (Mushonga et al., 2020; Schnitzer et al., 2011), where the prior crop affects the abiotic and biotic components of the soil that influence the subsequent crop generation (Bever, 1994; Ehrenfeld et al., 2005). PSF involves two steps: first, plants alter the composition of their related soil microbial community (Chanway et al., 1991; Kowalchuk et al., 2002); second, these plant-driven changes in the soil affect the performance of the next generation of plants (Bever et al., 1997; Thrall et al., 1997). Due to the PSF interactions, the growth rate of the subsequent crop may be higher than that of the previous crop, i.e. positive feedback, or lower than that of the previous crop, i.e. negative feedback.

Although the essence of PSF remains poorly understood, it is well known that the rhizosphere of plants contains both beneficial and detrimental microorganisms (Van der Putten, 2003). Positive PSF may be due to increased nutrient availability, such as legume-cereal (Chapman et al., 2006; Grayston et al., 1998; Wardle et al., 1999), or the enrichment of symbiotic mutualists in the rhizosphere (Klironomos, 2002). Nevertheless, it was found that the PSF is often negative (Bever, 1994; Bever et al., 1997; Klironomos, 2002; Olff et al., 2000). Accumulation of herbivores and soil pathogens in plant roots (Van der Putten, 2003), as well as the fixation or depletion of nutrients (Berendse, 1994), may cause negative feedbacks, and interspecies interactions can be influenced by negative feedbacks (Bonanomi et al., 2005; Van der Putten and Peters, 1997). However, most of the existing studies of PSF effects are from non-cropping systems, little is known about the ecological interactions and how the underlying mechanisms can also be utilized to enhance cropping systems productivity (Kumar et al., 2021). The research of combining spatio-temporal diversity (intercropping together with crop rotation) in cropping systems is needed.

Aims and hypothesis

In the first and second chapters a mesocosm experiments were conducted to investigate the effects of combining straw and NI Piadin$^{(R)}$ on soil N loss and utilisation at temperature regime mimicking winter and spring soil temperature and freezing-thawing cycle at field conditions. We quantified soil mineral N, N_2O and leached N, microbial biomass at different phases and N uptake by barley. We hypothesized that i) NI will reduce N losses in the short term and must be applied repeatedly, while straw addition will reduce N losses through long-term microbial sequestration of N over seasonal temperature fluctuations, and NI and straw in combination have positive additive effects on barley N uptake; ii) a significant proportion of sequestered microbial N is released after soil cooling-warming periods mitigating N limitation of subsequent crops; iii) freezing-thawing will induce strong N_2O and N leaching losses more than cooling-warming. The size of straw and NI effects was studied against three rates of mineral N applied as fertilizer.

In chapter three, we designed a field trial that comprised an intercropping phase followed by a rotation phase. Maize, faba beans and lupins were grown as monocultures or mixtures of the two as intercrops in the intercropping phase. Following the intercropping phase, we grew barley monocultures in the rotation phase. The objectives of the study were twofold: i) to identify intercropping crop combinations with higher total yields than monocropping; ii) to determine the effect of PSF in the intercropping phase on barley yield and soil biochemical properties during the rotation. Therefore, we hypothesized that: i) Intercropping will yield more than monocropping. ii) Intercropping would change soil properties and leave soil legacies, affecting the microbial properties and the performance of the following crop in the rotation via PSF effects. The conceptual diagram of the thesis is shown in Figure 1.

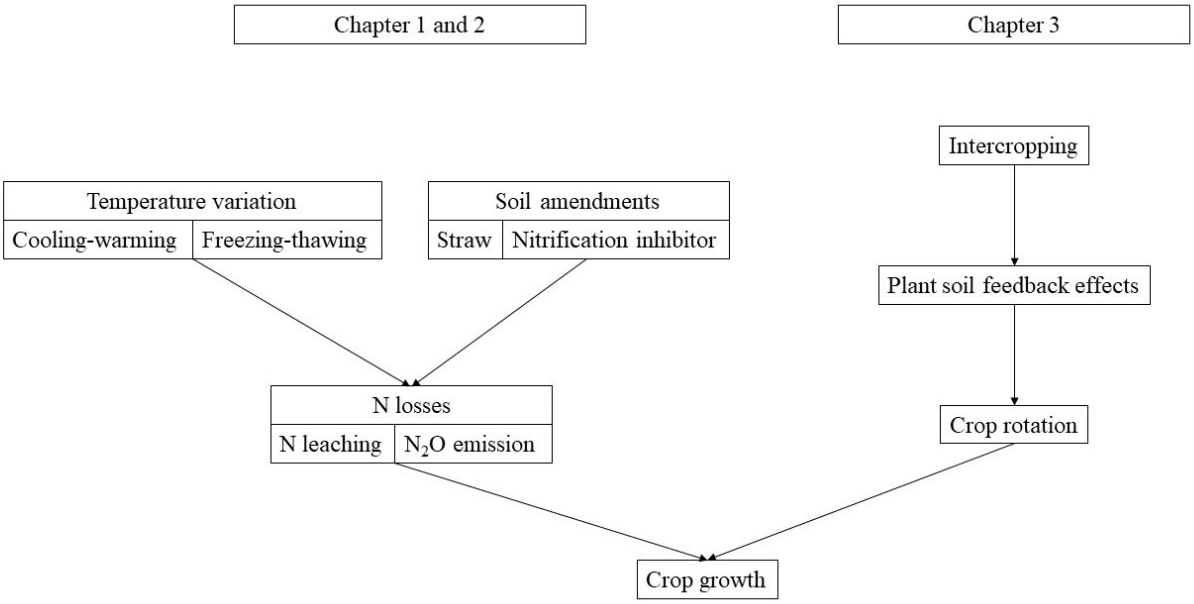

Figure 1. Conceptual diagram of the thesis.

Chapter summary

Chapter 1 Straw amendment in combination with nitrification inhibitor controls N losses and immobilization under soil cooling-warming cycle

Summary: Soil amendments including straw and nitrification inhibitors (NI) were used to reduce soil N losses and improve crop N uptake. But the combined effects of straw and NI under seasonal soil temperature variations were rarely studied. To ascertain whether the remineralization of the soil microbial immobilised N following the addition of straw will occur in time for the growth of the following generation of barley, and NI and straw application can be effectively combined to combat N losses from soils. It was also aimed to address the effects of seasonally varying temperatures on N cycling in arable soils. To simulate the dynamics of soil N cycling before and after crop cultivation, we designed a mesocosm experiment simulating winter temperature variation at three different N fertiliser application rates, incorporating two soil amendments, straw and NI. We monitored the amount of soil N lost as N_2O and leaching, and the uptake by the next generation crop of barley. It was expected that i) straw and NI have a superimposed effect and their combination maximises N uptake by barley; ii) straw significantly reduces soil N losses at any stage of temperature variation; iii) the cooling-warming process triggers the release of N fixed by soil microorganisms to supply subsequent barley growth.

Chapter 2 Straw application and nitrification inhibitor affect soil N losses during cooling-warming and freeze-thaw cycles

Summary: Soil N losses including N_2O emissions and NO_3^- leaching caused multiple negative effects including eutrophication of surface waters, destroying the ecological balance and posing challenges to human health. To combat these problems soil amendment with straw and NI are used in agriculture. Microbial immobilization of N stimulated by straw and NI addition can be altered by seasonally varying temperatures, but the research is still inadequate. Moreover, it is still not clear how the variation in soil temperature and moisture will modify the effect of NI on soil N_2O emissions. The divergent N_2O responses to straw addition have been attributed to differences in soil physicochemical properties and fertilization practices, however, the mechanisms responsible for the effect of straw on N_2O emissions are complex and still little understood. It was aimed to address the effects of cooling-warming and freezing-thawing cycling on N transformations in agricultural soils. A mesocosm experiment simulating seasonally varying temperatures was carried out to study the effects of the addition of wheat straw, NI and temperature variation (both freezing-thawing and cooling-warming) on N losses from soil as N_2O and leachate with and without N fertilization. It was expected that i) NI and straw addition in combination have additive effects on mitigating N losses and NI could dampen negative effect of combined N and C addition effect on acceleration of N_2O emission; ii) The addition of straw is an effective way of preventing N losses in cooling-warming and freezing-thawing cycles; iii) freezing-thawing will induce strong N_2O and N leaching losses, but cooling-warming alone will not.

Chapter 3 Gone and forgotten: facilitative effects of intercropping combinations did not carry over to affect barley performance in a follow-up crop rotation

Summary: Intercropping can increase the yield of each crop relative to monocultures. Indeed, little is known about the facilitation effects in various intercropping systems and their role in shaping the soil legacy, which can indirectly influence the subsequent crops in the rotation through PSF effects. It is unclear whether the crop species with enhanced yields from intercropping can produce a positive soil legacy, increasing soil nutrients and mutualistic bacteria populations and lowering pathogen levels to benefit the next crop in the rotation. To offer additional insights from cropping systems regarding the relative biomass production of various crop combinations with respect to the strength of the facilitative interactions that result in increased productivity. Select intercropping crop combinations with higher total biomass than in monocultures. It was also aimed to evaluate the PSF effect of intercropping on soil biochemical properties and barley biomass in crop rotation. We designed a field trial that comprised an intercropping phase followed by a rotation phase. Maize, faba beans and lupins were grown as monocultures or mixtures of the two as intercrops in the intercropping phase. Following the intercropping phase, we grew barley monocultures in the rotation phase to test PSF effects from intercropping phase. It was expected that: i) Intercropping will increase yield compared to monocultures. ii) Intercropping would change soil properties and leave soil legacies that would affect the microbial properties and performance of the subsequent crop in the rotation via PSF effects.

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Chapter 1 Straw amendment and nitrification inhibitor controlling N losses and immobilization in a soil cooling-warming experiment

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Straw amendment and nitrification inhibitor controlling N losses and immobilization in a soil cooling-warming experiment

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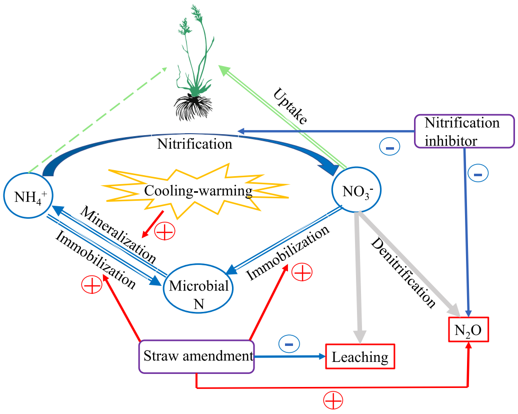
HIGHLIGHTS

- N losses from agriculture can be mitigated by combination of straw and nitrification inhibitor.
- Effect of straw and nitrification inhibitor application was not additive.
- Winter soil cooling-warming increased ammonification and suppressed nitrification.
- Straw application decreased the N leaching from soil, but increased N_2O emission.
- Straw amendment amplified N competition between soil microorganisms and plants during the early growing phase.

GRAPHICAL ABSTRACT



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ABSTRACT

It is common practice in agriculture to apply high-carbon amendments, e.g. straw, or nitrification inhibitors (NI) to reduce soil nitrogen (N) losses. However, little is known on the combined effects of straw and NI and how seasonal soil temperature variations further affect N immobilization. We conducted a 113-day mesocosm experiment with different levels of ^{15}N-fertilizer application (N0: control; N1: 125 kg N ha^{-1}; N2: 250 kg N ha^{-1}) in an agricultural soil, amended with either wheat straw, NI or a combination of both in order to investigate N retention and loss from soil after a cooling-warming phase simulating a seasonal temperature shift, i.e., 30 days cooling phase at 7 °C and 10 days warming phase at 21 °C. Subsequently, soils were planted with barley as phytometers to study ^{15}N-transfer to a following crop.

Straw addition significantly reduced soil N-losses due to microbial N immobilization. Although carbon added as straw led to increased N_2O emissions at high N fertilization, this was partly counterbalanced by NI. Soil cooling-warming strongly increased ammonification (+77 %), while nitrification was suppressed, and straw-induced microbial N

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immobilization dominated. N immobilized after straw addition was mineralized at the end of the experiment as indicated by structural equation models. Re-mineralization in N2 was sufficient, but still suboptimal in N0 and N1 at critical times of early barley growth. N-use efficiency of the ^{15}N tracer decreased with fertilization intensity from 50 % in N1 to 35 % in N2, and straw amendment reduced NUE to 25 % at both fertilization rates. Straw amendment was most powerful in reducing N-losses (−41 %), in particular under variable soil temperature conditions, but NI enforced its effects by reducing N_2O emission (−40 %) in N2 treatment. Sufficient N-fertilization coupled with straw application is required to adjust the timely re-mineralization of N for subsequent crops.

1. Introduction

N application to agricultural land has increased steeply in recent decades and is expected to further increase in order to meet the growing food demand (Shahzad et al., 2019). Yet, only 30–50 % of applied N fertilizer is taken up by crops, with the remainder being either emitted into the atmosphere as nitrous oxide (N_2O) or leached into aquatic systems (Chen et al., 2020). Excess N in agriculture translates into economic losses for farmers, as the N has to be replenished, but it also entails significant costs for the general public. The European Union estimates the costs of pollution by excess N at 70–320 billion Euro per year, more than twice the value that N fertilizer use adds to European farm income (Sutton et al., 2011). Thus, retaining more N in soil and its targeted supply to crops is one of the most important challenges for sustainable agricultural management.

Substantial amounts of N are lost between cultivation phases, where longer periods of variable soil moisture and temperature with periods of warming, cooling, freezing and thawing release soil N before it can benefit the next crop generation (Cookson et al., 2002; Sieling and Kage, 2006). Seasonal temperature fluctuations are identified as main drivers of N transformation processes in soil (Hong et al., 2019; Li et al., 2021), and there is detailed knowledge on long-term N transformation after soil incubation at different temperatures (Dai et al., 2020; Hu et al., 2020), or after short-term stresses like soil freezing-thawing cycles (Li et al., 2019; Monteux et al., 2020; Rosinger and Bonkowski, 2021; Rosinger et al., 2022). However, experiments addressing the effects of seasonal temperature fluctuations on N transformations in agricultural soils are scarce (Sieling and Kage, 2006).

Nitrification, i.e., the microbial conversion of ammonium (NH_4^+) to nitrate (NO_3^-) in soil is a major source of seasonal N losses in agriculture (Norton and Ouyang, 2019). Farmers strive to directly control soil nitrification rates by applying synthetic nitrification inhibitors (NI) to inactivate the microbial enzymatic conversion of NH_4^+ to NO_3^- (Lin and Hernandez-Ramirez, 2020; Zerulla et al., 2001). However, the persistence of NI strongly depends on soil temperature, moisture, and fertilization rate, which may restrict their effectiveness (Norton and Ouyang, 2019; Thapa et al., 2016). An indirect measure to prevent soil N losses in agriculture is the use of high organic C amendments (HCA) (Reichel et al., 2018). The incorporation of crop straw as HCA is one of the oldest, most feasible and economic practices worldwide to reduce soil N losses by improving the internal cycling of N (Diacono and Montemurro, 2011; Norton and Ouyang, 2019; Xia et al., 2018). As microbial growth in agricultural soil is strongly limited by the availability of C, the incorporation of HCA, such as straw with its wide C:N ratio, stimulates the microbial uptake of N for stoichiometric reasons (He et al., 2015). Farmers may apply the straw of pre-crops before sowing the next-generation crop in order to immobilize excess mineral N (N_{min}) in soil and microbial biomass (van Duijnen et al., 2018). Although microorganisms are very effective short-term competitors for N, plants may win in the longer term (Hodge et al., 2000; Kuzyakov and Xu, 2013; Sieling and Kage, 2006) due to the constant remobilization of microbial biomass N in the rhizosphere by the microbial loop and subsequent root or mycorrhizal uptake (Bonkowski, 2004; Koller et al., 2013a, 2013b; Rozmoš et al., 2022). However, intensive agricultural management may impede these processes (Roy et al., 2017). Significant parts of N may remain sequestered in soil microbial biomass and cause N shortage of crops if N is not released in time (Jingguo and Bakke, 1997), and even higher N fertilization may be needed to sustain optimal growth of the next crop generation. However, studies on the long-term effects of straw incorporation and its effects on the performance of subsequent crops are quite rare (Congreves et al., 2013). More precise management of the timing of N immobilization and mobilization by straw amendments could make a significant contribution to global efforts to reduce agricultural N losses and mitigate greenhouse gas emissions. At present, it is even unclear if direct (e.g., application of NIs) and indirect (e.g., application of straw) means can be reasonably combined to prevent N losses from soils.

Therefore, we designed a mesocosm experiment to simulate N dynamics between crop cultivation phases in soil using treatments with wheat straw incorporation in combination with the NI Piadin® at three different N fertilization rates. Variable incubation temperature represented winter and spring soil temperature changes under field conditions (Fig. 1). We quantified soil N_{min} and microbial biomass dynamics as well as N losses from soil as N_2O and leachate and the subsequent uptake of the remaining N into barley as next-generation crop. We hypothesized that i) a combination of NI and straw addition will be most effective due to additive effects on barley N uptake; ii) straw addition will be an effective means to reduce N losses over seasonal temperature fluctuations; iii) but soil cooling-warming periods stimulate mineralization of sequestered microbial N into soil to supply nutrients to subsequent crops.

2. Materials and methods

2.1. Experimental set up

Agricultural soil was sampled at the Experimental Farm "Hohenschulen" (University of Kiel, Germany, 54°18′N, 9°58′E). The soil was classified as Luvisol with 58 % sand, 29 % silt, 13 % clay content and pH 6.5, containing 1.07 % organic C, 0.11 % total N (Ni et al., 2018).

Mesocosms (pots with 22 cm height, 24 cm diameter, 10 L volume) were filled with 8 kg dry weight of sieved (1 cm mesh size) and well homogenized soil at a bulk density of 1.2 g cm^{-3} in the pot. A hole of 1 cm diameter at the bottom of the mesocosms, covered with a 1 mm gauze to prevent soil from falling out, served for collecting leachate.

One week before the experiment started, wheat straw (41.8 % C, 0.84 % N), cut into 1–2 cm pieces, had been mixed into the soil of half of the mesocosms, giving 2.4 g C kg^{-1} soil (eq. 14.4 t ha^{-1} wheat straw amendment, which is closed to the upper limit of conventional straw amendment in agriculture, see e.g., Liu et al., 2014). The experiment started after adding 2.09 atom % ^{15}N-enriched N-fertilizer ($(^{15}NH_4)_2SO_4$, VWR, Germany) at three rates: N0, no fertilizer added; N1, 36.5 mg N kg^{-1} soil (eq. to 125 kg N ha^{-1}); N2, 73 mg N kg^{-1} soil (eq. to 250 kg N ha^{-1}), and application of nitrification inhibitor (NI) at 2.04 μl kg^{-1} soil (eq. to 7 l ha^{-1}) to half of the mesocosms. The NI application was repeated on day 62 with a rate of 1.75 μl kg^{-1} soil (eq. to 6 l ha^{-1}, the start of the warming phase, see below), the application rate was selected according to manufacturer recommendation (https://www.piadin.de/en/#yield). As nitrification inhibitor served a commercial formulation (PIADIN®, SKW, Piesteritz, Germany), which contains approximately 3.1 % and 1.6 % of the two active pyrazole derivates 1H-1,2,4-triazole and 3-methylpyrazole, respectively (Barneze et al., 2015). The effect of PIADIN® starts immediately after fertilization, and the conversion of ammonium to nitrate is said to be delayed by six to ten weeks (https://www.piadin.de/en/#home). Soil water content was adjusted to 50 % WHC (equalling a gravimetric water content of

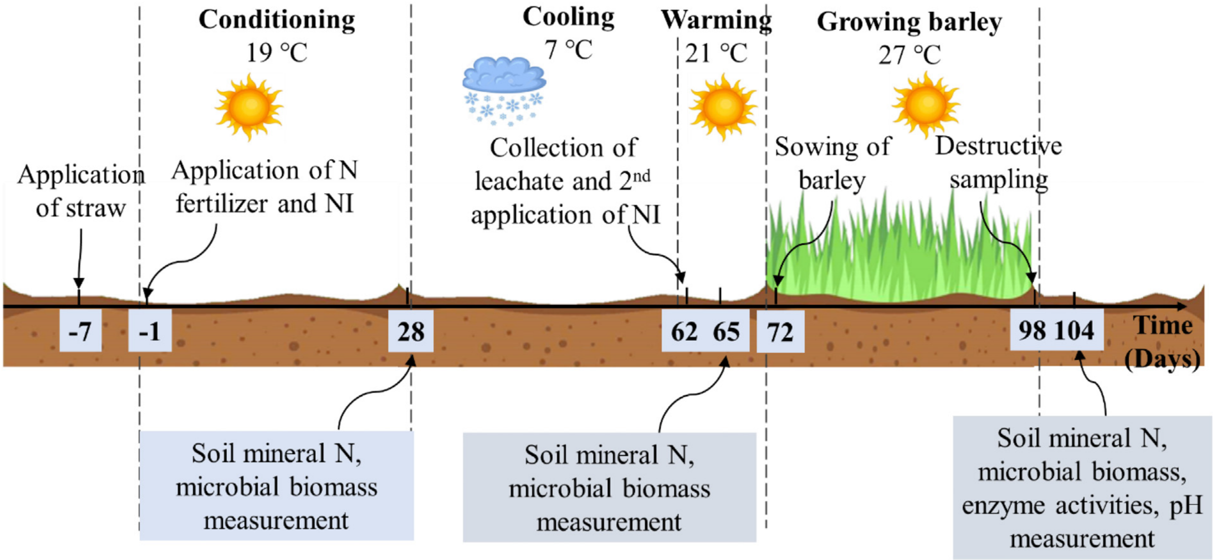

Fig. 1. Time plan of the experiment.

19 %) on the day of fertilizer application and monitored by regularly weighing the mesocosms (every two to three days during all phases).

Overall, the experimental treatments included three rates of N fertilizer (N0, N1 and N2), without (S0) and with (S1) wheat straw as a high-C amendment, and with and without a nitrification inhibitor (NI) to reduce post-harvest N-losses in a full factorial design. Each treatment had 7 replications totalling to 84 mesocosms (full description of treatments coding is found in Table S1).

The experiment was separated into three phases from June 2020 to September 2020 (Fig. 1): i) a conditioning phase from day 0 to day 28 simulating the summer and autumn season, during which mesocosms were incubated at stable daily average temperatures of around 19 ± 5 °C in a greenhouse, ii) a cooling-warming phase from day 29 to day 71, simulating mild winter soil temperatures of 7 °C over one month from day 29 till day 60 in a climate chamber, followed by a temperature rise to 21 ± 5 °C from days 61 to 71 simulating the typical temperatures in the spring and following summer, and finally iii) a growing phase (day 72 to 98), where each mesocosm was sown with 50 seeds (eq. to 940 seeds m^{-2}) of winter barley on day 72 and incubated at 27 °C in a temperature-controlled greenhouse until harvested at day 98. Winter barley served as phytometer to calculate the efficiency of ^{15}N-fertilizer transfer to the next crop generation.

2.2. Analytical measurements

Soil samples were taken on day 28 at the end of the conditioning phase, on day 65 at the end of the cooling-warming phase, and on day 104 at the end of the growing phase. Around 30 g soil was sampled from three locations in each mesocosm with a soil corer of 2 cm diameter to a depth of 10 cm.

Soil NH_4^+ and NO_3^- contents, and microbial biomass N and C were determined in all three phases. Soil enzyme activity and pH were measured at the end of the growing phase. For measurements of NH_4^+ and NO_3^- contents, 5 g fresh soil was extracted with 20 ml 0.01 M $CaCl_2$. After horizontal shaking for 30 min, subsequent centrifugation (5 min, 4500 rpm) and filtration through a Whatman 595 filter paper, NH_4^+ and NO_3^- concentrations were immediately determined using ELIT 8051 and ELIT 8021 ion-selective electrodes, respectively (Nico 2000 Ltd., UK). Soil N_{min} was calculated as the sum of NH_4^+- and NO_3^--N. Soil pH was determined in 0.01 M $CaCl_2$ at a ratio of 1:5 (*w/v*) (multi 340i pH meter, WTW GmbH, Weilheim, Germany) according to the ISO 10390 method (ISO, 2005).

Microbial biomass carbon (MB-C) and nitrogen (MB-N) were measured using the chloroform fumigation-extraction method (Vance et al., 1987). In brief, the soil sample was split into two parts of 4 g fresh weight each, one part was fumigated with ethanol-free chloroform in a desiccator for 24 h. Then, both fumigated and unfumigated soil samples were extracted with 16 ml of 0.5 M K_2SO_4. Extractable C and N were measured with a TOC/TN analyser (Multi N/C 2100S, Analytik Jena, Germany). MB-C and MB-N were calculated in fumigated samples subtracting the extractable C and N in the unfumigated samples with conversion factors of 0.45 and 0.54 for MB-C and MB-N, respectively (Joergensen and Mueller, 1996).

Potential extracellular activities of leucine aminopeptidase (LAP) and *N*-acetyl-glucosaminidase (NAG) were measured after the growth of barley (day 104) by applying standard fluorimetric techniques at 365 nm using a multiplate reader (Varioskan™, Thermo Fisher Scientific GmbH, Germany) with the substrate-conjugated fluorescent compounds, L-leucine-7-amino-4-methylcoumarin (AMC) and 4-methylumbelliferone (MUB) according to Marx et al. (2001) and German et al. (2011).

After barley growing, dry weight of shoots was determined (48 h, 60 °C) and total N content of milled shoot material was determined with a CN element analyser (Flash 2000, Thermo Fisher Scientific GmbH, Germany). Samples for ^{15}N analysis were sent to the Stable Isotope Facility, University of California, Davis (https://stableisotopefacility.ucdavis.edu/) for analysis.

The N fertilizer use efficiency (NUE) of barley shoots was calculated according to eq. (1) (Cabrera and Kissel, 1989).

$$\text{NUE}\ (\%) = \frac{p \times (c - b)}{f \times (a - b)} \times 100.$$

where p = moles of N of plant material, f = moles of N of fertilizer, c = atom % ^{15}N abundance of plants, b = atom % ^{15}N of plants in unfertilized soil, a = atom % ^{15}N of fertilizer.

N_2O fluxes were measured weekly and biweekly using the closed chamber method (Dobbie et al., 1999). Gas samples were taken four times over one week (i.e. 4 technical replicates) after the application of fertilizer and NI, as well as throughout the warming phase. Opaque PVC gas sampling chambers of 20 cm × 55 cm (diameter × height) were used to enclose the whole plants for gas sampling. The chambers were inserted into 5 cm soil depth to ensure gas-tightness. Sampling time was always between 11 am and 1 pm throughout the observation period. For each gas sampling, 20 ml of headspace gas samples were collected 30, 60 and 90 min after the chambers were inserted into the soil. As control, five ambient air samples were collected, and their average N_2O concentrations served as baseline N_2O measurements. N_2O were measured with a gas chromatograph, equipped with an electron capture detector and a flame ionization detector (GC-ECD/FID, Clarus 580, PerkinElmer, Rodgau, Germany) (Reichel et al., 2018).

To obtain leachate, mesocosms were watered with 600 ml water on day 62, the second day of the warming phase. The volumes of leachate were recorded and concentrations of NO_3^- and NH_4^+ were measured with ion-sensitive electrodes as described above.

2.3. Statistical analysis

All statistical analyses were performed in R 3.6.3 (R Core Team, 2020), and graphs were prepared with Origin Pro 8.1 (Origin Lab, Northampton, MA, USA). Data was analysed by 3-way analysis of variance (ANOVA) to test for the effects of N fertilizer, straw and NI. Comparisons between means were performed at $p < 0.05$ by a Tukey test.

Structural equation models (SEM) were calculated using the 'lavaan' package in R (Rosseel, 2012) in order to investigate potential microbial mobilization of N_{min} by enzyme activity and fluxes into barley and microbial biomass at the end of the experiment. The best fitting model was selected by step-wise removal of non-significant paths. The data were square root-transformed before SEM analysis, considering non-dimensional expressions. The standardized total effects of each variable on shoot N were calculated by summing up all direct and indirect effects. PCA was used to extract a major principal component (PC1, explaining 87 % of total variation) for SEM analysis from soil MB-C and MB-N (Fig. 2) as a representative parameter describing soil microbial biomass dynamics. The criteria for evaluation of the SEM fit, such as the Chi-square/degree values (CHI/DF), goodness-of-fit index (GFI) and standardized root mean squared residual (SRMR) were adopted according to Shen et al. (2021).

3. Results

3.1. N-mineralization and soil microbial biomass after wheat straw amendment

Straw addition led to a 63 % increase of MB-C ($F_{[1,67]} = 28.1, p < 0.001$), but MB-N increased by 144 % ($F_{[1,58]} = 39.6, p < 0.001$, Fig. 2A, D). The disproportionate N-uptake in microbial biomass led to an overall strongly reduced MB-C:N ratio. The incorporation of N into MB-N was also influenced by N-fertilization rate and NI (significant N × NI interaction, $F_{[2,58]} = 3.6$, $p = 0.033$), but to a lesser extent. On average, NI slightly decreased MB-N in N0 and N1 by 12 and 16 %, respectively, but slightly increased MB-N (+16 %) in N2.

Accordingly, the resulting MB-C:N ratio was influenced by straw in concert with N-fertilization rate and NI (significant N × S × NI interaction, $F_{[2,56]} = 6.3, p < 0.003$). Straw always reduced MB-C:N to low values of 5–6, while in absence of straw the MB-C:N ratio was 10, 9 and 10 in N0, N1 and N2 levels, respectively (Fig. 2G).

Despite the increase of soil N_{min} by N fertilization from an average of 26 mg N kg^{-1} in N0 to 30 and 44 mg N kg^{-1} in N1 and N2, respectively ($F_{[2,67]} = 17, p < 0.001$), addition of straw always decreased N_{min} by half of the -straw treatments ($F_{[1,67]} = 12.1$, $p < 0.001$; Table S2). Soil NO_3^- contributed by far the most (67–92 %) to N_{min} content, and straw showed its greatest effects on reducing NO_3^- (38.7 and 16.9 mg N kg^{-1} in -straw vs. +straw, respectively), while the proportion of NH_4^+ increased in

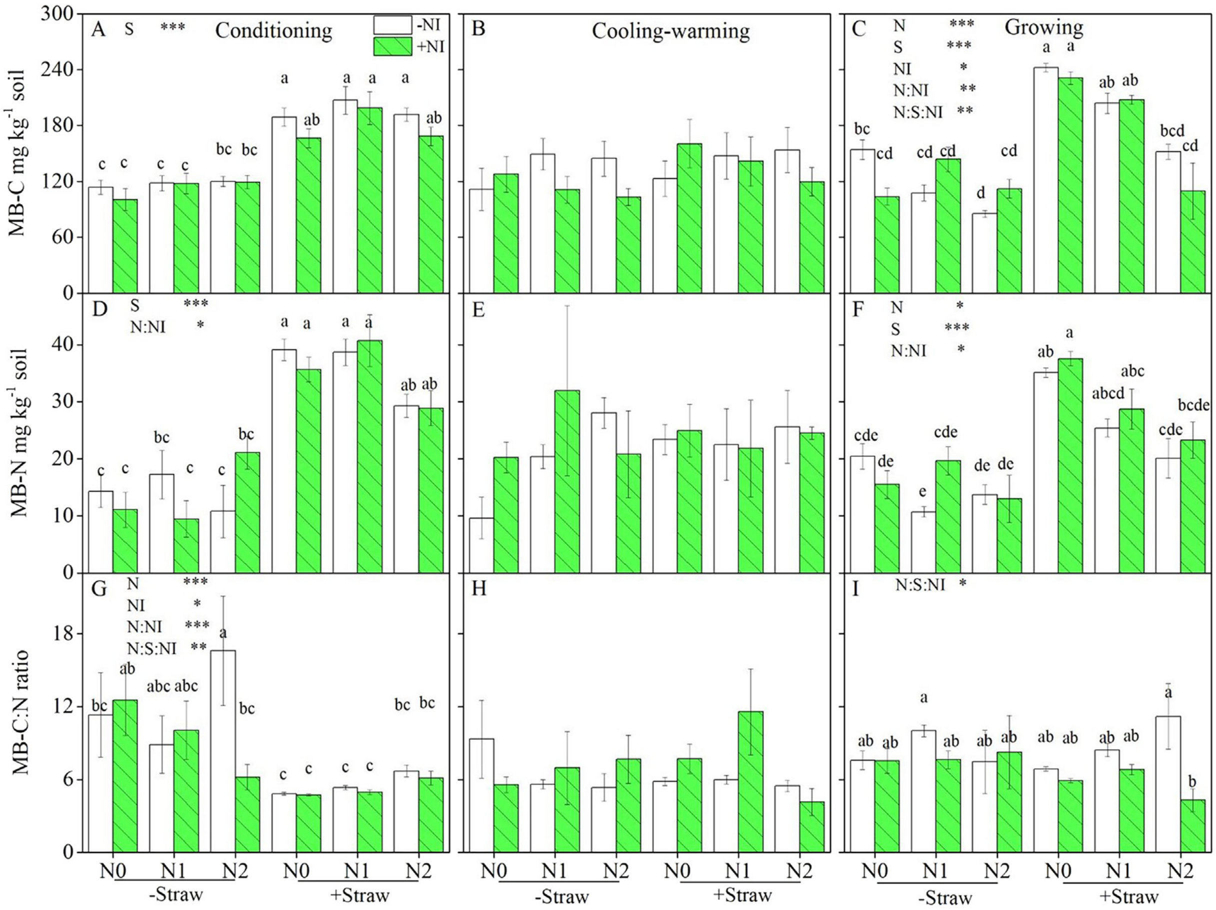

Fig. 2. Soil microbial biomass C (MB-C), N (MB-N) and their ratio (MB-C:N) under different treatments in the conditioning, the cooling-warming and the growing phase. The treatments N0, N1 and N2 refer to the N fertilization rates of 0, 125 and 250 kg N ha^{-1}, respectively; S and NI refer to application of straw and nitrification inhibitor, respectively. Given is the mean ± 1SE ($n = 7$). Different lowercase letters indicate significant differences between treatments at the $p < 0.05$ level. Significant treatment and interaction effects as revealed by 3-way ANOVA are given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

+straw treatments (+ 24 %, $F_{[2,66]} = 7.8$, $p < 0.01$). In comparison, the influence of NI on soil NH_4^+ and NO_3^- levels was rather small in the conditioning phase and affected only the N2 fertilization rate (N × NI interaction, Table S2).

3.2. N-mineralization during the cooling-warming phase

The amount of NH_4^+ had increased on average by 77 % after cooling-warming (Fig. 3, Table S2), with an increase by 57 and 94 % without and with NI addition, respectively. In the absence of NI, the relative increase of soil NH_4^+ levels due to cooling-warming was reduced at higher N fertilizer rates (with 83 %, 48 % and 44 % increases of NH_4^+ in N0, N1 and N2, respectively), but this was not the case in the presence of NI. NI application strongly enhanced the total amount of NH_4^+ in N1 and N2 fertilization treatments, but not in N0 (N × NI, $F_{[2,67]} = 24.9$, $p < 0.001$), while straw strongly reduced the positive effect of NI on the availability of NH_4^+ during the cooling-warming phase (significant N × S × NI interaction, $F_{[2,67]} = 11.3$, $p < 0.001$).

The positive effect NI on NH_4^+ and NO_3^- content was restricted to treatments with high fertilization (N2) without straw (significant N × S × NI interaction; Table S2). Accordingly, soil NO_3^- content was strongly enhanced during cooling-warming phase in N2 fertilization treatments and further increased with NI addition (38 % vs. 112 % increase in N2 and N2-NI, respectively) compared to conditioning phase. Moreover, the increase of NO_3^- was higher in absence of straw, NO_3^- content increased in N2 by 47 % and 133 % without and with NI, respectively.

3.3. N-mineralization and uptake during successive crop growth

During growing phase, MB-N was increased in +straw treatments on average by 78 % ($F_{[1,62]} = 14.7$, $p < 0.001$) as compared to non-straw-amended soils, indicating that still significant amounts of N were locked up in microbial biomass. MB-C and MB-N remained approximately at constant levels in treatments without straw, while straw caused a doubling of MB-C and MB-N in N0. However, this effect decreased with increasing N-fertilization until MB-C and MB-N in N2 with straw was equal to N2 without straw (Fig. 2C, F). Effects of NI on MB-C were low and variable between treatments during growing phase (N × S × NI, $F_{[2,61]} = 4.7$, $p < 0.05$).

As expected, soil N_{min} contents of growing phase were strongly reduced, owing primarily to N uptake by plants (Fig. 3, Table S2). Compared to N0 and N1, the high N2 fertilization rate led 2-fold and 1.7-fold higher residual soil N_{min}, respectively ($F_{[2,67]} = 13.2$, $p < 0.001$), matching the high soil NO_3^- contents in N2 ($F_{[2,68]} = 15.0$, $p < 0.001$). Straw in contrast increased the availability of NH_4^+ during barley growth, but the effect was strongly dependent on N-fertilization rates. Straw led to increased soil NH_4^+ contents only in N0 (2-fold) and N1 (1.7-fold) treatments as compared to the average of all the other treatments (N × S interaction; $F_{[2,68]} = 20.2$, $p < 0.001$).

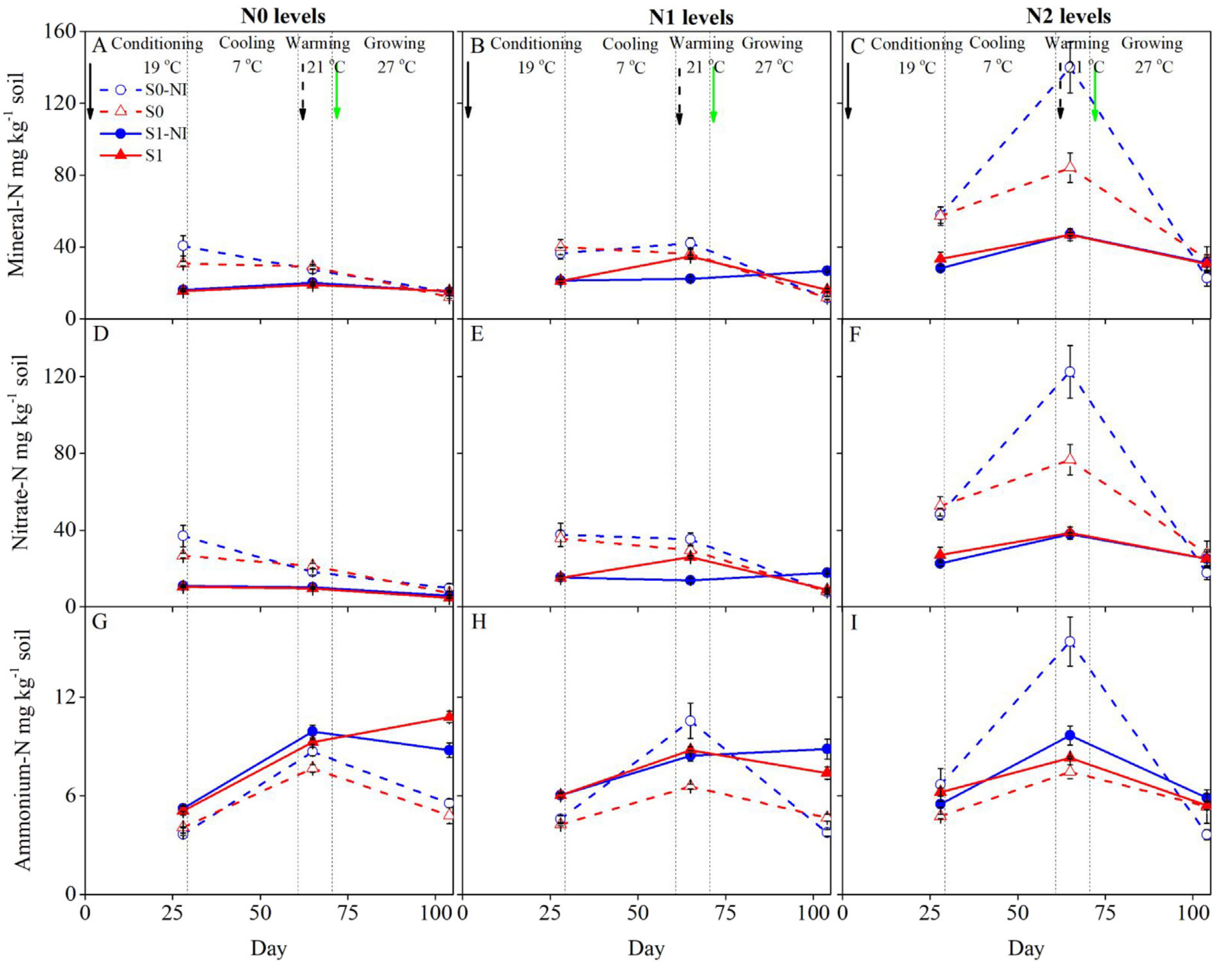

Fig. 3. Soil NH_4^+, NO_3^- and mineral N contents under different treatments in the conditioning, the cooling-warming and the growing phase. The treatments N0, N1 and N2 refer to the N fertilization rates of 0, 125 and 250 kg N ha^{-1}, respectively; S0 and S1 refer to the treatments without or with straw application, S0-NI and S1-NI refer to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n = 7). Black solid arrows indicate the time of fertilization and first NI application, black dashed arrows indicate the time of second NI application, while green arrows indicate the time for sowing the seeds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Due to the restricted size of the mesocosms, the barley plants had to be harvested premature, well before the plants started to sprout. Shoot biomass of barley correlated well with shoot N uptake ($R^2 = 0.83$, $p < 0.001$), indicating that crop growth was mainly limited by the availability of N. Accordingly, treatment effects on shoot biomass were similar to the treatment effects on shoot N content. Compared to unfertilized control soils (N0), N-fertilization led to higher shoot biomass and N-uptake at both N1 and N2 fertilizer levels. However, while straw reduced shoot biomass by 23–34 % and shoot N by 28–46 % in N0 and N1treatments, growth of barley at N2 fertilization rates was not negatively affected by straw (N × S, $F_{[2,66]} = 4.1$, $p < 0.05$, Fig. 4A, C).

^{15}N-labelling of fertilizer allowed for calculation of plant-uptake efficiency of applied N as affected by straw and NI. Despite the higher total uptake of fertilizer N by barley in N2, barley NUE was significantly higher in N1 (50%) than in N2 (35%), and straw reduced NUE to 25 % at both N1 and N2 fertilization rates (N × S, $F_{[1,48]} = 47.1$, $p < 0.001$).

3.4. N lost from soil

Final total N losses, comprising N_2O-N emissions and leachate N, were 0.17–2.56 mg N kg^{-1} soil (Fig. 5), which is much less than the soil N_{min} content (12–33 mg N kg^{-1} soil) and barley shoot N (17–48 mg N kg^{-1} soil). Despite a significantly higher release of N_2O with straw at high N-fertilization (N2), overall N losses were reduced by 41 % in +straw compared to -straw treatments ($F_{[1,36]} = 22.4$, $p < 0.001$), because N leaching losses were 43–91 % lower. While straw increased N_2O losses in N2 rates, therefore the effects of straw on total N losses differed between N rates (N × S, $F_{[1,36]} = 4.9$, $p < 0.05$). NI led to a significant (−40 %) reduction of N_2O losses at high N application (N2) and this effect was independent of straw application (Fig. 5, $p < 0.001$).

3.5. Soil enzyme activity

The N-acquiring enzyme activities were influenced by straw and N fertilization, but the effects differed between enzymes (Fig. 6). Straw led to increased LAP activities of 58–88 %, while N fertilization decreased LAP activity. In the presence of straw, LAP decreased by 30 % in N2 as compared to N1, and by 20 % in N1 as compared to N0. Without straw, LAP decreased in N2 by 50 % as compared to N0 (Fig. 6A, $p < 0.001$). NAG activity was increased by 113 %, 151 % and 161 % with straw in N0, N1 and N2 treatments, respectively (Fig. 6B, $p < 0.001$).

3.6. Structural equation model

The SEM explained 70 % of variation of total shoot N uptake (Fig. 7A). It showed that after the cooling-warming phase a significant part of the soil NH_4^+ had been converted to NO_3^- ($\lambda = 0.45$, $p < 0.001$). Soil NO_3^- measured after the cooling-warming phase showed a strong positive correlation with shoot N uptake ($\lambda = 0.51$, $p < 0.001$), indicating that NO_3^- was the major N source for plant nutrition. Shoot N uptake in contrast showed a negative correlation to soil NH_4^+ ($\lambda = -0.19$, $p < 0.01$) after the cooling-warming phase. Also, the NO_3^- contents in the growing phase were

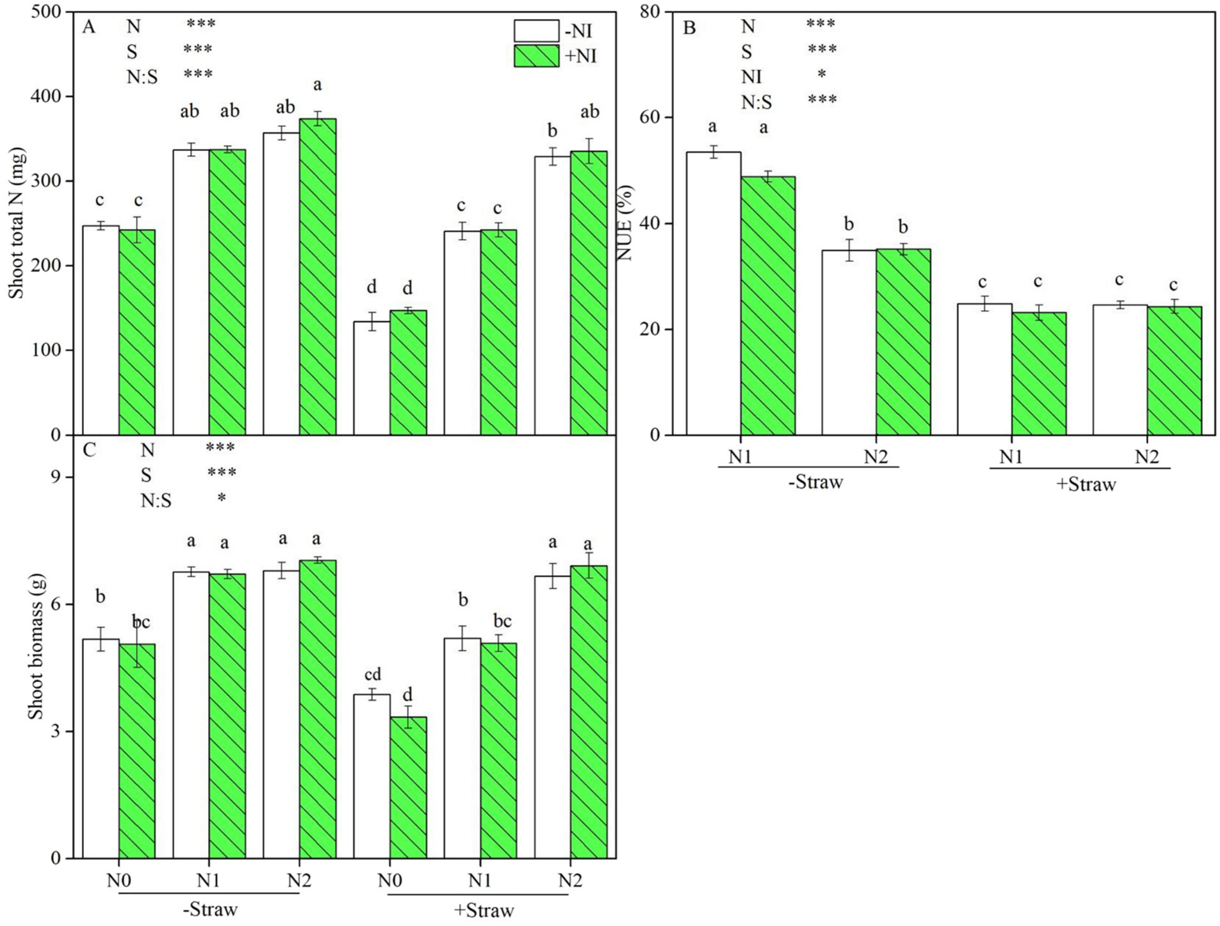

Fig. 4. Barley shoot N content (A), NUE (B) and shoot biomass (C) under different treatments. The treatments N0, N1 and N2 refer to the N fertilization rates of 0, 125 and 250 kg N ha^{-1}, respectively; S and NI refer to application of straw and nitrification inhibitor, respectively. Given is the mean ± 1SE (n = 7). Different lowercase letters indicate significant differences between treatments at the $p < 0.05$ level. Significant treatment and interaction effects as revealed by 3-way ANOVA are given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

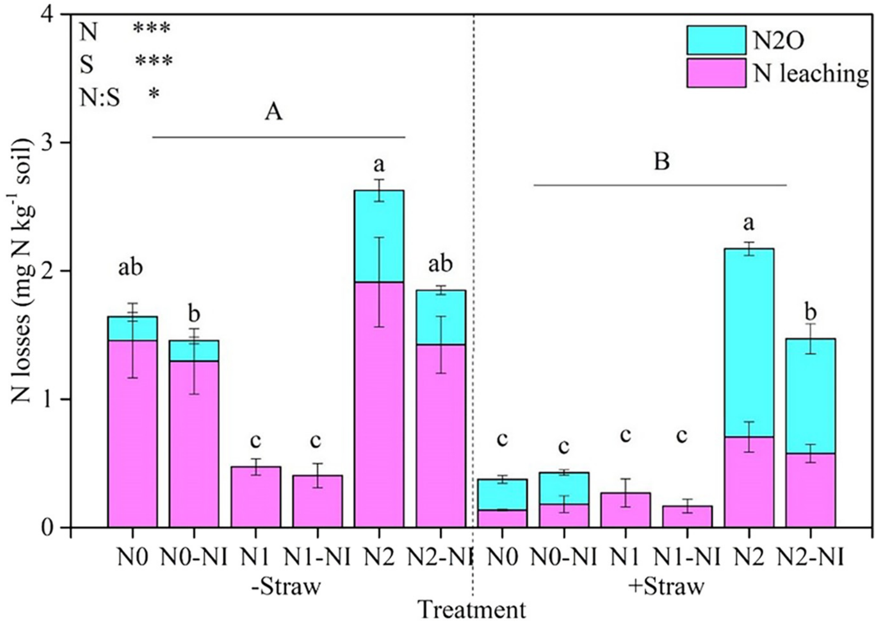

Fig. 5. N losses from soil as N_2O and leachate under different treatments of the whole experiment phases. The treatments N0, N1 and N2 refer to the N fertilization rates of 0, 125 and 250 kg N ha^{-1}, respectively; S refers to straw application; N0-NI, N1-NI and N2-NI refer to the treatments with nitrification inhibitor application. Given is the mean ± 1SE ($n = 4$). Different lowercase letters indicate significant differences between treatments within - straw and + straw treatments at the $p < 0.05$ level, while different capital letters indicate significant differences between treatments grouped by straw at the $p < 0.05$ level. Significant treatment and interaction effects as revealed by 3-way ANOVA are given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

positively correlated to NO_3^- levels measured after the cooling-warming phase ($\lambda = 0.47, p < 0.001$), but correlated much less with shoot N uptake ($\lambda = 0.16, p < 0.05$). After the cooling-warming phase soil microbial biomass and soil NO_3^- contents showed a strongly negative correlation ($\lambda = -0.61, p < 0.001$), while soil NH_4^+ contents of growing phase were positively correlated with microbial biomass ($\lambda = 0.21, p < 0.05$), indicating ongoing mineralization. Measurements at the end of the experiment indicate that soil microbial biomass had stimulated the activity of the N-mining enzyme LAP ($\lambda = 0.42, p < 0.001$), but less at high soil NO_3^- contents after the cooling-warming phase ($\lambda = -0.58, p < 0.001$). A strongly positive correlation of LAP activity and NH_4^+ ($\lambda = 0.68, p < 0.001$) indicates that the enzyme released NH_4^+ from organic material. The positive correlation between NH_4^+ and NO_3^- remaining during the growing phase ($\lambda = 0.28, p < 0.001$) indicates that ammonium, being a substrate for nitrification, still was in excess to fuel this process. The standardized total effects of the SEM (Fig. 7B) show that NO_3^- was the most influential N stock for plant uptake after the cooling-warming phase, and confirm negative relationships between barley N uptake and microbial biomass, LAP and NH_4^+ after the growing phase.

4. Discussion

We investigated the combined effects of straw and a nitrification inhibitor (PIADIN®) to prevent N mobilization in agricultural soil during a non-vegetated phase over variable temperatures and its mobilization and plant uptake into a simulated next barley crop generation. One month

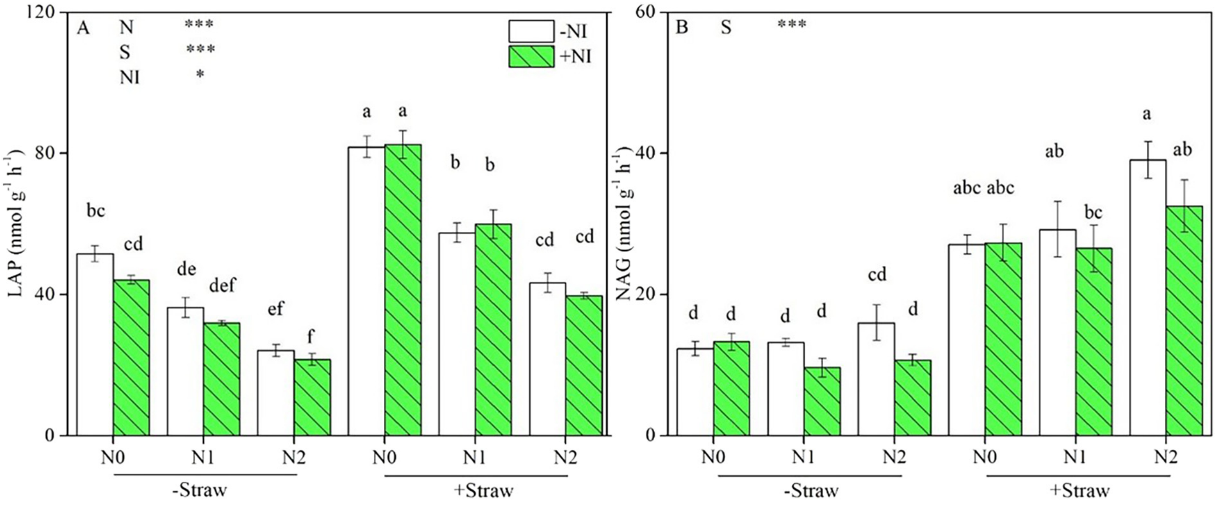

Fig. 6. Enzyme activities of leucine aminopeptidase (LAP) and *N*-acetyl-glucosaminidase (NAG) under different treatments after the growing phase. The treatments N0, N1 and N2 refer to the N fertilization rates of 0, 125 and 250 kg N ha^{-1}, respectively; S and NI refer to application of straw and nitrification inhibitor, respectively. Given is the mean ± 1SE ($n = 7$). Different lowercase letters indicate significant differences between treatments at the $p < 0.05$ level. Significant treatment and interaction effects as revealed by 3-way ANOVA are given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

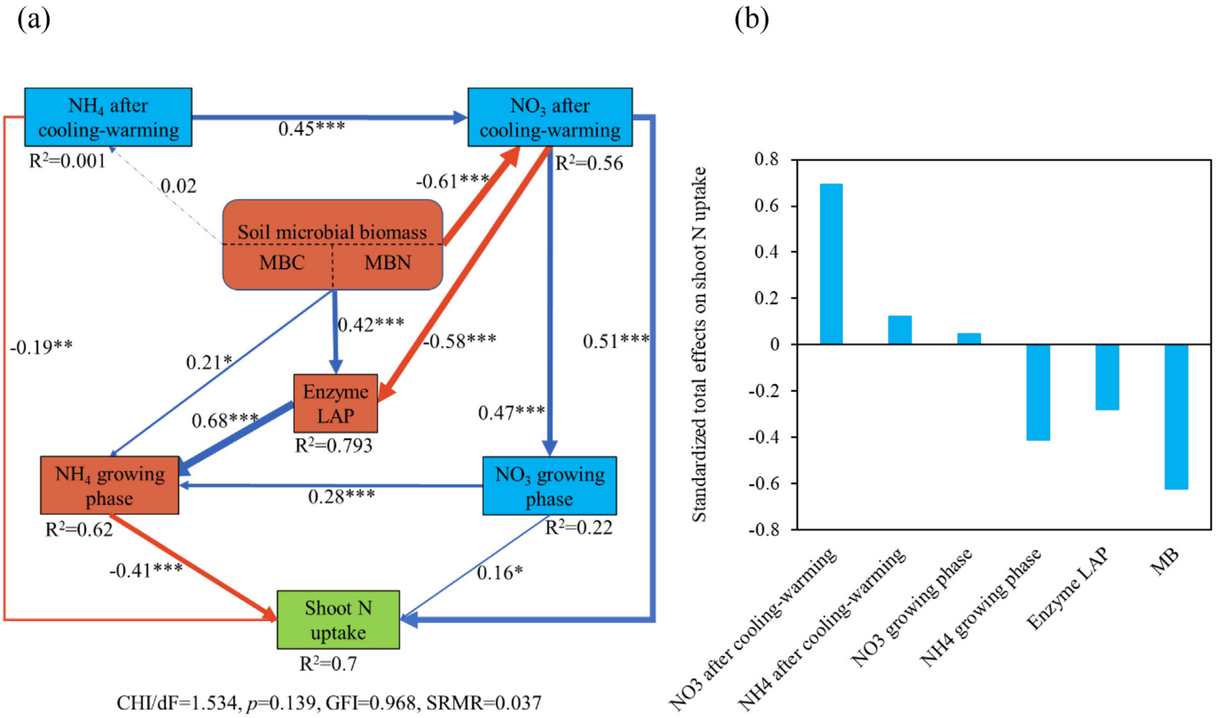

Fig. 7. Structural equation model explaining the multivariate effects on barley shoot N uptake (A) and the total effects of controlling variables on the barley shoot N uptake (B). The numbers adjacent to arrows are standardized path coefficients, which reflect the effect size of the relationship. Arrow width is proportional to the strength of path coefficients, the blue and red arrows indicate positive and negative relationships, respectively; the continuous and dash arrows indicate significant and non-significant relationships, respectively. The proportion of variance explained (R^2) appears alongside each response variable. Significant levels are as follows: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$. Goodness-of-fit statistics are shown underneath the modeling frames. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conditioning at 25 °C had led to the conversion of most (86–93 %) of the fertilizer-NH_4^+ to NO_3^- (Fig. 3), irrespective of the fertilizer application rates (N1 or N2), showing that the direct suppression of nitrification by NI was short-lived (Barneze et al., 2015; Federolf et al., 2016). The effectiveness of most NI products rapidly decreases with increasing soil temperatures due to an increased activity of nitrifiers (Thapa et al., 2016). Accordingly, NO_3^- was the dominant form of mineral N remaining in soil after the conditioning phase. During soil cooling N mineralization processes continue, but gross nitrification rates respond quite sensitive to low temperatures as compared to N mineralization and immobilization (Björsne et al., 2014; Schütt et al., 2014). Differences in soil NH_4^+ contents between N fertilization rates (N0,N1,N2) after the cooling-warming phase therefore reflect the mineralization of previously immobilized N and a parallel suppression of nitrification in the cooling period. For example, Cookson et al. (2002), showed that decreasing soil temperatures from 15 to 2 °C for 56 days led to a transient accumulation of NH_4^+ followed by a strongly delayed nitrification. In our experiment, the accumulation of NH_4^+ during cooling-warming cycle continued due to a second NI application after the cooling phase, where in the soils without straw but with NI, the NH_4^+ contents were steeply enhanced with increasing N fertilization rates. This demonstrates a legacy-effect of NI after the cooling-warming phase where high amounts of N_{min} were released at high N fertilization long after NI application. The soil cooling effect was not sufficiently strong to suppress net nitrification at high N application rate (N2), leading to an almost exponential increase of NO_3^- with increasing N fertilization rates in soils without straw. The fact that soil NO_3^- levels were further enhanced by NI (significant N × S × NI interaction) indicates that NI legacy caused only a delay, but did not prevent the nitrification processes.

In contrast to NI, straw led to a rapid immobilization of N and reduced potential mineralization losses by more than half reductions of soil NO_3^- contents in the conditioning as well as in the cooling-warming phase. However, due to optimal watering of plants in our experiment the amount of N leaching losses accounted only for 1 % of fertilizer N, which is rather low compared to losses under natural field conditions (Abdalla et al., 2019). Under natural seasonal rainfall dynamics, N retention by straw is expected to be much more effective than what we simulated in our mesocosm study.

Straw applied after crop harvest, has a high potential to immobilize available soil N in microbial biomass (He et al., 2015; Xia et al., 2018). Straw provides soil microorganisms with a source of readily available C and stimulates microbial growth and reproduction (Pan et al., 2016; Reichel et al., 2018). Accordingly, N immobilization in the conditioning phase was strongly reflected by microbial nutrient contents, with straw increasing MB-C and MB-N 1.6-fold and 2.4-fold, respectively. The strongly negative correlation between soil microbial biomass and soil NO_3^- contents after the cooling-warming phase in the SEM indicates the microbial uptake of NO_3^-. The fact that NI affected soil NH_4^+ and NO_3^- in the absence of straw during the cooling-warming phase, shows that N immobilization by straw dominated over the effect of NI (Ma et al., 2019). Straw on the other hand induced higher N_2O emissions in N2 (Fig. 5), probably by providing additional C for denitrifiers (Akhtar et al., 2020; Xu et al., 2021). The combined application of NI with straw was shown to mitigate N_2O emissions caused by HCA (Wu et al., 2017) and this was confirmed at high fertilization (N2) in our experiment. The overall low N losses from soil as N_2O and leachate (0.17–2.6 mg N kg^{-1}) in the present study had, however, little effect on barley N uptake and the total N budget of the whole system (Fig. 5). Overall, under natural conditions the application of straw and its combination with NI, especially at high fertilizer rates, has a high potential to reduce N losses from agricultural systems (Di and Cameron, 2002; Wu et al., 2017).

However, a great challenge of straw application is the timing of N-release for the following crop. The mineralization of N from straw can occur weeks or months after its application (Cao et al., 2018; Shindo and Nishio, 2005). N-mineralization is mainly a function of substrate stoichiometry which can be influenced by N fertilization at times of HCA incorporation (van Duijnen et al., 2018). High N sequestration by microbial biomass occurs at C:N ratios of >1:30 (Hodge et al., 2000), but the rates of net N mobilization will increase with decreasing substrate C:N ratio towards a critical threshold value at C:N ~ 20–25 for microbial mineralization (Aoyama and Nozawa, 1993; Kuzyakov et al., 2000). Fertilization of the applied straw decreased the C:N ratio of soil amendments from 49 in N0 to 28 and 22 in N1 and N2, respectively, which generally meets the stoichiometric requirements for net N mobilization at some later stage. High activity of both N-liberating enzymes, LAP and NAG in +straw treatments (Fig. 6) indicates still high microbial demand for N in all +straw treatments (Kumar et al., 2017; Sinsabaugh et al., 1993). Since the activity of NAG mainly reflects the mineralization of fungal biomass (Sinsabaugh and Moorhead, 1994), we included only LAP in our SEM model. Enhanced LAP activity correlated positively to higher microbial biomass (Fig. 7). However, in agreement with stoichiometric theory, the LAP activity decreased at higher N fertilization (Fig. 6), demonstrating that the N demand of soil microorganisms induced by straw was partly alleviated by N fertilizer application. Accordingly, straw led to the re-mineralization of N in the barley growth phase, where straw increased soil NH_4^+ contents by 2-fold in N0 and 1.7-fold in N1 (Fig. 3, Table S2). Soil NH_4^+ content is the main indicator of ammonification and re-mineralization in our experiment. NO_3^- content depends on the further N transformations like nitrification and denitrification. This demonstrates that microbial mineralization of straw acted as a slow-release fertilizer during plant growing. Our hypotheses of a rapid, temporal sequestration of N directly after incorporation of straw; and the potential of straw to release NH_4^+ later for plant uptake after subsequent soil cooling-warming periods was hereby confirmed. It must be noted, however, that the size of the mesocosms strongly restricted the growth of the root system and that the barley therefore had to be harvested prematurely. The NUE of shoots with 50 % and 35 % in N1 and N2, respectively, decreased with increasing fertilization rate, but in +straw treatments NUE was only 25 % at this premature state. The N gain of crops from decomposition of straw and subsequent release of N might last significantly longer under natural field conditions (Li et al., 2015). However, according to the SEM, the soil NO_3^- released after the cooling-warming phase contributed by far the most to shoot N uptake (Fig. 7), indicating that the availability of N at the seedling stage was most critical to plant performance. Apparently, the late mineralization of straw -immobilized N was not sufficient to compensate for the N demand of barley at its early growth stage. Still, we cannot exclude that a continuation of N mineralization in the +straw treatment over a longer growth period would have led to a better nutrient balance. In the absence of straw, the N1 fertilization rate was sufficient for barley growth as shoot biomass and N content did not differ between N1 and N2 fertilizer rates (Fig. 4). In presence of straw, a higher fertilization rate (N2) was necessary to reach a similar plant biomass (Fig. 4). Apart from its direct effects on N-immobilization, straw also has indirect beneficial long-term effects. For example, straw application in long-term trials (>10-years) across the major agricultural zones of China increased crop yield by 7 % due to improved soil biophysical and physico-chemical properties, such as increased SOC contents (Wang et al., 2015). Therefore, it is likely that fertilization rates might be even reduced after long-term conditioning of soils by straw amendment. Overall, our results show that straw in combination with NI was highly efficient at preventing immediate N losses as well as N losses over a period of changing soil temperatures from agricultural soil. Given a sufficient stoichiometric balance between straw and N supply, straw acts as a slow-release fertilizer and can provide sufficient N for following crops (Cao et al., 2018). The timing of N-mineralization at the seedling stage, however, turned out to be most critical and deserves more attention in subsequent studies.

5. Conclusions

Straw application had immediate, strong and long-term effects on the immobilization of soil N that lasted until the cooling-warming phase. Although straw stimulated N_2O emissions at high N fertilization, this might be alleviated by a combined application of HCA and NI. During the cooling phase, the nitrification rate was more suppressed than N immobilization by microorganisms, while cooling-warming cycle induced strong ammonification. Compared to the strong N-immobilization potential of straw, NI caused only a relatively short delay of N-mineralization and nitrification that did not last long enough to affect barley growth or N-uptake. Although N was partly re-mineralized until barley growing in +straw treatments, N was not sufficiently delivered during the critical early growth stage after the cooling-warming phase, resulting in reductions of barley growth under no N (N0) and low N fertilizer (N1) rates in its early growing phase. Thus, while straw amendments led to strongly reduced N losses, our study shows that the synergistic application of straw and NI has great potential to reduce N losses from agricultural soils between cropping seasons, but the timing of straw application and manipulation of its C:N stoichiometry by fertilization appear most critical to match N mineralization after winter periods with the demand of following crops.

CRediT authorship contribution statement

Hao Chen: Investigation, Formal Analysis, Data Curation, Writing – Original draft; **Christoph Rosinger:** Investigation, Conceptualization, Methodology, Data Curation, **Sergey Blagodatsky:** Conceptualization, Data curation, Writing - Review and Editing. **Rüdiger Reichel:** Investigation, Methodology. **Bo Li:** Methodology, Writing - Review and Editing; **Amit Kumar:** Methodology, Formal Analysis; **Steffen Rothardt:** Resources; **Jie Luo:** Formal Analysis, Visualization, Software; **Nicolas Brüggemann:** Project administration, Supervision; **Henning Kage:** Resources; **Michael Bonkowski:** Conceptualization, Supervision, Writing- Reviewing and Editing.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162007.

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Chapter 2 Straw application and nitrification inhibitor affect soil N losses during cooling-warming and freeze-thaw cycles

Chapter 2

Straw application and nitrification inhibitor affect soil N losses during cooling-warming and freeze-thaw cycles

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Abstract

Nitrogen (N) lost from the agricultural field as leachate and nitrous oxide (N_2O) cause water pollution, global warming and decreases N use efficiency by plants. To combat these problems soil amendment with straw and application of nitrification inhibitors are used in agriculture. Microbial immobilization of N stimulated by straw addition and nitrification can be moderated by seasonal temperature variation, but this interfering factor is insufficiently studied. A 99-days mesocosm experiment that simulated the seasonal temperature variation was conducted, to investigate the effects of wheat straw amendment, nitrification inhibitor (NI) and temperature variation (both freezing-thawing and cooling-warming) on N losses from soil as N_2O and leachate with and without N fertilization. We observed N_2O emission peak immediately after applying straw and N fertilizer that increased the N_2O emission three-folds by stimulating denitrification. NI effectively reduced N_2O emission after fertilization by 57.6 %. Cooling-warming strongly induced N mineralization and caused N_2O emission peak even in the absence of freezing-thawing, while freezing-thawing increased the N_2O emission furtherly (38 %). Straw addition and NI had no legacy effect on N_2O emission during this phase that was caused mainly by denitrification according to the SEM analysis. Straw addition (70 %) and freezing-thawing (66.3 %) reduced N leaching, thus mitigating the total N losses as N_2O and leaching by 26 % and 31.6 %, respectively. We showed that temperature variation during winter season strongly affect the processes leading to N losses from soil and this factor need to be considered when measures preventing N losses, as straw and NI application are planned.

1. Introduction

Globally, soils are the largest anthropogenic source of N_2O, and agricultural activities are responsible for about 52% of the anthropogenic N_2O emissions (Tian et al., 2020). The main N flux escaping agroecosystems is nitrate leaching, especially prominent in intensive agriculture (Kumar et al., 2020). These losses causing multiple negative effects such as eutrophication of surface waters, destroying the ecological balance and posing challenges to human health (Cui et al., 2020).

Substantial amounts of N are lost from soil between plant cultivation phases, where longer periods of variable soil moisture and temperature with e.g. warming, cooling, freezing and thawing periods in temperate climate release soil N before it can benefit the next crop (Cookson et al., 2002; Sieling and Kage, 2006). Despite seasonal temperature fluctuations are identified as main drivers of N mobilising processes in soil (Li et al., 2021), and there is detailed information on long-term N transformation and losses after soil incubation at different stable temperatures (Dai et al., 2020; Duan et al., 2018), or after short-term stresses like soil freezing-thawing cycles (Li et al., 2019; Monteux et al., 2020; Rosinger and Bonkowski, 2021; Rosinger et al., 2022), experiments on seasonal N transformations and losses in agricultural soils are needed (Sieling and Kage, 2006).

Nitrification inhibitors (NIs) are widely used to reduce N loss and improve crops NUE (Wu et al., 2017). NIs are compounds applied to prevent the bacterial oxidation of soil ammonium (NH_4^+) to nitrite (NO_2^-) by inhibiting the activity of ammonia-oxidizing bacteria in the soil (Zerulla et al., 2001). Application of NIs has repeatedly been shown to lower N_2O emissions from agricultural soils; however, their effectiveness varies greatly (Prasad and Power, 1995; Qiao et al., 2015; Ruser and Schulz, 2015). Soil temperature, moisture, and fertilization level may affect the longevity of NIs and reduce their effectiveness (Norton and Ouyang, 2019; Thapa et al., 2016). It is known that the inhibitory capacity of NIs decreased with increasing temperature (Irigoyen et al., 2003; McGeough et al., 2016), while Menéndez et al (2012) reported a higher persistence of the NI in soil at higher water contents, and the effect of soil temperature on the efficiency of NI was dependent on soil water content, under 80% WFPS, the efficiency in reducing N_2O emissions decreased with temperature, but at 40% of WFPS, the percentage of reduction of N_2O emissions after DMPP application increased with temperature (Menéndez et al., 2012). Therefore, it is still not clear how the variation in soil temperature and moisture will modify the effect of NI on soil N_2O emissions.

The incorporation of crop straw as high organic carbon soil amendment is one of the world's oldest, most practical and economical methods of reducing soil N losses by increasing internal N cycles (Diacono and Montemurro, 2011; Norton and Ouyang, 2019; Xia et al., 2018). By retaining the N and restraining its losses from soil, straw has the potential to combat N losses in agriculture (Wu et al., 2017). Straw mitigates N leaching by immobilizing N in microbial biomass (Reichel et al., 2022; Reichel et al., 2018; van Duijnen et al., 2018; Wei et al., 2020; Zhao et al., 2021) as shown both in incubation (Congreves et al., 2013a; Zavalloni et al., 2011) and field studies (Congreves et al., 2013b; Török et al., 2014). However, concerns have been raised regarding potential increase of N_2O emissions under straw incorporation (Huang et al., 2017; Yang et al., 2015; Zhang et al., 2019). The effects of straw addition on soil N_2O emissions appear inconsistent, ranging from positive (Li et al., 2013; Xia et al., 2018) to neutral (John et al., 2020; Malhi and Lemke, 2007), and negative (Shan and Yan, 2013; Yao et al., 2017). The divergent N_2O responses to straw addition have been attributed to differences in soil physicochemical properties and fertilization practices (Chen et al., 2013; Yu et al., 2019), suggesting that the underlying mechanisms driving straw effects on N_2O emissions are complex and still little understood (Wu et al., 2020). The positive effect of straw amendment on N losses with leachate due to increased N immobilization can be counterbalanced by acceleration of N_2O emission via denitrification. A critical role in this case will be played by the dynamics of soil water content and oxygen availability, which could support anaerobiosis. In this case, NI could suppress the provision of NO_3^- for denitrification and its combination with straw could potentially lead to the overall positive effect. However, this hypothetical interaction needs to be proven under controlled conditions. It was reported that the efficiency of NI in reducing N_2O emissions was negatively correlated with soil organic matter content (Lin and Hernandez-Ramirez, 2020; McGeough et al., 2016), while Wu et al (2017) reported that NIs mitigate N_2O emissions more effectively under straw-induced conditions. At present it is even unclear if direct (i.e. application of NIs) and indirect (i.e. application of high organic carbon soil amendment) means can be reasonably combined to prevent N losses from soils, especially under temperature variation and freezing-thawing.

Thus, we set up a mesocosm experiment to simulate soil N dynamics in treatments with wheat straw incorporation combined with the NI Piadin® at varying temperature regimes/cooling-warming and freezing-thawing cycle and quantified N losses from soil as N_2O and leachate. We hypothesized that i) NI and straw addition in combination have additive effects on mitigating N losses and NI could dampen negative effect of combined N and C addition effect on acceleration of N_2O emission; ii) Straw addition will be an effective mean to reduce N losses over seasonal temperature fluctuations; iii) freezing-thawing will induce strong N_2O and N leaching losses, but cooling-warming alone will not; iv) nitrification and denitrification contribute differently to the N_2O emission during different phases of experiment and this can be used in planning of NI application.

2. Materials and Methods

2.1 Experiment design

We performed a mesocosm experiment simulating seasonal variations comprising two typical between-crop cultivating phases: i) a conditioning phase simulating the incorporation of crop residues into soil after harvest (phase 1), ii) a cooling-warming phase simulating winterly temperature fluctuations (phase 2). First phase continued from day 0 to day 28, all pots were incubated in a greenhouse with daily average temperature around 19 °C. Second phase continued from day 29 to day 71, all pots were incubated in a climate chamber at 7 °C from day 29-60, then from day 58 to day 60, 7 repetitions with fertilized pots (as detailed below) were frozen in -20°C freezer as freezing treatments (Fert-F1), the other half repetitions of the fertilized pots were not frozen (Fert-F0), and all pots were incubated in greenhouse again from day 61 to day 71 with daily average temperature around 21 °C. On day 62, the soil water content was adjusted to 69.8% water holding capacity to obtain leachate (Figure 1).

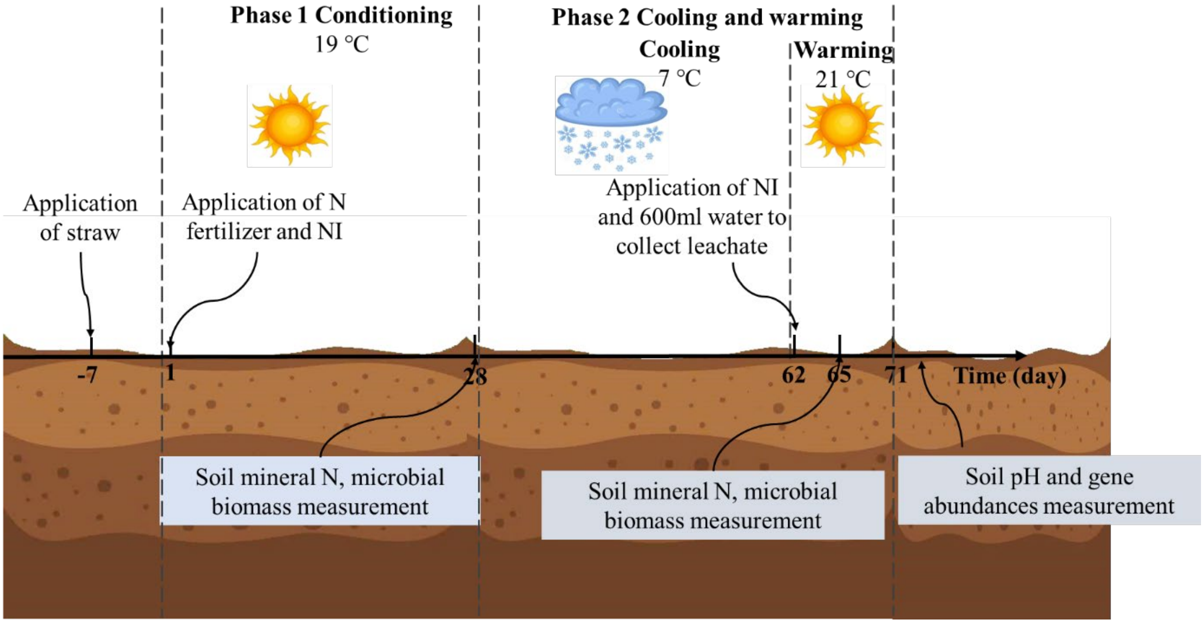


Figure 1 Time plan for the experiment

The mesocosms consisted of 10L buckets with a tube in central hole at the bottom to collect leachate. The buckets were filled with 8 kg dry-weight agricultural soil irrigated regularly with tap water to keep 50% water holding capacity during the whole experiment. The agricultural soil used in this study was obtained from the Experimental Farm Hohenschulen, which is affiliated to the University of Kiel, Germany (54°18′N, 9°58′E) in June 2019. The soil is classified as Luvisol, with the following properties: Bulk density 1.37 $g\ cm^{-3}$, pH 6.5, total organic C 1.07%, total N 0.11%, sand 58%, silt 29%, clay 13%. The soil gravimetric water content was 7.2% (w/w, about 19.4% water holding capacity) before incubation. The soil was sieved (1 cm mesh size) and homogenized for the experimental set up.

The treatments including two levels of N fertilization (Ctrl, no N added; Fert, 73 mg N kg^{-1} soil added as $(NH_4)_2SO_4$, which is equal to 250 kg N ha^{-1}), two levels of wheat straw (W0, no straw; W1, eq. to 20 t ha^{-1}) and two levels of nitrification inhibitor (no NI and NI, eq. to 13 l ha^{-1}) were applied in a multifactorial design. The 4 treatments of Ctrl levels have 7 repetitions and the 4 treatments of Fert levels have 14 repetitions (i.e., 84 pots in total). These additional fertilized pots were used to test the effect of freezing (forth factor), as detailed in the first paragraph. 25 cm of soil tillage layer was used for calculating N fertilizer, straw and Piadin application amount, the amount of straw application is 10 t ha^{-1} in the field of Germany normally, but the residues of root and shoot from the former crops are left in the field; in our study the soil was not grown with plants for years, so we choose 20 t ha^{-1} to simulate the organic C amendments from straw and crops residues. Each pot contained 8 kg dry weight soil, irrigated regularly with tap water to keep 50% water holding capacity during the whole experiment.

Straw was added into the soil and mixed homogenously one week before the starting of experiment. As NI we used Piadin® (SKW, Piesteritc, Germany), a commercial formulation containing the two active pyrazole derivatives1H-1,2,4-triazole and 3-methylpyrazole at inclusion rates of approximately 3.1% and 1.6%, respectively, which is a product to enhance N use efficiency and improve crop yields when applied to soils with livestock manure (Barneze et al., 2015). The effect of PIADIN® starts immediately after fertilisation. The conversion of N from stable NH_4^+ to the mobile NO_3^- form is delayed by six to ten weeks (https://www.piadin.de/en/#home). The NI (Piadin) was applied twice, the first time (eq. to 7 l. ha^{-1}) together with N was applied on day 0 (the start of the experiment), soil water content was adjusted to 50% water holding capacity on the same day. The second application of Piadin (eq. to 6 l. ha^{-1}) occurred on day 62 (the beginning of warming phase).

2.2 Repeated measurements of soil biological activity during the incubation experiment

Gas samples for the determination of net N_2O and CO_2 emissions were taken at regular intervals with 4 replicates in each treatment using the static-opaque chamber method (Chen et al., 2023; Dobbie et al., 1999). Chambers for gas sampling consisted of PVC tubes (20 cm diameter, 55 cm height) and were closed by an airtight lid with tubing and a three-way cock for gas sampling. For gas sampling, the chambers were inserted to a depth of 5 cm into the soil surface of mesocosms to ensure gas tightness. During the barley growth phase, the chambers enclosed the whole plants during gas sampling. During each sampling, three samples were collected from the headspace with a 20 ml syringe 30, 60 and 90 min after the chambers were inserted into the soil. As control, 5 ambient air samples were collected on each sampling occasion, and the average N_2O concentration from these samples were used as “time-zero” N_2O measurement. Sampling time was always between 11 a.m. and 1 p.m.

In addition to gas analysis, soil samples were collected on day 28 at the end of the conditioning phase (phase 1), on day 65 cooling-warming phase (phase 2). The samples collected after the warming-thawing were immediately frozen at -20°C and later used for functional gene abundance analysis. At each time point, approximately 30 g of soil was sampled from 3 opposite locations in each mesocosm using a soil corer with a diameter of 2 cm to a depth of 10 cm. Soil NH_4^+-N and NO_3^--N contents were measured at days 28 and 65.

For the determination of soil NH_4^+-N and NO_3^--N contents 5 g fresh wt soil was extracted with 20 ml 0.01 M $CaCl_2$ under shaking (30 min). After subsequent centrifugation (for 5 min at 4500 rpm) the supernatant was filtered (Whatman 595 filter paper) and NH_4^+-N and NO_3^--N concentrations were immediately determined using ion-selective electrodes (Nico 2000 Ltd, UK). Total potentially available soil mineral N (Nmin) was calculated as the sum of NH_4^+-N and NO_3^--N. Soil pH was determined with a pH meter (multi 340i, WTW GmbH, Weilheim, Germany) according to ISO 10390 guidelines (ISO, 2005) from a suspension of 5 g fresh wt soil in 1 M KCl at 1:5 (w/v) after shaking for 2 h.

Functional marker genes involved in nitrification (AOB and AOA amoA) and denitrification (bacterial nirK, nirS, nosZ), were amplified by quantitative real-time PCR (qPCR) using SYBR Premix Ex Taq (TaKaRa Biotechnology Co. Ltd., Dalian, China) and the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, CA, U.S.A.). All nucleic acid extracts were diluted to decrease the impact of inhibition for successful amplification and increased reaction sensitivity. The RT-qPCR reaction mixtures contained 5µL of 2×SYBR Green qPCR Master Mix (TaKaRa Biotechnology, Otsu, Shiga, Japan), 0.2 µL10 µ M each of forward and reverse primer, 1 µL of cDNA template, and 3.6 RNAase/DNase-free water to a final volume of 10 µL. Melting curve analyses were performed at the end of each run to confirm the reactions specificity. The RT-qPCR data presented in this study were derived from independent extractions of six replicates. Standards of 108 to 101 gene copies µL-1 were prepared from linearised plasmids with target gene fragments inserted. Amplification efficiencies varied between 90.9% and 101.2% and R2 values were > 0.991 for all genes.

2.3 N leachate

To collect leachate, the mesocosms were irrigated with 600 ml water on day 62, the second day of the warming phase. Leachate volumes were recorded and concentrations of NO_3^- and NH_4^+ determined using ion-sensitive electrodes as described above.

2.4 Calculations and statistical analysis

The N_2O emission factors were calculated as the amount of net N_2O-N emissions in +N treatments minus the net emissions in no fertilization treatment (background N_2O emissions) as the percentage of the fertilizer N applied for the period between gas samplings. The yield-scaled N_2O emissions were related to barley biomass at harvest as in Van Groenigen et al. (2010) and were calculated as the amount of cumulative N_2O emissions per dry shoot biomass. The global warming potential (GWP) of total greenhouse gas emissions was calculated with CO_2 as reference gas, where an increase or reduction in emissions of N_2O were converted into 'CO_2-equivalents' by means of their GWPs (Wei et al., 2022; Wei et al., 2018). We used the following equations to calculate the GWP (g CO_2 equivalent/kg soil):

$$\text{GWP} = CO_2\ (\text{g } CO_2/\text{kg soil}) + 298\times N_2O\ (\text{g } N_2O/\text{kg soil})$$

The presented values in graphs and tables are means ± standard errors. All the statistical analyses were performed in R 3.6.3 (R Core Team, 2020) and graphs were prepared with the Origin Pro 8.1 (Origin Lab, Northampton, MA, USA). Treatment effects were analysed by a prior contrast. Linear regression analyses were used to evaluate the relationships of the parameters from soil, microorganisms, and N_2O emission.

Structural equation modelling (SEM; 'lavaan' package; (Rosseel, 2012)), was used to identify the potential relationships between barley shoot N, soil microbial biomass, soil mineral N content, N_2O emissions, soil enzyme activities and abundance of microbial functional genes responsible for nitrification and denitrification. Prior to the SEM procedure, principal component analysis (PCA) as implemented in the vegan package (Oksanen et al., 2013) was conducted to remove variables with collinearity. Hypothetical relationships between the variables in the models were established according to the results of correlation analyses. The best fitting model was selected by step-wise removal of non-significant paths. The data were square root-transformed before the SEM analysis considering nondimensional expression.

The criteria for evaluation of the structural equation model fit, such as the Chi-square/degree values (CHI/DF), adjusted goodness-of-fit index (AGFI) and root mean square error of approximation (RMSEA) were adopted according to Duan et al. (2018).

3. Results

There were two N_2O emission peaks during the experiment: one at the beginning of the conditioning phase just after N fertilizer application, and the other during the warming phase after soil cooling or freezing. Since these two emission peaks dominated (90%) the total cumulative N_2O emission, we present the results on N_2O and other soil properties separately for two phases: first - conditioning phase (section 3.1) and second - cooling-warming phase (section 3.2). Freezing-thawing (FT) occurred in the transition period between the cooling and warming phases for half of the fertilized samples.

3.1 N immobilization and N_2O emission during conditioning phase

The application of straw increased the cumulative CO_2 emission in the conditioning phase by an eightfold compared to the variants with no straw ($p<0.001$, Fig. S1 A).

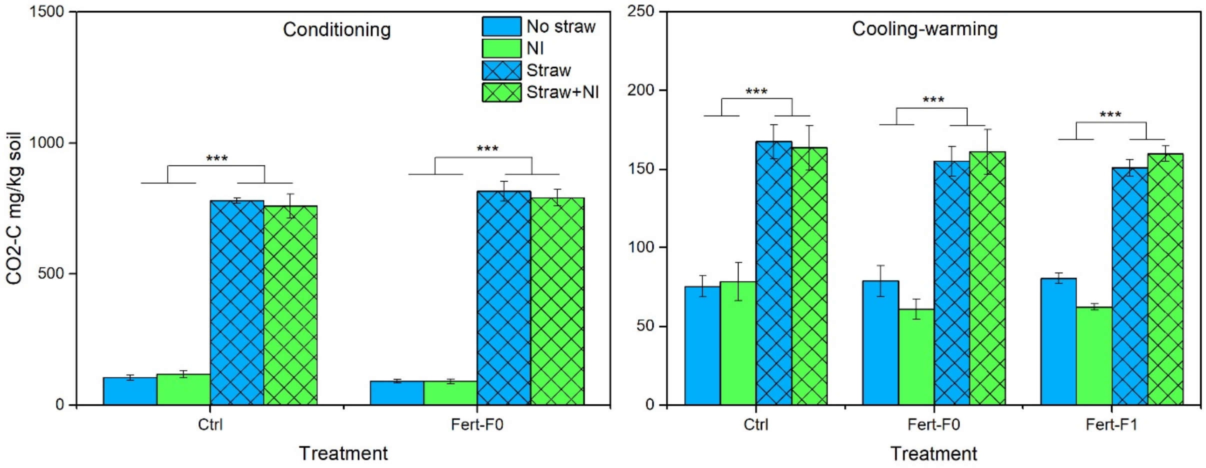


Figure S1 Cumulative CO_2 emission as affected by straw and NI application, fertilization and freezing-thawing separated according to experiment phases. The treatments Ctrl and Fert refer to the unfertilized and N-fertilized pots, respectively; Fert-FR refer to the Fert levels experiencing freezing-thawing. Straw refers to the treatments with straw application, NI refer to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n=4). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

As a result of microbial activity and N immobilization stimulated by straw addition, amounts of total soil mineral N (Nmin) decreased by half in straw addition treatments (Figure 2, $p<0.001$), and this effect was independent of N-fertilisation and corresponding average decrease of Nmin ($p<0.001$) was 25.8 mg N kg^{-1} in no fertilization and 44.1 mg N kg^{-1} in fertilization treatments, respectively. Application of the NI increased Nmin content by 32.1 % in no fertilization level in the absence of straw addition (Figure 2, $p<0.05$). Since soil NO_3^- contributed by far most (67-92 %) to the total Nmin content, straw addition showed its greatest effects on reducing NO_3^- (41.1 and 17.8 mg N kg^{-1} in no straw vs. straw addition, respectively), while the proportion of NH_4^+ slightly increased in straw addition treatments (+ 14.6 %, $p<0.001$), specifically, straw addition increased NH_4^+ in no fertilization levels ($p<0.001$). Addition of NI led to significant increase of NH_4^+ only in fertilized microcosms in the absence of straw (39.6 %, $p<0.001$), NI increased soil NO_3^- by 38.6 % in no fertilization level in the absence of straw addition ($p<0.05$).

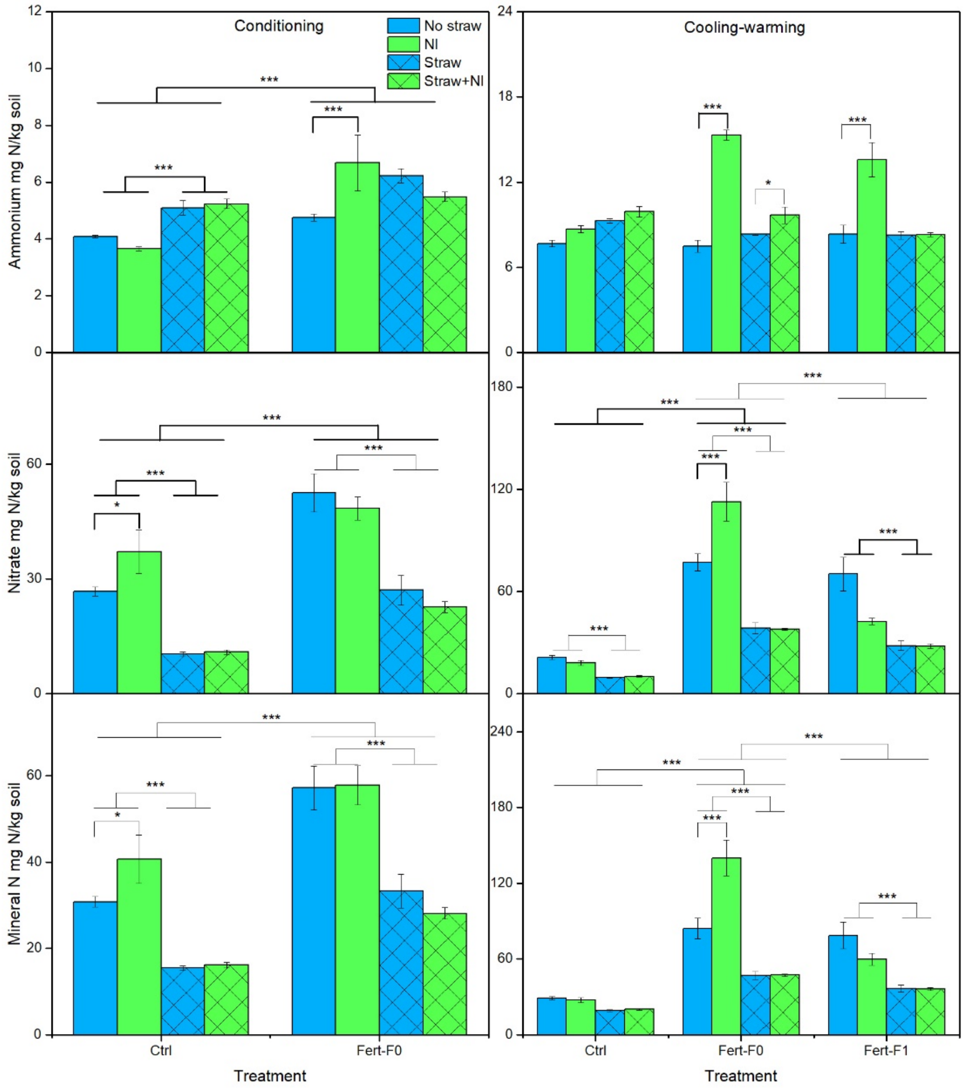


Figure 2 Soil NH_4, NO_3 and mineral N contents under different treatments of conditioning and cooling-warming phases. The treatments Ctrl and Fert refer to the unfertilized and N-fertilized pots, respectively; Fert-FR refer to the Fert levels experiencing freezing-thawing. Straw refers to the treatments with straw application, NI refer to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n=7). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

Once after application of N fertilizer and/or NI, N_2O emission peaked 1.5 times in no fertilization and 5.5 times in fertilization treatments, comparing treatments with and without straw. (Figure 3). Accordingly, fertilization increased the cumulative N_2O emission during the conditioning phase by an average of 6.5 times. (Figure 4, $p<0.001$). Straw addition increased it by 5-folds ($p<0.001$), while NI decreased N_2O emissions by 58.6 % in fertilized pots (Figure 4, $p < 0.01$). These general effects were confirmed by ANOVA with contrasts as shown in the Table 1.

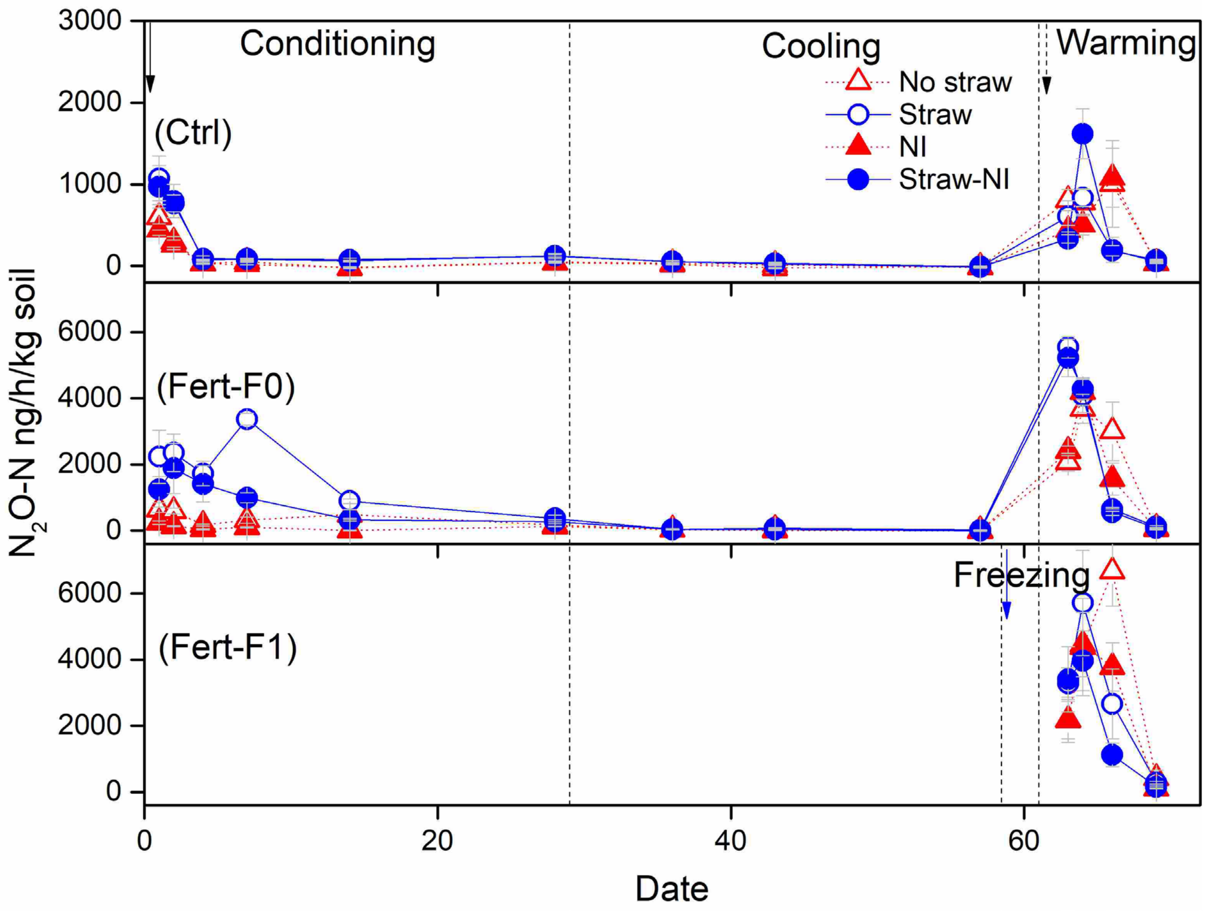


Figure 3 Dynamics of N_2O emission under different treatments during the incubation experiment, the top sub-figure is the Ctrl level treatments, the middle sub-figure is the Fert level without freezing-thawing treatments, the bottom sub-figure is the Fert level with freezing-thawing treatments, given is the mean ± 1SE (n=4), the dash lines separate the different phases of experiment, the solid arrow indicates application of N fertilizer and NI, the dash arrow indicates application of NI only.

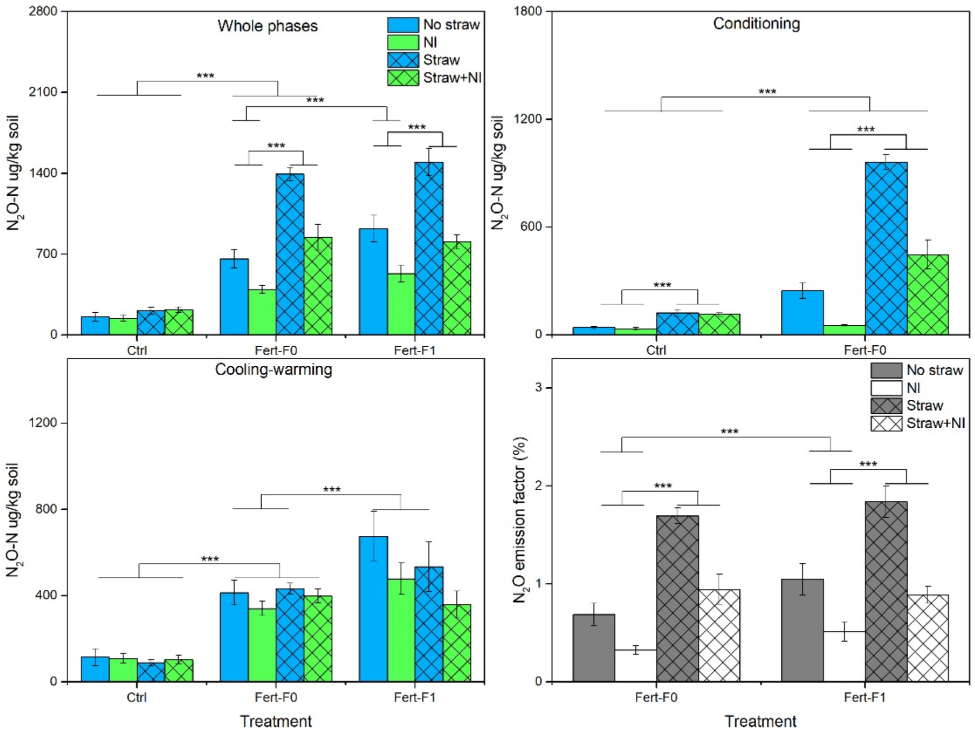


Figure 4 Cumulative N_2O emission under different treatments during the whole experiment (top left) and split by phases (conditioning phase – top right, cooling-warming phase – bottom left) and emission factors (bottom right). The treatments Ctrl and Fert refer to the control and with N fertilizer, respectively; Fert-F0 and Fert-F1 refer to the Fert levels without and with freezing-thawing, respectively. Straw refers to the treatments with straw application, NI refer to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n=4). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

3.2 N remineralization and N_2O emission after cooling-warming and freezing- thawing

Straw addition increased cumulative CO_2 emissions throughout the cooling-warming phase (two-folds, $p<0.001$, Fig. S1 B), but the increase was significantly less than that observed in the conditioning phase, reflecting exhausting of available C sources in straw addition treatments during the conditioning phase.

During the cooling-warming phase, total Nmin showed little difference in magnitude from the conditioning phase, except after N fertilization, where Nmin levels doubled (fertilization) and tripled (fertilization-NI) in treatments without straw addition (Figure 2). This effect was mainly due to the strong increase of NO_3^- levels in the fertilized pots. N fertilization increased soil NO_3^- by 5.5 times ($p<0.001$), while straw addition reduced it by half ($p<0.05$). The effect of NI depended on N fertilization and straw addition: NI increased NO_3^- by 46 % in the absence of straw addition when fertilized ($p<0.001$).

Total amounts of NH_4^+ had increased by 80.4% on average after cooling warming and freezing-thawing without significant differences between no-frost and frost treatments. NI application strongly enhanced the amounts of NH_4^+ in fertilization levels ($p<0.001$), while straw addition strongly reduced the positive effect of NI on the availability of NH_4^+ ($p=0.09$).

Similar to CO_2, N_2O showed two emission peaks, but in contrast to CO_2, the one at the onset of the conditioning phase was approximately half of the massive peak occurred directly after the end of the cooling phase (Figure 3). The first (day 1-14 in conditioning phase) and second (day 61-67 in warming phase) N_2O emission peaks contributed 33 % and 56 % of the total cumulative N_2O emission, respectively, therefore these two N_2O emission peaks dominated the pattern of total cumulative N_2O emission.

Straw addition, fertilization and NI had variable effects on cumulative N_2O emissions of different phases (Figure 4). Fertilization increased the cumulative N_2O emissions of cooling-warming phase by 5-folds compared to no fertilization treatments ($p<0.001$), freezing-thawing increased it by 29.2 % compared to cooling-warming treatments ($p<0.001$), while not in the case of having both straw addition and NI ($p=0.388$). NI decreased N_2O emissions under fertilization for the cooling-warming and freezing-thawing temperature treatments by 12.6 and 30.9 %, respectively ($p<0.001$).

3.3 N transformation gene abundances

The gene abundances of denitrifiers were higher than those of nitrifiers, and the nirS abundances was in general lower than abundancies of nirK and nosZ (Figure 5). Denitrifiers were affected mostly by freezing-thawing treatment, it increased nirK, nirS and nosZ by 3-folds ($p<0.001$), 9-folds ($p<0.001$), and 1.5-folds ($p<0.001$), respectively, in comparison with cooling-warming treatment. The freezing-thawing also increased AOB by 1.5-folds ($p<0.001$) and was dependent on straw application ($p<0.001$). Straw addition increased gene abundances of denitrifiers nirK, nirS and nosZ under no fertilization levels by 3-folds ($p<0.01$), 2.6-folds ($p<0.001$) and 2.7-folds ($p<0.001$), respectively. Straw addition increased AOA by 2.7-folds if unfertilized treatments without NI ($p<0.001$) are compared. N fertilization decreased AOB, nirS and nosZ by 67 % ($p<0.001$), 86.5 % ($p<0.001$) and 13.1 % ($p<0.05$), respectively. In the absence of straw addition and freezing-thawing, NI increased the AOA, AOB, nirK, nirS and nosZ by 42.9 % ($p<0.001$), 19.6 % ($p<0.05$), 68.2 % ($p<0.05$), 60.3 % ($p<0.01$) and 36.5 % ($p<0.01$), respectively. NI increased AOA by 13 % on average (F[1,57]=5, $p<0.05$).

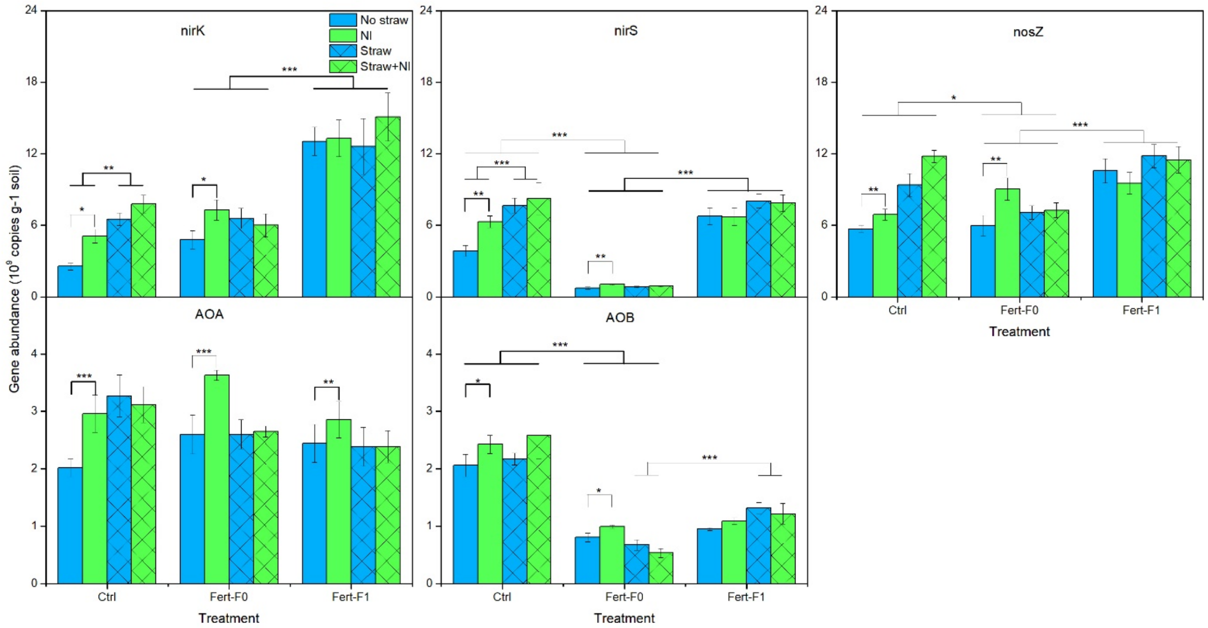


Figure 5 N cycling functional gene abundances of ammonia-oxidizing bacteria (AOB) and archaea (AOA), NO_2^- reductase nirK and nirS, nitrous oxide reductase nosZ under different treatments. The treatments Ctrl and Fert refer to the control and with N fertilizer, respectively; Fert-F0 and Fert-F1 refer to the Fert levels without and with freezing-thawing, respectively. Straw refers to the treatments with straw application, NI refer to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n=7). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

3.4 Reduction of N losses due to leaching and N_2O emissions

As hypothesized, total N losses (gaseous N_2O-N emissions plus leached mineral N) increased by half with N-fertilisation ($p<0.001$), whereas total N losses were reduced by both NI and straw addition ($p<0.001$). Overall, NI reduced N-losses under both fertilisation treatments (no-frost and freezing-thawing) by 35.5 % on average ($p<0.001$). However, the effect of straw additionwas dependent on N-fertilisation with N-losses decreasing by 72.8 ($p<0.001$) and 20.6 % ($p<0.001$) in no fertilization and fertilization-no frost treatments, respectively. Freezing-thawing decreased the total N losses by 31.6 % on average ($p<0.001$) and this happened mainly due to the relative decrease of leachate volume if frost and no-frost fertilized treatment are compared (Supplementary Fig. 2).

When the factors were considered separately, fertilization increased N lost through leaching by 44.9% ($p < 0.001$) and straw addition reduced N leaching by an average of 70% ($p < 0.001$). NI reduced the N leaching if all treatments with and without NI were compared ($p < 0.001$), the only significant NI effect on N leaching was in fertilization-no frost treatments in the absence of straw addition ($p < 0.001$). Freezing-thawing reduced it by 66.3 % on average ($p < 0.001$), if fertilization-no frost and fertilization-frost treatments are compared.

N fertilization increased the total cumulative N_2O emissions by 5.5-folds ($p < 0.001$), straw addition increased it by 2.8-folds in fertilized microcosms ($p < 0.001$), and NI decreased it by 42.3 % in fertilized treatments ($p < 0.001$). As a result, straw addition increased the N_2O emission factors during the whole experiment by 3 times ($p < 0.001$), while NI decreased them by half ($p < 0.001$).

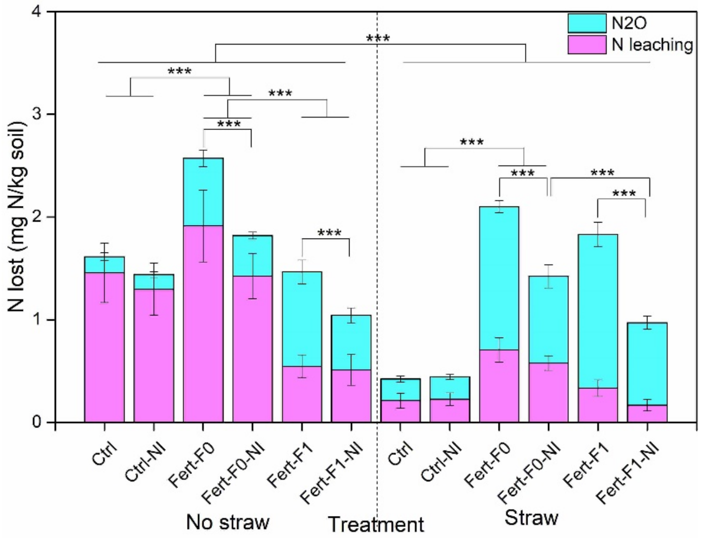


Figure 6 N lost from soil as N_2O and leachate under different treatments during the whole experiment. The treatments Ctrl and Fert refer to the control and with N fertilizer, respectively; NI refer to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n=4).

Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

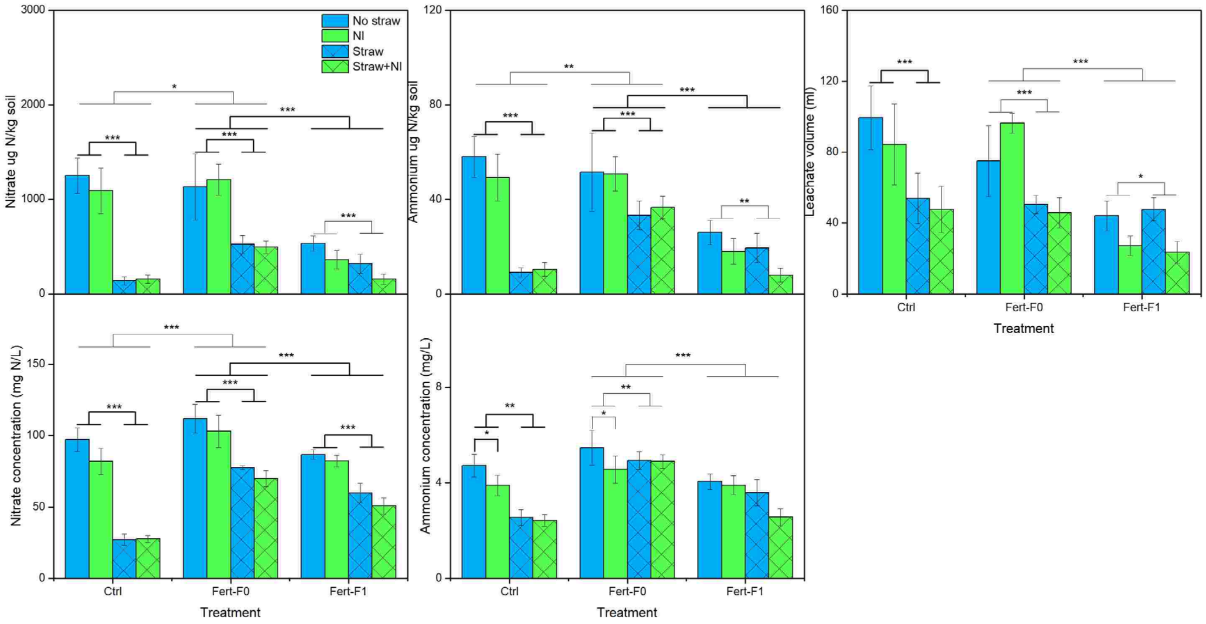
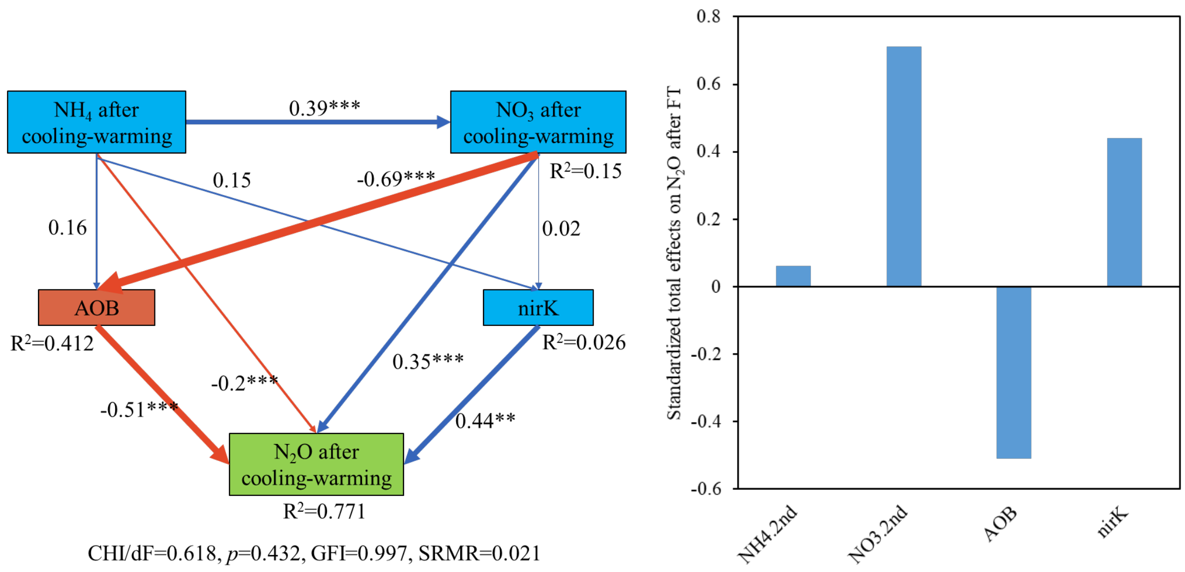


Figure S2 Amount of leaching N from ammonium, nitrate, volume of leachate, concentration of leaching N from ammonium, nitrate under different treatments. The treatments Ctrl and Fert refer to the control and with N fertilizer, respectively; Fert-F0 and Fert-F1 refer to the Fert levels without and with freezing-thawing, respectively. Straw refers to the treatments with straw application, NI refers to the treatments with nitrification inhibitor application. Given is the mean ± 1SE (n=7). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

3.5 Pathways leading to N losses as disclosed by structural equation model

The SEM explained 77 % of variation of N_2O emission during cooling-warming phase. Soil NH_4^+ decreased N_2O emission directly ($\lambda=-0.2$, $p<0.001$), but increased soil NO_3^- by nitrification ($\lambda=0.39$, $p<0.001$). Soil NO_3^- increased N_2O emission directly ($\lambda=0.35$, $p<0.001$), but decreased AOB ($\lambda=-0.69$, $p<0.001$). nirK increased N_2O emission ($\lambda=0.44$, $p<0.001$) and AOB decreased N_2O emission ($\lambda=-0.51$, $p<0.001$), respectively. As a result, the N_2O emission after freezing-thawing was contributed mostly by soil NO_3^- of cooling-warming phase (0.71) and nirK (0.44), but decreased by AOB (-0.51), soil NH_4^+ (0.06) had tiny effect on the N_2O emission.



CHI/dF=0.618, *p*=0.432, GFI=0.997, SRMR=0.021

Figure 7 Structural equation model (SEM) of conditioning phase showing the hypothesized causal relationships between N_2O emission and soil mineral N, gene abundance of AOB and nirK. The NH4 2nd and NO3 2nd indicate the soil mineral N of cooling-warming phase.

3.6 Global warming potential of GHG

When computing the GWP of two gases, CO_2 and N_2O, the CO_2 emission was dominant, therefore the pattern is the same as the total cumulative CO_2 emission, straw addition increased the GWP by 4.6-fold ($p < 0.001$). Because of the contribution of N_2O, N fertilization increased GWP by 14.7 % ($p < 0.001$) in the presence of straw addition, NI decreased GWP by 4.6 % ($p < 0.001$) in the presence of N fertilization.

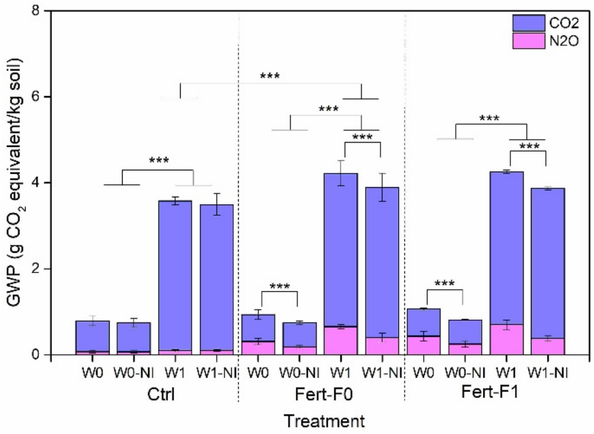


Figure S3 Global warming potential of CO_2 and N_2O gases. Given is the mean ± 1SE (n=4). Significant treatment and interaction effects as revealed by ANOVA with contrast is given on the top, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

4. Discussion

The cumulative N_2O emission in our experiment was increased by fertilizer and straw addition, and decreased by the addition of nitrification inhibitor, however, the effects of these treatments differ between phases of our experiment (Figure 3, Figure 4).

4.1 N2O emission before cooling-warming

During the conditioning phase, straw addition strongly increased soil N_2O emission (Figure 3, Figure 4, $p < 0.001$). Numerous reports have shown that the addition of soil organic matter with readily decomposable organic C can stimulate denitrification by increasing respiration (through the creation of anoxic microsites) and by supplying energy to denitrifiers (Burford and Bremner, 1975; Firestone and Davidson, 1989; Köster et al., 2015; Weier et al., 1993). Straw addition resulted in dramatically increased CO_2 and N_2O emissions compared to the non-straw-amended soils (Figure S1), indicating stimulated microbial activity and greater denitrification rate; denitrification was considered to be the main process leading to increased N_2O fluxes specifically in straw-amended soils (Wu et al., 2017).

However, the reports of straw addition affect soil N_2O emission are inconsistent, straw addition could have positive, neutral or negative effects on N_2O emission, which depends on the quality of straw, soil and fertilization management (Wu et al., 2020). Straw application under reduced N fertilizer and drip irrigation neither increased the N_2O emission nor decreased the crop N uptake compared to the treatments without straw (Zhao et al., 2021). However, under flooding irrigation conditions, adding organic matter stimulated N_2O losses (Wang et al., 2018). Generally, straw amendment in conjunction with NO_3^- can cause higher soil N_2O emissions under conditions favouring denitrification (Wu et al., 2018). We argue that the effects of straw addition on N_2O emission could be highly affected by soil water content and N content, for example, in wet soil with enough mineral N, the straw addition easily induced anaerobic environment by increasing soil respiration, which stimulates denitrification strongly and increases N_2O emission; while in dry soil, straw decomposed slowly and increased soil respiration slightly, the soil is still aerobic and unfavourable for denitrification, but straw addition stimulated the growth of microbes and retained soil mineral N, thus decreased N_2O emission by reducing available N for nitrification. It has been revealed that N2O emissions in response to straw addition were largely regulated by the timing of N fertilizer and straw application: the simultaneous application of straw and urea could boost the fast development of anoxic zones caused by the rapid degradation of straw together with ammonium nitrification, thus induced higher N_2O emission; but the N fertilization delayed for 3 weeks could untangle the coupling effects of straw and N fertilizers on N_2O stimulation (Ye et al., 2023). We recommend that apply the straw enough time before fertilization, so when the straw is decomposed, there is not enough N resource for denitrification and N_2O emission, which is same with the situation of no fertilization treatments during the whole experiment phases (Figure 4).

NI reduced N_2O emission significantly, we assumed that at the beginning of the experiment, after adding fertilizer $(NH_4)_2SO_4$ and NI, NI inhibited nitrification, decreased soil NO_3^- while increased NH_4^+ compared with treatments without NI, which can be also indicated by the obvious decrease of N_2O emission in this phase (in the first two weeks). NI reduces N_2O emission by directly decreasing the nitrification rate and furtherly reducing the NO_3^- concentration as substrate for denitrification (Chen et al., 2019). NI decreased N_2O emission of fertilization levels in conditioning phase, while NI did not reduce the N_2O emission of no fertilization levels, because no N fertilizer was added in no fertilization levels (Figure 4). However, NI had no strong effect on soil mineral N during conditioning phase, because the mean soil nitrification rate was 3.82 mg kg^{-1} day^{-1} in croplands, and positively related with temperature (Li et al., 2020). After 4 weeks when we measured the soil mineral N contents at the end of conditioning phase, most of soil NH_4^+ was already transferred to NO_3^-, thus, no significant effects of NI on soil mineral N were detected (Figure 2).

We assumed that during the first two weeks of the experiment when the first emission peak happened, both nitrification and denitrification contributed to the N_2O emission. Because straw addition leads to a simultaneous emission peak of CO_2 and N_2O, and the increase in CO_2 indicates a decrease of O_2 content in the soil, which favours denitrification. While NI inhibited N_2O emission in this phase efficiently and N_2O emission was positively corelated with soil NH_4^+ content, which means that nitrification was also a pathway of N_2O emission.

4.2 N_2O emission after cooling-warming

During the cooling-warming phase of the experiment we observed an N_2O emission peak and the increase in soil NH_4^+ and NO_3^- content differing between N fertilization variants (no fertilization, fertilization) reflecting thus the remineralization of previously immobilized N (Figure 2, Figure 3). It is reported that elevated temperature drives microbial N cycling from anabolic processes (i.e. speculated from decreased microbial biomass N) to catabolic (i.e. increased N mineralization) processes, regardless of the presence or absence of plants (Dai et al., 2020). Thus, less N was converted to microbial biomass and a relatively larger fraction of organic N was released as NH_4^+ by N mineralization. This change leads to increased inorganic N availability (e.g. NH_4^+) and its subsequent processing (e.g. nitrification, denitrification) in the soil environment (Wang et al., 2020b). Similar effect was observed by Cookson et al (2002) that decreasing soil temperature from 15 to 2 °C caused an initial increase in mineral-N and quickly followed by rapid immobilization of mineral-N, microbial biomass N and gross immobilization increased with decreasing soil temperature; increasing temperature from 2, 5 or 10 °C to 15 °C caused a rapid increase in soil NO_3^- concentration and gross mineralization and nitrification rates, but less mineral-N was released if incubated at a constant 15 °C (Cookson et al., 2002). It indicates a potential risk of N losses during the substantial cooling-warming cycle, which happens in early spring frequently (Cameron et al., 2013). Generally, experimental warming significantly increased the net mineralization, net nitrification and denitrification rates (Yin et al., 2012), increased N_2O emission. On the other hand, the soil water content was increased from 50 % water holding capacity to 70 % water holding capacity before collecting leachate, which increased the soil denitrification (Thilakarathna and Hernandez-Ramirez, 2021). The N_2O emission rate of all treatments was similar during cooling, but during warming, the N_2O emission rate of Fert levels was obviously higher than that of no fertilization levels, indicate that the N fertilizer stimulated the emission peak during warming. We assumed that the significant higher soil NO_3^- of fertilization levels provided substrate for denitrification, which produced more N_2O than that of no fertilization levels. Qiu et al (2018) reported that the increased N_2O emission was caused by warming enhancement of soil NO_3^--N substrate and the microbial community, significantly higher abundances of nirK and nirS provided direct evidence illustrating that warming stimulated denitrifiers, and nirK communities were more sensitive to temperature changes than nirS communities, which is similar with our research that nirK was positively correlated with N_2O emission during cooling-warming.

In the meanwhile, compared with conditioning phase, the soil NO_3^- was increased during cooling-warming phase in fertilization levels but not in no fertilization levels, we assumed that the increased soil NO_3^- came from mineralized soil NH_4^+ through nitrification. However, the nitrification was not the major pathway of N_2O emission in this phase (SEM, Figure 7), denitrification contributed mostly of N_2O emission during this phase, because soil NO_3^- and nirK contributed mostly to N_2O emission but soil NH_4^+ and AOB decreased N_2O emission (Figure 7). In addition, the soil NH_4^+ of no fertilization and fertilization levels were similar during cooling-warming, but the soil NO_3^- of fertilization levels were higher than no fertilization levels, indicating that soil NO_3^- was the factor that induced difference of N_2O emission between different N levels.

Freezing-thawing increased N_2O emission compared to the treatments without freezing (Figure 4, $p < 0.01$). This may be due to (i) degradation of diffusion barriers after thawing; (ii) increased soil moisture during thawing under anaerobic environment; and (iii) strong acclimation of denitrifiers to the changing environment and rapid recovery during thawing. (Gao et al., 2018). Freezing-thawing increased the gene abundances of nirk, nirS (Figure 5, $p < 0.01$), which explained the effect of freezing on N_2O emission. However, the increase of N_2O (5.5-37.9%) caused by freezing in our experiment was less than that found by many researches (Song et al., 2017; Wagner-Riddle et al., 2017). We assume that on the basis of alternating cooling-warming, freezing-thawing would not strongly increase N_2O emission as compared with treatments experienced cooling-warming. If there were treatments without cooling phase but incubated continuously in the greenhouse, freezing would have a large increase of N_2O emission compared to them. Because the N_2O emission rates of all treatments were quite low at the end of conditioning phase (Figure 3), if no fluctuation of soil temperature or moisture, no N_2O emission peak should be in the next phases of experiment.

Straw addition had no effect on N_2O emission after cooling (Figure 4), we assumed that the straw was decomposed already before warming phase, thus did not increase the soil CO_2 emission, did not reduce the soil O_2 content and provided no additional substrate for denitrification.

During warming-thawing, a slight downtrend of N_2O emission after cooling was observed with NI amendment (this trend was more pronounced under freezing thawing, figure 4), we assumed the reason is either nitrification contributed much less than denitrification to N_2O production during this phase, or the NI was leached into subsoil, thus the effects was diminished.

During the cooling-warming phase, NI had effect on soil NH_4^+ and NO_3^- in the absence of straw addition (NIxStraw interaction), indicating straw addition inhibiting the effects of NI by immobilizing N (Ma et al., 2019). NI increased soil NH_4^+ in fertilization levels but not in no fertilization levels (NIxN fertilizer interaction), because there was exogenous N in fertilization treatments.

4.3 N losses from soil

N lost as N_2O and leachate was reduced by straw addition which resulted from the decreased N leaching (Figure 6, $p < 0.001$). Straw addition increased the N lost from N_2O emission, but the combination of NI could mitigate the increased N_2O emission caused by straw addition. In our experiment, the leaching N (accounted for around 1% of fertilizer N) was much less than the field situation (Abdalla et al., 2019), we assumed that straw should have stronger mitigation of N losses under the field conditions. Contrary to the former researches that freezing-thawing increased N leaching (Gao et al., 2018; Wang et al., 2020a), in our study the freezing-thawing decreased N leaching, thus decreased total N losses especially in the absence of straw addition. Freezing-thawing increased the volume and porosity of the soil (Xie et al., 2015), thus, increased the ability of soil water retention, since we added water to a constant soil water content in all the treatments, freezing-thawing decreased the leachate volume. Freezing decreased leached N because it also decreased first, leachate volume, second, N concertation of leachate (data not shown), resulting in decreased N amount in leachate (Hentschel et al., 2008). In the field, freezing reduce the soil water evaporation and during thawing the meltwater increase soil water content results in leaching (Kreyling et al., 2020). While in our study we adjusted the soil water content constantly, counteracted the effects of increased soil moisture during thawing. The ammonia volatilization is negatively correlated with soil moisture below 60% water holding capacity (McGarry et al., 1987) and correlated with the drying-rewetting of soil surface (Hargrove, 1988), the soil was kept constantly at 50% water holding capacity in our study, which reduces the potential of ammonia volatilization. In our reach, we collected the leachate 2 months after fertilization when most of the soil NH_4^+ was transferred into NO_3^-, so the effect of NI on N leaching was weaker than straw addition. But still, NI decreased the N leaching under fertilization level in the absence of straw addition and freezing-thawing. WSA also inhibited the capacity of NI in soil mineral content and all the gene abundance in our study, which is similar with the other research that straw application decreased the effects of NIs because NIs can be adsorbed on organic matter and clay mineral surfaces (Lin and Hernandez-Ramirez, 2020). But in the field situation, the rainfall and irrigation after fertilization will cause obviously N leaching, and the NI will reduce the N leaching from soil when the soil NH_4^+ content is enough.

Conclusions

In the whole experiment phase, straw increased N_2O emission by stimulating denitrification and NI reduced N_2O emission by inhibiting nitrification once after application, indicating both nitrification and denitrification contributed to the N_2O emission before cooling-warming cycle. Cooling-warming induced strong N mineralization and inspired N_2O emission peak and freezing-thawing increased the N_2O emission furtherly by denitrification, but straw addition and NI had no effect during this phase. Straw addition mitigated the total N losses by reducing N leaching, we suggest applying straw when the soil inorganic N and moisture is under low content, could avoid causing N_2O emission from denitrification.

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Chapter 3 Gone and forgotten: facilitative effects of intercropping combinations did not carry over to affect barley performance in a follow-up crop rotation

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ORIGINAL ARTICLE

Gone and forgotten: facilitative effects of intercropping combinations did not carry over to affect barley performance in a follow-up crop rotation

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Abstract

Aim Intercropping often leads to improved productivity of individual species compared to monocultures. We have practically little knowledge of facilitation effects in different intercropping systems and their importance in creating soil legacies that can indirectly affect the succeeding crop in a crop rotation through plant-soil feedback (PSF) effects.

Methods To test this, we used a two-phased field experiment where we combined intercropping and crop rotation. During intercropping, we grew maize, faba bean, and lupine in monocultures or two-species crop combinations. The following season, we grew winter barley on the soil previously used for intercropping to test PSF effects under field conditions.

Results We found evidence for facilitative effects on aboveground biomass production that were species-specific with faba bean and maize biomass benefitting when intercropped compared to their expected biomasses in monocultures. Lupine, in contrast, performed best in monocultures. After the intercropping phase, total soil mineral nitrogen was higher in legume monocultures creating soil legacies but this did not affect soil microbial parameters and barley biomass production in the follow-up rotation phase.

Conclusions We found support for species-specific positive and negative interactions in intercropping. Our results also demonstrated that soil legacies play no significant role under moderately high nutrient environments.

Keywords Soil legacies · Plant-soil feedback effects · Arbuscular mycorrhiza colonization · Enzyme activities · Microbial biomass · Belowground interactions

Introduction

To meet the projected food demand by 2050, agricultural production must increase by 60–110% and this increase should be environment-friendly through reduced usage of synthetic pesticides and fertilizers and increased ecological intensification (Tilman et al. 2011; Wezel et al. 2014). In this regard, Gurr et al. (2016) showed evidence that ecological intensification can be promoted by crop diversification. Through crop diversification, increasing the positive

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biodiversity effects, that is, higher productivity in mixed cultures than the corresponding monocultures, may help us enhance the ecological intensification. We have demonstrated knowledge where increasing plant species richness has shown to increase multiple ecosystem functions in forest (Huang et al. 2018) and grassland (Isbell et al. 2017) ecosystems. This knowledge may be applied in cropping systems to boost agricultural productivity. However, differences in experimental designs and management practices in both forest & grassland ecosystems and cropping systems make it difficult to apply the knowledge gained from the former to cropping systems. For instance, in cropping systems, different intercropping types (relay-, strip- and mixed intercropping) and planting densities have been used, whereas, in most of the biodiversity-ecosystem functioning experiments, planting densities remain constant whereas the proportion of plant species vary. Further, the cropping systems remain intensively managed as compared to forest and less intensive grasslands. Therefore, we require more evidence from cropping systems on relative biomass production with different crop combinations as regards the strength of facilitative interactions leading to enhanced productivity. In situations where facilitation is particularly strong, one could envisage that its effects may even carry over into a subsequent crop.

In cropping systems, crop diversification can be achieved either spatially by growing more than one cultivar or crop simultaneously in close proximity (intercropping) or temporally by growing different consecutive crops (crop rotation). The positive effects of intercropping are mediated through trait complementarity and plasticity as well as the facilitative effects of interacting plant species (summarized in Li et al. 2014) whereas, for crop rotation, such effects are mediated through indirect feedback interactions (Schnitzer et al. 2011; Mushonga et al. 2020). There is more evidence on the positive spatio-temporal crop diversification on plant productivity (Li et al. 2014; Gaudin et al. 2015; Zhang et al. 2017; Dong et al. 2018), but negative effects have also been observed (Polley et al. 2003; Bukowski et al. 2018). This suggests that such effects appear to be species-specific and to a larger extent depend on soil biotic and abiotic properties as well as environmental conditions (Van der Putten et al. 2013; Craven et al. 2016; Png et al. 2019). For instance, by growing 4 barley cultivars (Prague, Spire, Waggon, and Krystal) and 3 legumes (*Trifolium subterranaeum*, *Ornithopus sativus*, and *Medicago truncULata*) in monocultures and possible intercropping combinations, Darch et al. (2018) showed that, compared to monocultures, barley-legume intercropping resulted in an up to 40% increase in overall biomass production (combined of both crops in intercropping). This increase was dependent on soil P availability, with the highest gain occurring at or below the sub-critical P demand for barley. They further showed that intercropping of different cultivars of barley did not change their productivity compared to when growing in monocultures. Su et al. (2014) showed that even though the total chlorophyll content (chlorophyll a + chlorophyll b) of two soyabean cultivars increased in a relay intercropping with maize, the photosynthetic activity decreased as compared to their monocultures. This decreased photosynthetic activity was attributed to shading effects of maize. In an another wheat/maize relay intercropping system, the SPAD values (measure of leaf greenness) of maize decreased when intercropped with wheat (Li et al. 2020). This suggests that it is not always the bigger plant in the mixed cultures that suppress the growth of the 'subordinate' plant. The underlying mechanisms still need to be identified.

The cornerstone of crop rotation practices lies on the assumption of plant-soil feedback (PSF) effects, that is, a preceding plant alters the soil abiotic and biotic components that may ultimately affect succeeding plant performance (Bever 1994; Ehrenfeld et al. 2005). It has been shown how PSF effects contribute to overyielding in intercropping and the succeeding crops by altering soil microbial communities (Wang et al. 2017, 2020). As microbiome assemblages in the soil appear to be generally plant species-dependent (Panke-Buisse et al. 2015; Uroz et al. 2019), it is believed that having phylogenetically distinct preceding and succeeding plant species may disrupt the species-specific pathogen accumulation in soil, thereby resulting in negative PSF effects (better plant performance in soil previously grown with different species) (Bever 2003; Miller et al. 2019; Heinen et al. 2020). This ideology is not strongly supported by either empirical (Fitzpatrick et al. 2017; Ingerslew and Kaplan 2018; Kaplan et al. 2020) or synthesis (Mehrabi and Tuck 2015) evidence. For example, Ingerslew and Kaplan (2018) demonstrated using the PSF approach that the succeeding plant biomass (tomato) strongly depended on the identity of 36 plant species that previously trained the soil. However, this effect was independent of phylogenetic relatedness of tomato with the preceding plant species.

These findings urge us to identify optimal crop species combinations in intercropping as well as in the rotation with an overall positive interaction effect on both above- and belowground yields and processes, irrespective of their phylogenetic relatedness. As most of the biodiversity-ecosystem functioning and PSF effects knowledge is derived from non-cropping systems, we have limited knowledge if these ecological interactions and underlying mechanisms can also be utilized in cropping systems to enhance productivity through ecological intensification. More specifically, there have been limited attempts to combine spatio-temporal diversity (intercropping together with crop rotation) in cropping systems (Karpenstein-Machan and Stuelpnagel 2000; Scalise et al. 2015; Wang et al. 2017, 2020; Kaplan et al. 2020). It is also not clear if different crop species that are performing better when intercropped would also create a positive soil legacy by improving soil nutrient contents, dilution of soil borne pathogens, and increased abundance of mutualists (e.g. arbuscular mycorrhizal fungi) that later would benefit the next crop in the rotation. It has recently been shown that identity of previous crop may lead to changes in AMF communities in soil that may persist over time to affect the follow-up crop (Roy et al. 2021). To fill this knowledge gap, we performed a field experiment comprising of two phases: an intercropping phase followed by a crop rotation phase. The intercropping phase consisted of monocultures and intercrops (a combination of two crops) of maize, faba bean, and lupine. The rotation phase had barley monocultures grown on soils from intercropping phase. The overall aim was two-fold: (1) identify the crop combinations in intercropping with overall enhanced biomass production relative to their expected biomasses in monocultures, and (2) to test the PSF effects of intercropping on soil biochemical parameters and barley biomass production in the rotation. Therefore, we hypothesized that:

1. Compared to monocultures, intercropped species will have a greater aboveground biomass production.
2. Intercropping would alter soil properties and create soil legacies which, in turn, affect the microbial parameters & the performance of the next crop in the rotation through PSF effects.

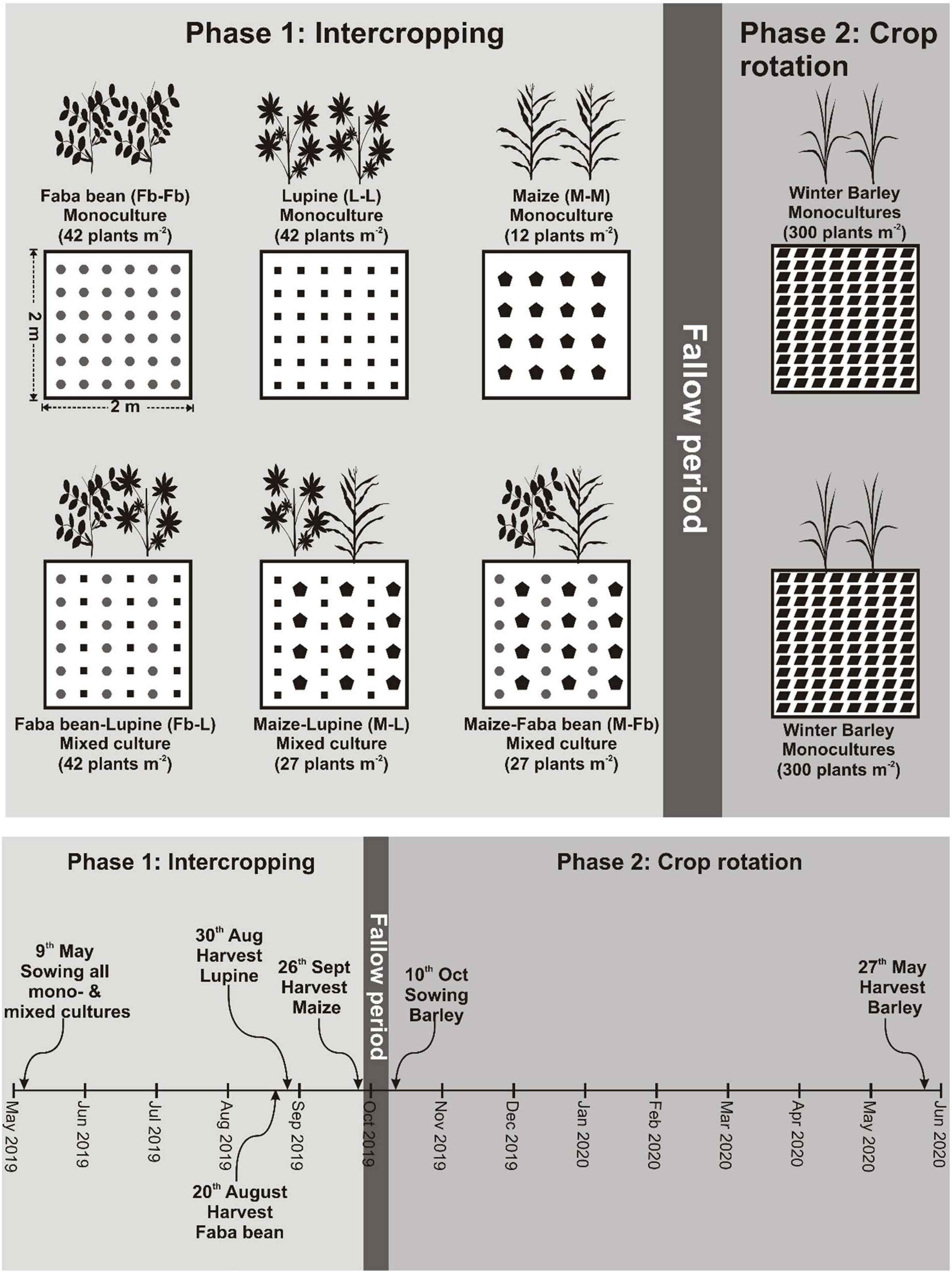
Materials and methods

Experimental design and management

The field experiment started in May 2019 in an agricultural field in Lüneburg (53° 12′ N and 10° 22′ E), Germany. The climate is typical of temperate regions with mild summers and cold winters. The daily mean temperature and precipitation are shown in Supplementary Fig. 1. The agricultural field was under conventional practices with 800 kg ha^{-1} chalk lime, 470 HAS (Harnstoff-Ammonsulfat) solution containing 20% N and 6% S, and 300 kg ha^{-1} Caralonkali containing 12% P 30% K, 6% Mg, and 4% S applied for summer barley in 2018. Soil was slightly acidic (pH$_{H20}$ 6) and classified as Cambisol and contained around 2.1% total C and 0.2% total N. The experiment comprised of block design in which five blocks were placed parallel to each other and six plots of 2 × 2 m were randomly placed inside each block, yielding a total of five replicates per monoculture and intercrop combination. In each block, plots were 1 m apart from each other to avoid edge effects. The experiment consisted of two phases: an intercropping phase and a rotation phase.

Phase 1: intercropping phase

Maize (*Zea mays* L. cv. Colisee), faba bean (*Vicia faba* L. cv. Tiffany), and white lupine (*Lupinus albus* L. cv. Energy) were grown in monocultures and intercrops of two species combinations (Fig. 1). Crops were grown in rows and intercrops had alternating rows of each species. Monocultures of maize (M-M), faba bean (Fb-Fb), and lupine (L-L) had planting densities of 12, 42, and 42 plants m^{-2}, respectively. In intercropping (maize + faba bean (M-Fb), maize + lupine (M-L), and faba bean + lupine (Fb-L)), the planting density of each species was reduced to half (6, 21, and 21 plants m^{-2} for maize, faba bean, and lupine, respectively). All crop species were sown simultaneously within 2 days (9th and 10th May 2019) and crop weeds were removed weekly during the growing season.


Phase 1: Intercropping
Fallow period
Phase 2: Crop rotation
Faba bean (Fb-Fb) Monoculture (42 plants m^{-2})
Lupine (L-L) Monoculture (42 plants m^{-2})
Maize (M-M) Monoculture (12 plants m^{-2})
Winter Barley Monocultures (300 plants m^{-2})
2 m
2 m
Faba bean-Lupine (Fb-L) Mixed culture (42 plants m^{-2})
Maize-Lupine (M-L) Mixed culture (27 plants m^{-2})
Maize-Faba bean (M-Fb) Mixed culture (27 plants m^{-2})
Winter Barley Monocultures (300 plants m^{-2})
Phase 1: Intercropping
Fallow period
Phase 2: Crop rotation
9th May Sowing all mono- & mixed cultures
30th Aug Harvest Lupine
26th Sept Harvest Maize
10th Oct Sowing Barley
27th May Harvest Barley
20th August Harvest Faba bean
May 2019
Jun 2019
Jul 2019
Aug 2019
Sep 2019
Oct 2019
Nov 2019
Dec 2019
Jan 2020
Feb 2020
Mar 2020
Apr 2020
May 2020
Jun 2020

◂**Fig. 1** Upper panel shows the experimental design and layout with all the crop combinations and planting densities in monocultures and in the intercropping phase and that of barley in the subsequent rotation phase. The lower panel shows sowing and harvesting times for both the phases

SPAD measurements

Approximately on the 80th day after sowing, leaf greenness was measured as a proxy of chlorophyll content using a SPAD 502 Plus Chlorophyll Meter (SPAD-502-, Minolta Camera, Tokyo, Japan). We chose this time period as all the crop species were fully developed and were in their reproductive phase. For this, we randomly selected five plants from each crop species from both monocultures and intercrops. SPAD values were taken from two youngest yet fully developed healthy leaves at 10 points along the leaf length by avoiding edges and mid ribs. For faba bean and lupine, the measurements were distributed over the leaflets per leaf. Doing this, we had 4500 measurement points.

Harvest and soil sampling

On 20th and 30th August 2019, we harvested faba bean and lupine, respectively, whereas maize was harvested on 26th September 2019. This differential harvest date was chosen to allow complete maturity of each crop at harvest. We had initially planned to separate the grain yield from the total aboveground biomass at harvest, but due to a rust pathogen infection on the faba bean, we had to harvest before grain maturity. Since we did not have this separation in the faba bean, we decided in order to be consistent to treat all three species in the same way by measuring aboveground biomass. We randomly harvested 5, 10, and 10 plants of maize, faba bean, and lupine, respectively by cutting stem from soil surface towards the center of each plot to avoid edge effects at plot level. The harvested biomass was dried at 60 °C for 5 days to measure dry biomass and extrapolated to kg m^{-2}. The total aboveground biomass in intercrops for each species was calculated as difference between observed and expected values in intercrops compared to their respective monocultures to identify either positive or negative effects of intercropping on biomass production. For the expected aboveground biomass estimation for each crop species in intercrops, we halved their respective biomasses in monocultures to correct for planting density using paired monocultures and intercrops per block to account for block effects.

After maize harvest on 26th September 2019, all the plots experienced a fallow period of 12–13 days before rotation phase started (Fig. 1 lower panel). Prior to sowing winter barley in the rotation phase, we collected soil samples for loss-on-ignition and soil mineral N measurements to assess soil legacies created by intercropping phase.

Loss-on-ignition (LOI) was used as a proxy for soil organic matter (SOM). Pre-weighed fresh soil samples were first oven dried at 105 °C for overnight to remove the moisture content. Pre- and post-ignition (500 °C for 24 h) soil weight was recorded. Percent LOI was calculated as below:

$$LOI(\%) = 100 \times \frac{pre \cdot ignition\ weight(\mathrm{g}) - post \cdot ignition\ weight(\mathrm{g})}{pre \cdot ignition\ weight(\mathrm{g})}$$

For ammonium (NH^{4+}) and nitrate (NO^{3-}), 5 g of fresh soil were extracted in 20 ml of a 0.01 M $CaCl_2$ solution. After horizontal shaking for 30 min and subsequent centrifugation (for 5 min at 4500 rpm) and filtration (through a Whatman 595 filter paper), ammonium and nitrate concentrations were immediately determined using ion-selective electrodes (Nico 2000 Ltd, UK).

Phase 2: rotation phase

We grew winter barley (*Hordeum vulgare* vr. Meridian) in the same plots which were used for the previous intercropping phase to investigate if barley performance is affected by soil legacy created by intercropping through PSF effects. For this, barley seeds were hand sown in rows on 10th and 11th October 2019 at a planting density of 300 seeds m^{-2}. As it was impractical to hand-sow 30 plots of 2×2 m^2, we reduced the sowing area to 1×2 m (2 m^2) per plot in the rotation phase. To facilitate sowing, we superficially ploughed all plots (~ 10 cm deep) and barley seeds were placed at 4–5 cm soil depth.

Harvest and soil sampling

Barley was harvested on 27th May 2020 from an area of 0.5×0.25 m^2. After harvesting barley, we randomly took 4 soil cores (4 cm inner diameter and 10 cm depth) from the harvested area by placing the soil cores on the cut stem. This allowed us to collect soil and barley roots. Four cores were then pooled together to make one composite sample per plot and stored at 4 °C overnight before sieving (2 mm sieve) the next day. After the sieving process, the roots were transferred to 250 ml plastic bottles containing distilled water and shaken overnight to remove soil adhering to roots. Afterwards, roots were carefully washed and stored in 90% glycerol for later counting for root length colonization by arbuscular mycorrhizal fungi.

Microbial biomass and potential enzyme activities

Sieved soil samples were used to measure microbial biomass C and N by chloroform-fumigation-extraction with modifications (Vance et al. 1987; Witt et al. 2000). Two sets of subsamples (5 g) were taken from fresh samples. One set was horizontally shaken in 25 ml of 0.5 M K_2SO_4 for 1 h and thereafter centrifuged for 5 min at 4500 rpm. Subsequently, 3 ml of the supernatant were transferred to another plastic vessel and stored frozen until they were analysed for dissolved organic C (DOC) and total dissolved N (TDN) with a TOC analyser (multi N/C 2100S, Analytik Jena, Germany). The other set of samples was fumigated with 50 ml of ethanol-free chloroform for 24 h. After fumigation, soil extractions and C and N measurements were performed as described above. Soil microbial biomass C and N were determined as the difference of fumigated and non-fumigated DOC and TDN, respectively. Microbial biomass C and N were corrected by extraction efficiency factors of 0.45 (Vance et al. 1987) and 0.54 (Brookes et al. 1985), respectively.

$$Microbial\ biomass\ C\ (or\ N) = \frac{DOC(or\ TDN)_{fumigated\ soil} - DOC(or\ TDN)_{non-fumigated\ soil}}{K_{EC(0.45) or\ EN(0.54)}}$$

Potential activities of leucine aminopeptidase (LAP), N-acetyl-*β*-D-glucosaminidase (NAG), *β*-glucosidase (GLU), and phosphomonoesterase (PHO) were measured fluorometrically according to the method described in Marx et al. (2001) and German et al. (2011). Briefly, 0.5 g of soil was suspended in 50 ml sterile deionized water, homogenized for 1 min in a sonication bath, and aliquots of 200 µl were subsequently pipetted under constant stirring into black 96-well microplates (Puregrade, Germany). Optimal substrate concentrations and incubation times for substrates were evaluated ahead. 50 µl of substrate solution were added to each well, followed by a 120 min incubation in the dark at 20 °C. Fluorescence was measured using a Perkin Elmer EnSpire multiplate reader with an excitation of 365 nm and an emission of 450 nm. Potential enzyme activity was expressed in units of nmol MUB/AMC cleaved g^{-1} dry soil h^{-1}.

Barley root length colonization by AMF

AMF abundance was determined as root length colonization in percent. Fresh roots stored in 90% glycerol were cut into 1–1.5 cm fragments and cleared in 10% KOH for 20 min in a water bath at 80 °C. Afterward, roots were washed 4 times with distilled water and acidified for 10 min with 1% HCl and placed in a 2% blue ink in 1% HCl for 30 min at 80 °C before clearing them overnight in lactoglycerol (1:1:1) (Phillips and Hayman 1970; Vierheilig et al. 1998). Cleared root fragments were mounted on glass slides and the percent of root length colonization was quantified with the intersection method (McGonigle et al. 1990).

Statistics

All the statistical analyses were performed within R environment (Team 2020) and graphs were prepared with the 'ggplot2' (Wickham 2016) and 'ggpurb' (Kassambara 2020) libraries. The measured variables are presented as means with confidence intervals (CIs) of 95% that were computed by using non-parametric bootstrap resampling with 10,000 iterations. To avoid common statistical errors, we followed the step-wise protocol for data exploration (Zuur et al. 2010). The mean–variance relationship was visually checked from residual plots. We used 'glmmPQL' function from 'MASS' library (Venables and Ripley 2002) to fit generalized linear mixed models (GLMMs) followed by Type III ANOVA and Tukey's test for multiple contrasts to test if intercropping phase affected LOI, mineral N, barley shoot biomass, root length colonization with AMF, microbial C and N, and the potential activity of GLU, LAP, NAG, and PHO enzymes. For SPAD values, separate GLMMs were fit for each crop species followed by Type III ANOVA and Tukey's test for multiple contrasts as mentioned above. The absolute mean, bootstrap mean, and upper & lower CIs of the measured values were computed with ‘rcompanion’ library (Mangiafico 2020). We refer to significant differences at the $p < 0.05$ level but based on recent discussion on the significance and null hypothesis testing using $\alpha = 0.05$, we refrain from using the word ‘significant’ and mostly mention the mean differences between the treatments and effect sizes wherever possible (Ho et al. 2019; Rillig et al. 2019).

Results

Phase 1: intercropping phase

Aboveground biomass production and SPAD values

Total aboveground biomass was affected in intercrops relative to their monocultures and this effect was crop-specific. Aboveground biomass of faba bean and maize increased when intercropped whereas that of lupine decreased in intercropping irrespective of crop combinations. This increase was 65% and 47% for faba bean when intercropped with maize and lupine, respectively (Fig. 2, Supplementary table 2). Similarly, maize aboveground biomass increased by 135% and 131% in intercropping with faba bean and lupine, respectively. On the contrary, the aboveground biomass of lupine decreased by 28% and 36% when intercropped with maize and faba bean, respectively. At the species level, we found greatest SPAD values for lupine and the values remained similar in both monoculture and when intercropped. On the other hand, faba bean and maize had greater SPAD values in their monocultures compared to their intercropping independent of species combination (Supplementary Figure 2).

Legacy effects on soil properties

The total SOM content was unaffected after intercropping phase (Fig. 3a). The mineral N in the soil, however, showed the legacy effects created from the intercropping phase (Fig. 3b). For instance, compared to maize monoculture, legume monocultures had higher mineral N content in the soil (38% higher in Fb-Fb monoculture and 46% higher in L-L monoculture), whereas that of intercrops was in between.

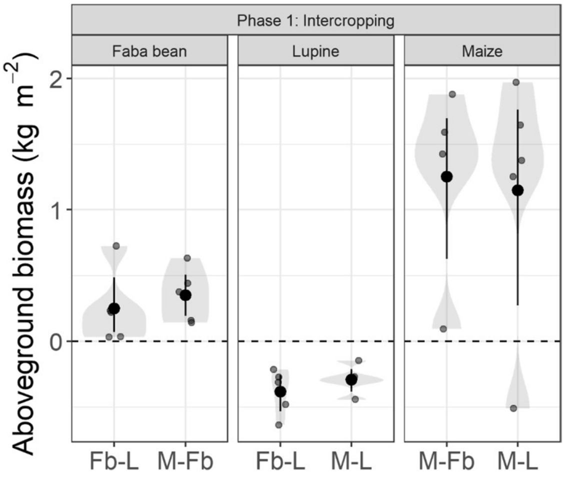


Fig. 2 Effect sizes of crop species combinations on observed aboveground biomass production in intercropped combinations. Dashed lines indicate aboveground biomass of the respective monoculture. Values are the absolute differences between intercrops and monocultures for each crop species. Positive and negative values for each species represent greater or lower aboveground biomass in intercropping than its corresponding monoculture. Values are the means and 95% confidence intervals. Small dots represents individual replicates. Fb–L: faba bean + lupine intercrop, M-Fb: maize + faba bean intercrop, M–L: maize + lupine intercrop

Phase 2: rotation phase

Microbial biomass and potential enzyme activities

In the rotation phase, microbial biomass C and N remained similar and did not vary depending on the intercropping phase. The same was true for microbial biomass C:N ratios (data not shown). Similarly, the potential activities of four measured enzymes were not dependent on the soil legacies from the intercropping phase (Fig. 4).

Root length colonization by arbuscular mycorrhizal fungi

The percent root length of barley colonized by AMF (as determined by staining technique) was affected by the intercropping phase (Fig. 5). Barley grown in soil previously trained by maize-faba bean (M-Fb) crop combination had the highest root length colonization (61%) followed by faba bean-lupine (Fb-L)

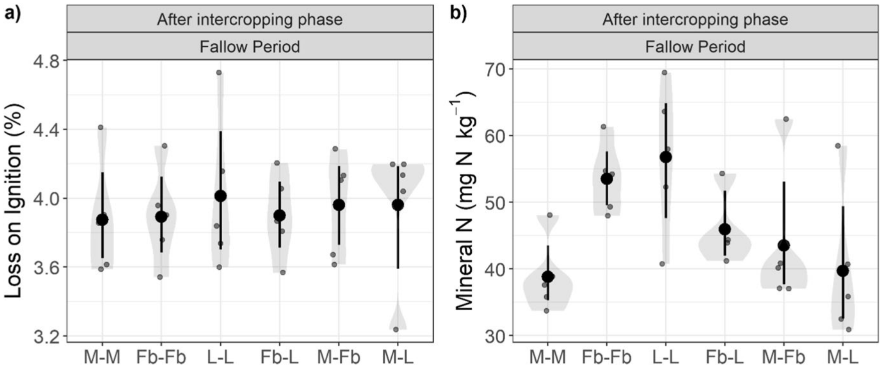


Fig. 3 **a**) Loss on ignition (%) as a proxy for soil organic matter (SOM) and **b**) soil mineral N (mg N kg^{-1} soil) after the intercropping phase. Values are the means and 95% confidence intervals. Small dots represents individual replicates. M–M: maize monoculture, Fb–Fb: faba bean monoculture, L–L: lupine monoculture, Fb–L: faba bean + lupine intercrop, M–Fb: maize + faba bean intercrop, M–L: maize + lupine intercrop. Refer to Supplementary table 1 for descriptive statistics

intercropping (56%). Barley root length colonized by AMF was lower when grown in soil from monocultures and the maize-lupine (M-L) intercrops.

Barley aboveground biomass production

Barley aboveground biomass varied from 0.25 to 0.37 kg m^{-2} but the soil feedback effects from the intercropping phase had no effect on barley biomass production (Fig. 6).

Discussion

Positive effects of intercropping on aboveground biomass production are species-specific

In support of the first hypothesis, we showed positive effects of intercropping on aboveground biomass production and such effects were crop-specific and dependent on the exact combinations of species grown together (Fig. 2). Maize benefitted the most from facilitative interactions with the legumes. The mineral N accumulation that occurred only in legume monoculture plots underlines the importance of legume-grass interactions as strong candidates for creating facilitative interactions. Such crop-specific effects of intercropping on aboveground biomass production have previously been shown to be likely mediated by interspecific interactions and soil type (Dissanayaka et al. 2015; Gou et al. 2016; Chen et al. 2019), with the underlying mechanisms varying with crop species identity. In intercropping systems of two crops growing simultaneously in close proximity, multiple scenarios may arise in terms of biomass production. For instance, in intercropping, (1) both crop species may benefit from each other thereby increasing their biomasses, (2) one crop species may benefit without affecting the performance of other species, (3) one species may benefit on the expense of other species, and (4) no benefit of intercropping on biomass production of both crop species.

In our study, we found that faba bean and maize had greater aboveground biomass production in in intercropping than their expected biomasses in monocultures (Fig. 2). Such stimulated productivity of maize and faba bean biomass has been attributed to inter-specific rhizosphere interactions, in which, root exudates from maize act as signaling molecules to induce faba bean root nodulation and consequently higher rates of biological N fixation (Li et al. 2016). Maize, on the other hand, gets access to soil nutrients such as N that is spared by faba bean but also to increased P availability through faba bean mediated by rhizosphere acidification (Li et al. 2007; Zhang et al. 2016). It should also be noted that even though maize is found to be benefitting when intercropped with legumes (Sileshi et al. 2008; Chai et al. 2014; Latati et al. 2014), the aboveground biomass of maize

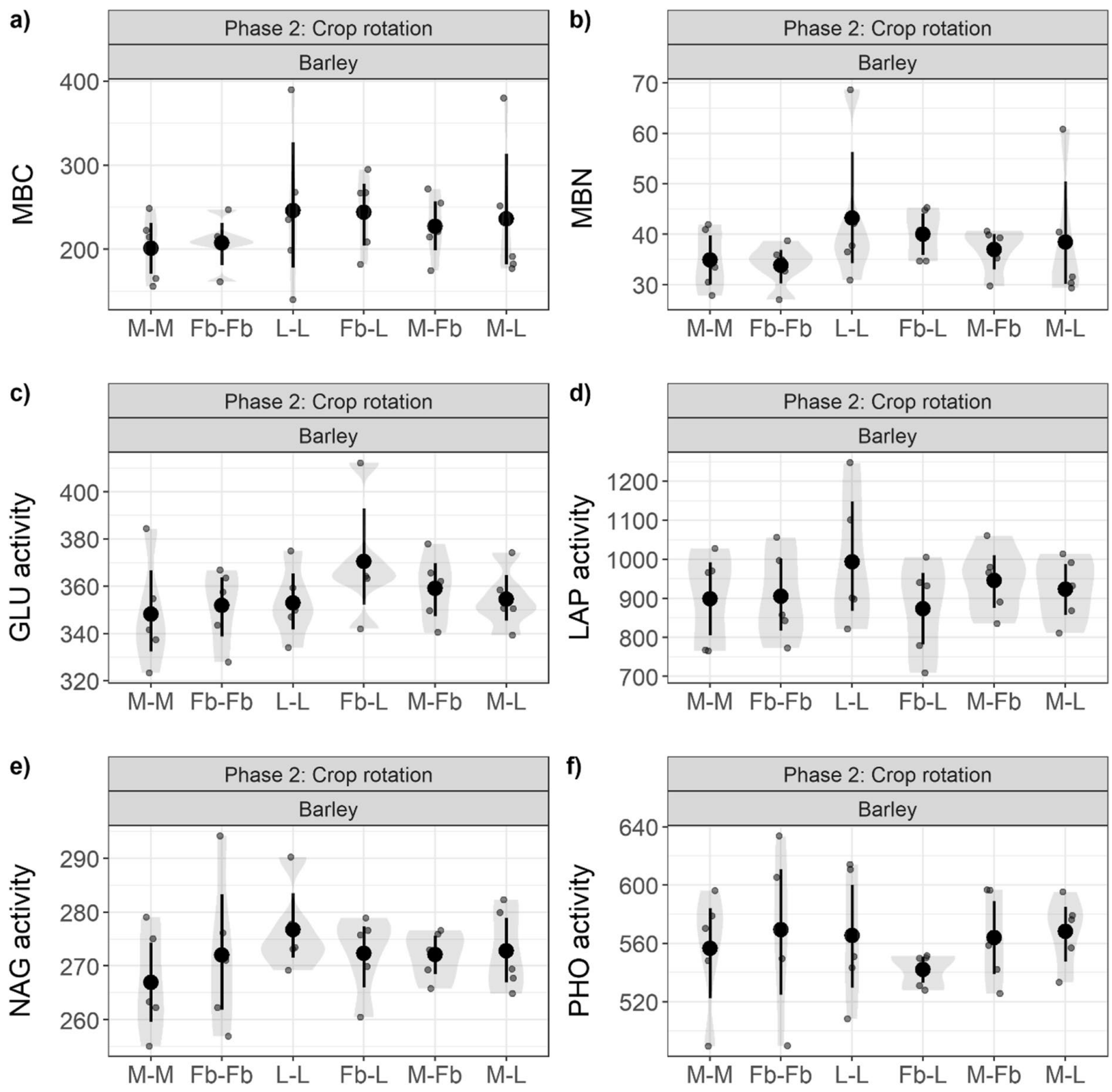


Fig. 4 **a**) Microbial biomass C (MBC, mg C kg^{-1} soil), **b**) microbial biomass N (MBN, mg N kg^{-1} soil), **c**) β-1,4-glucosidase (GLU, nmol MUB cleaved g^{-1} soil h^{-1}) activity, **d**) L-leucine aminopeptidase (LAP, nmol AMC cleaved g^{-1} soil h^{-1}) activity, **e**) β-1,4-N-acetylglucosaminidase (NAG, nmol MUB cleaved g^{-1} soil h^{-1}) activity, and **f**) phosphomonoesterase (PHO, nmol MUB cleaved g^{-1} soil h^{-1}) activity in the rotation phase when grown on soils trained from intercropping phase. Values are the means and 95% confidence intervals. Small dots represent individual experimental replicates. M-M: maize monoculture, Fb-Fb: faba bean monoculture, L-L: lupine monoculture, Fb-L: faba bean + lupine intercrop, M-Fb: maize + faba bean intercrop, M-L: maize + lupine intercrop. Refer to Supplementary table 1 for descriptive statistics

was exceptionally high in intercropping in the present study. Along with facilitation, this increase may additionally be attributed to the lowered competition for resources as a result of early harvest of faba bean and lupine than maize as well as reduced maize planting density when intercropped. Such temporal differentiation due to different harvesting period has been shown to significantly contribute to

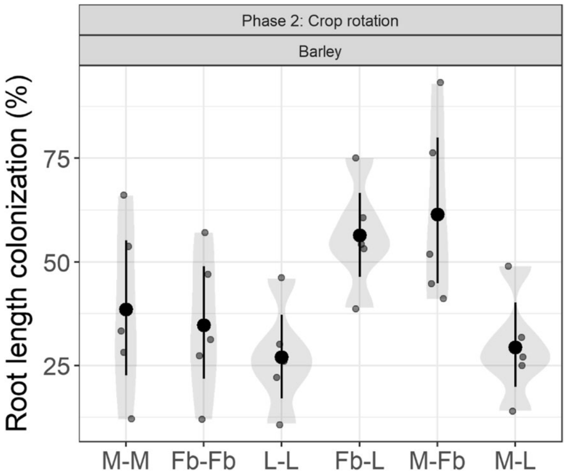


Fig. 5 Barley root length colonization (%) by arbuscular mycorrhizal fungi in the rotation phase when grown on soils trained from intercropping phase. Values are the means and 95% confidence intervals. Small dots represent individual experimental replicates. M–M: maize monoculture, Fb–Fb: faba bean monoculture, L–L: lupine monoculture, Fb–L: faba bean + lupine intercrop, M–Fb: maize + faba bean intercrop, M–L: maize + lupine intercrop. Refer to Supplementary table 1 for descriptive statistics

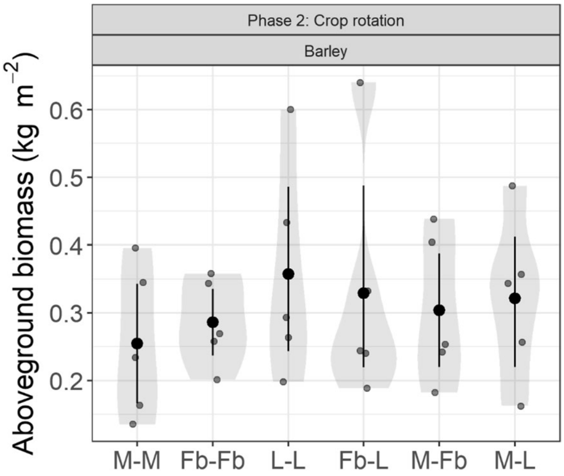


Fig.6 Barley aboveground biomass (kg m^{-2}) in the rotation phase when grown on soils trained from intercropping phase. Presented are the means and 95% confidence intervals. Small dots represents individual replicates. M–M: maize monoculture, Fb–Fb: faba bean monoculture, L–L: lupine monoculture, Fb–L: faba bean + lupine intercrop, M–Fb: maize + faba bean intercrop, M-L: maize + lupine intercrop. Refer to Supplementary table 1 for descriptive statistics

yield advantages in intercropping systems (Yu et al. 2015; Dong et al. 2018).

Next, we showed that when intercropped with lupine, the aboveground biomass production for faba bean and maize was greater (Fb-L and M-L) than their corresponding monocultures, whereas, the aboveground biomass of lupine decreased as compared to its monoculture. Lupines in nature tend to grow in large stands that dominate the surrounding vegetation, and hence evolutionarily-speaking our results seem to underline this habit, in that it did not benefit from intercropping. The lower performance of lupine when intercropped also hints toward antagonistic inter-specific interactions negatively affecting lupine growth and is very likely that the competition for resources severely constrained lupine growth when intercropped. This notion is supported by the smaller SPAD values (a proxy for chlorophyll content) for faba bean and maize leaves in the intercropping than their corresponding monocultures whereas SPAD values were the highest and remained similar for lupine in both monoculture and intercropping with maize and faba bean. This finding indicated that energy and resource investments for photosynthetic activity was generally greater for lupine than both maize and faba bean and did not change depending on monocultures and intercropping. On the contrary, smaller SPAD values for maize and faba bean when intercropped may suggest reduction in inter-specific competition for resources and efficient resource investments in biomass production for both maize and faba bean. We are aware that SPAD values are only the measure for leaf greenness and the actual rate of photosynthesis in both monocultures and intercrops may vary. It has been shown that intercropping maize with lupine resulted in higher maize biomass production but there was a tendency of lower biomass for lupine (although not significant) compared to their monocultures (Dissanayaka et al. 2015). In support of our results, Hauggaard-Nielsen et al. (2008) found that narrow-leafed lupine (*L.angustifolius* L.) performance was lowered in intercropping with barley with a reduction in atmospheric N-fixation from 15 to 5–6 g N m^{-2}. Further investigations are required to quantify C costs for resource acquisition and biomass production for lupine before adopting lupine as a viable companion crop in intercropping and mixed cultures.

Soil legacies from intercropping phase did not affect soil microbial parameters and barley aboveground biomass production in the rotation phase

We showed that variation in soil mineral N was dependent on the intercropping phase, thereby, created soil N legacies (Fig. 3). Mineral N was greater in legume monocultures (both Fb–Fb and L–L) compared to maize monoculture, with in-between effect for intercropped combinations. This is very likely a result of residual N in soil from decomposition of high-N plant residues that was reported previously (Freschet et al. 2012). Lower C:N ratios of legume residues make them faster to decompose by microbes. Legumes are also known to increase soil N availability through rhizodeposition (Fustec et al. 2009) and biological N fixation (Jensen and Hauggaard-Nielsen 2003) that leads to facilitative effects on neighbors (Temperton et al. 2007). Temperton et al. (2007) found that legume presence across a gradient of grassland plant diversity in the Jena Experiment facilitated a grass and a forb species, but the exact effect was largest for the grass, with the forb only increasing leaf N but not growing larger with legumes. Contrary to our expectation (i.e. higher mineral N in intercropped combinations than maize monoculture) we found comparable amounts of mineral N in maize monoculture (M-M) and all the intercrop combinations. This contrasts with previous findings showing that plants in mixed cultures extracted more nutrients from soil than those in monocultures due to complementarity in resource acquisition (Levine and HilleRisLambers 2009; Yang et al. 2013; Hacker et al. 2015; Wang et al. 2015). However, in the present study, this could be an artifact as we only measured the mineral N (NO_3^- and NH_4^+) after the intercropping phase which does not represent all N pools in soil. Depending on cropping systems, discrepancies in total and (in)organic N pools have been reported suggesting alteration in soil N pools after intercropping (Cong et al. 2015; Wang et al. 2015). Future studies would need to also measure organic N pools and mineralization rates to better understand soil N dynamics in relation to relative importance of organic and mineral N in intercropping settings. Similar to mineral N, we expected that faster decomposition of legume residues would increase the fraction of their residues that becomes a part of the SOM thereby increasing the total SOM content in soil. However, we found that the total SOM remained similar after intercropping phase. This finding suggests that the legume-derived SOM fraction decomposed quickly without affecting the total pool of SOM. This is plausible as one would expect an increased pool of particulate organic matter during early decomposition stages which is characterized by faster decomposition than the mineral associated organic matter pool. We suggest that future studies directly quantify the litter decomposition and its contribution in the formation of stable SOM from different cropping systems.

Soil legacies from intercropping phase neither affected the microbial parameters measured nor the aboveground biomass of barley in the rotation phase, thereby rejecting our second hypothesis. We found that, in the rotation phase, microbial biomass C and N, and their potential enzyme activities remained unchanged, suggesting an absence of strong PSF effects. In agreement with our findings, Wang et al. (2015) showed in a decade long mixed cropping experiment that even though the soil chemical parameters such as soil pH, exchangeable potassium, and cation exchange capacity varied depending upon cropping systems (monocultures versus continuous and rotational mixed cultures), the soil biological parameters such as activities of urease, phosphomonoesterase, and nitrate reductase remained largely unaffected. In an another experiment under rainfed conditions, Scalise et al. (2015) showed that legume-cereal intercropping had rather low impact compared to soil type and environmental factors on succeeding durum wheat productivity. Our findings are in contrast with results from Barel et al. (2019), where the identity of preceding crop affected the microbial biomass in the succeeding cropping phase. These discrepancies may arise from different plant species and nutrient availability in different soil types under investigation. For example, total N and total P content was higher in the present study than that found in Barel et al. (2019), and soil nutrient availability has strong regulation on microbial community composition and their activities (Olander and Vitousek 2000; Bell et al. 2015; Kumar et al. 2018) as well as the PSF effects (in't Zandt et al. 2019; Klinerová and Dostál 2019).

No change in barley aboveground biomass production in the rotation phase suggests that PSF effects are context dependent and edaphic factors may, in part, play a significant role. It further suggests that the generally observed positive plant-microbial interaction and plant performance in nutrient-limited soils may fade with higher nutrient availability. Our results are supported by a recent study (in't Zandt et al. 2019), where PSF effects on shoot biomass production of four grassland species were neutralized under increased nutrient availability. Interestingly, we found variation in the barley root length colonized by AMF but this did not lead to a measurable benefit (higher biomass production) for barley. This is in line with the long-held view that under higher nutrient availability, plants are less dependent on AMF for nutrient acquisition (Treseder 2004; Camenzind et al. 2016). Altered AMF colonization of barley roots may be due to changes in their community composition from intercropping phase. AMF communities have been shown to co-vary with their host plant community composition and diversity (Schmid et al. 2020; Smilauer et al. 2020). Therefore, it is very likely that soil harbored differential AMF communities from intercropping phase, which may have varied in the degree of root colonization potential.

Conclusions

We found evidence for good intercropping species combinations (maize and faba bean) as well as not so effective intercropping combinations (with lupine), with species-specific increases in biomass production in intercropping despite relatively high nutrient content in the agricultural soil. This suggests that inter-specific interactions overwhelmed the soil nutrient availability. Density-dependent relaxation in competition with maize in the intercropped combinations may have further resulted in increased biomass production but this was the case only for faba bean and not for lupine. Although belonging to the same plant functional group (i.e. legume), our study underlines that faba bean and lupine have a different potential in intercropping systems for biomass production. Further, we showed that the feedback effects of intercropping did not lead to improved barley biomass production even if there were changes in residual mineral N after the intercropping phase. These effects were also similar for other biological parameters (microbial biomass and their potential enzyme activity) in the rotation phase. Even though we showed that intercropping did not lead to significant PSF effects in our study, such effects may become important in management practices promoting reduced external mineral inputs or in soils with low fertility.

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Author contribution AK and VMT designed the experiment. AK collected field data and led lab measurements. CR and HC measured mineral N, microbial biomass, and potential enzyme activities. SP took SPAD measurements for leaf greenness. AK analyzed all the data and prepared the first draft. All coauthors read and contributed to finalize the manuscript, and gave their permission for submission.

Data availability Should the manuscript be accepted for publication, the presented data will be submitted to BonaRes data repository (https://datenzentrum.bonares.de/data-portal.php).

Declarations

Conflict of interest Authors declare no conflict of interest for this submission.

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General discussion

Losses of N (N leaching and N_2O) in winter period between crop growing

Temperature changes strongly affects N losses from soil, but to our knowledge few studies have investigated N transformations and losses during cooling-warming cycling in winter period. We have observed the remineralization of previously immobilized N and suppression of nitrification at low temperature after the cooling-warming phase of the experiment. These mineral N contributed to the growth of barley afterwards, especially during the critical N uptake time for the young barley seedlings. However, as a side effect, the cooling-warming cycle induced a potential risk of N losses, as confirmed by the N_2O emission peak in all treatments of the experiment.

Freezing-thawing cycle may occur if the temperature drops below freezing during the cold period in winter, which is a critical time for N loss. Freezing-thawing increased N_2O emission compared to the treatments without freezing. However, the increase of N_2O (5.5-37.9%) caused by freezing in our experiment was less than that found by many studies (Song et al., 2017; Wagner-Riddle et al., 2017). Furthermore, we found no research comparing N_2O emissions during freezing-thawing with cooling-warming conditions. We assume that freezing-thawing would not strongly increase N_2O emission as compared with treatments experienced cooling-warming. If there were treatments without cooling phase but incubated continuously in the greenhouse, freezing would have a large increase of N_2O emission compared to them.

Methods of reducing soil N losses and improving crop N uptake

By immobilizing soil N, straw application reduced the total N lost from soil, which was due to the mitigation of N leaching (chapter 1 and 2). However, the N leaching (accounted for around 1% of fertilizer N) was much less in our mesocosm experiment than the field situation (Abdalla et al., 2019). We assumed that under field conditions with significant N leaching, straw should have stronger mitigation of N losses. However, straw application strongly increased soil N_2O emission during conditioning phase in high N fertilizer levels by stimulating denitrification (chapter 2). The stimulatory effect of straw on N_2O emissions can be avoided, for example, straw application under reduced N fertilizer and drip irrigation did not increase the N_2O emission, because of lower levels of chemical N fertiliser and a more stable soil moisture situation (Zhao et al., 2021). In summary, straw amendment in conjunction with NO_3^- can cause higher soil N_2O emissions under conditions favouring denitrification (Wu et al., 2018), which was the case in the conditioning phase. We recommend adding the straw well in advance of fertilization so that when the straw decomposes, there is not enough NO_3^- resource for denitrification and N_2O emission.

Straw application was a main factor affecting processes of soil N cycle, i.e. immobilization, nitrification, remineralization and denitrification in our experiment. The mineral N was immobilized after straw application during pre-incubation and cooling-warming phases and remineralized in planting phase (chapter 1). The soil NO_3^- content decreased after straw application during pre-incubation and cooling-warming phases, but not in the planting phase, and the soil NH_4^+ increased after straw application (58-135 %) at no and low N fertilizer levels in the planting phase (chapter 1). N immobilization started immediately after straw application, and remineralization was weeks or months after (Cao et al., 2018; Shindo and Nishio, 2005). Therefore, straw application decreased shoot biomass and N contents in no and low N fertilizer levels treatments. The remineralization of immobilized N (induced by straw application) missed the N demand of early barley growth stage, the microbial N uptake caused N shortage for barley during its early growth stage. The soil NO_3^- of cooling-warming phase contributed more to the shoot N than the soil NO_3^- measured at the end of planting phase. It means that the critical time for barley N absorption was closer to the end of cooling-warming phase than the end of planting phase.

Differing from our results, straw could increase the crop yield by increasing SOC and improving soil's biophysical and physico-chemical properties (Wang et al., 2015), or reducing NO_3^- leaching (Yang et al., 2018). With sufficient N fertilization, straw incorporation led to the yield increase (e.g. van Duijnen et al., 2018) by the additional nutrient inputs or the remineralization of immobilized N during the growing season (Di and Cameron, 2002). Straw application did not decrease shoot N content in high N levels in our study, because of sufficient N supply both for soil microbes and barley. We assumed that with ample supply of soil inorganic N, either from fertilizers or because of accelerated organic N mineralization, the straw application will not decrease crop yields in the early growth stage, while the remineralized N from microorganisms may increase the crop's biomass in the late growth stage (Cao et al., 2018).

Compared to straw, the effect of NI is short-lived but effective in reducing N_2O emissions. When straw was combined with NI, the total N loss reduction was stronger in high N levels because NI reduced the N_2O emission and straw application reduced N leaching (chapter 1 and 2). However, the effects of NI on soil mineral N were quite weak (chapter 1 and 2). Therefore, no effects of NI on barley N uptake were detected 70 days after fertilization.

Besides straw and NI, there are other soil amendments, with biochar receiving the most attention. Regarding the impact of applying biochar to farmland on soil N loss, the time of application is crucial for its effect. Additionally, the impact of biochar on crop output and N loss is also influenced by the amount of fertiliser used (Wei et al., 2020). Although there is substantial evidence that applying biochar along with N fertiliser can increase the effectiveness of N utilisation, further research is necessary to determine the appropriate application rates for biochar and how these rates affect its economic viability in comparison to traditional fertilisers (Gao et al., 2022). Moreover, the N adsorption of biochar declined with longer treatment times, which could eventually result in an ecological risk (Zhang et al., 2021). Biochar application can also degrade soil properties, which should be noted if specific biochar is used to upgrade certain soil properties (Shaaban et al., 2018). In comparison, straw has stronger but shorter-term effects on the soil N cycling, biochar has longer-term but more subtle effects, and the production and transport of biochar requires additional human and material resources (Wu et al., 2019).

Among the soil amendments, sawdust is a relatively slow decomposing soil additive, and sawdust addition does not reduce NO3 leaching regardless of plant species (Esperschuetz et al., 2016). High rates of N fertiliser can cause soil acidification, but the addition of sawdust or mulch slows N fertiliser-induced acidification, pre-plant integrated sawdust also inhibited plant growth (White, 2006). Addition of untreated sawdust significantly reduced corn growth, dry matter production, and uptake of N and other nutrients (Olayinka and Adebayo, 1985). As a result, sawdust is not as effective as straw and biochar and is less commonly used on farmland.

The effects of different soil amendments on soil N fixation differed, with wheat straw application promoting rapid fixation of excess N by soil microorganisms, followed by sawdust, and pure lignin failing to promote microbial fixation of N. Compared with soil amended with straw, spruce sawdust-amended soils released more N_2O, whereas wheat straw-amended soils produced more CO_2 (Reichel et al., 2018). Diversification of C amendments may be good where specific materials are locally available and low cost, but their efficiency and mitigation of N losses should be tested to select the optimal soil amendment based on soil, climate and crop species.

We found that the effects of intercropping on crops growth were specie dependent, there were good intercropping species combinations e.g. maize and faba bean (chapter 3). Moreover, intercropping faba bean and maize had higher above-ground production of biomass in intercropping than in monoculture. Besides of good intercropping species combinations, there was also ineffective intercropping combinations, when faba beans or maize were intercropped with lupins, faba beans and maize gained more above-ground biomass, while lupins gained less above-ground biomass (chapter 3). Intercropping had a negative effect on lupin, suggesting the result of interspecific antagonism, possibly due to strong competition for resources during intercropping. However, this effect is soil and site specific and crop combinations will perform differently in different soil and climate types.

The enhanced effectiveness of intercropping on crop yields have been widely reported (Li et al., 2023; Li et al., 2021). However, intercropping has some disadvantages. These include lower yields of the main crop and high labour inputs (Gliessman, 1985). Because intercropped plants compete with each other for light, soil nutrients and water, yields of major crops in intercropping systems will not be as high as in monocropping (Willey, 1979). Another possible disadvantage is the high cost of maintenance, especially weeding, which may have to be done by hand. In countries where there is a surplus of labour, this is not a serious problem, and since growing monocultures is more vulnerable to natural disasters, intercropping sets can reduce the risks associated with growing monocultures and thus increase the incomes of smallholders; but in countries where there is a shortage of labour, intercropping can lead to an increase in costs (Gebru, 2015). Small-scale farmers tend to favour monoculture, while large farms prefer intercropping to reduce production costs (Ghazali et al., 2016).

Crop yields are shown to rise by 20% on average with crop rotation compared to continuous monoculture in a review of studies (Zhao et al., 2020). Crop rotation significantly boosts agricultural yields without the use of additional inputs; it encourages beneficial soil microorganisms and their interactions, breaks disease cycles and reduces weed populations, all of which enhance the physical and chemical properties of the soil and boost crop yields and land-use efficiency (Shah et al., 2021). However, one of the biggest challenges to the adoption of crop rotation is financial, as incorporating additional crops into the normal rotation may require farmers to make significant upfront investments, such as purchasing new machinery, and incur additional short-term costs (Shah et al., 2021). To optimise agronomic and environmental benefits, each crop rotation system should be carefully planned taking into account the local climate, soils, crops, and management practises. (Zhao et al., 2020). And soil amendments should be used in conjunction with farming practices, such as intercropping and crop rotation, to counter the limitations of using each practice alone.

Intercropping creates PSF effects to influence the growth of subsequent crop rotations. Although PSF could have positive or negative effects on the next generation crop growth (Kulmatiski et al., 2008; Kulmatiski and Kardol, 2008), during the rotation phase in our experiment, however, although intercropping alters soil mineral N, its feedback effect did not increase barley biomass production (chapter 3). However, it was proposed that in resource-rich environments, plants are dominated by competition and negative PSFs, whereas in resource-poor environments plants may receive a net benefit from mutualists in the soil, shifting interactions between individuals from mainly negative to mainly positive (Lekberg et al., 2018). Thus, under low resource conditions, plants may benefit from facilitation and positive PSF. Although intercropping had no significant PSF effects in our study, PSF effects may be important in farming practices with reduced mineral inputs or on low fertility soils.

Contribution of this study to the science and agriculture

Although the effects of soil amendments on N loss and crop N uptake varied considerably, the differences were due to variations in soils, climatic conditions and field management. It was reported that straw application several months or one season before fertilization and sowing crop seed (Yang et al., 2020; Zhang et al., 2015; Zhang et al., 2016) could improve the crop yield. Good crop management presumes addition of the fertilizer separately from (e.g. after) straw application to meet the demand of crop growth (Xu et al., 2019; Yang et al., 2020). Carefoot (1997) suggests that fresh plant residues stimulate microorganisms to immobilise large amounts of N, but after two months of decomposition, the immobilised N is remineralised and available for plant use. For example, straw application in the autumn after harvesting crops can keep the inorganic N in soil and immobilise it, mitigating N leaching and emission. The immobilized N could be released in the next early spring when substantial warming occurs after (cold) winter, during the cooling-warming cycle the strong mineralization of N would supply the growth of crops. In addition, application of straw with N fertiliser at sufficiently long intervals also avoids significant N_2O emissions, such as the no N fertiliser treatments, no substantial increase in N_2O emissions by straw application. Another approach is to control the quantity of straw applied or the C/N ratio of the straw, we found that the soil N sequestration capacity caused by straw was significantly reduced when the total C/N ratio of the soil amendments was below 25. Finally, the critical time for N uptake in barley is the early stage of planting, so it is essential to ensure the supply of N at this stage, to prevent low yields due to insufficient N supply in the early stages of barley growth.

Since N_2O emission peaks are common during the initial stages of fertilisation (Chen et al., 2019), we recommend applying NI together with N fertiliser. NI can be used concurrently with the straw to reduce the N_2O emissions inspired by the straw application. Previous studies showed that application of NI (e.g. PIADIN®) promoted the crop growth (Barneze et al., 2015; Federolf et al., 2016). The effects of NI on crops growth are determined in large amount on the mitigation of N leaching. It was founded that when NI mitigating N leaching effectively, it provides more N for crop and increase the crop growth. For example, during the time of strong precipitation and irrigation especially after the application of NH_4^+ fertilizer, the addition of NI could retard the nitrification and reduce the amount of easily leaching NO_3^-, retain soil N for the later crop uptake. Therefore, better results in reducing N loss and increasing yield can be achieved by applying NI during the fertiliser application period and during the heavy rainfall phase.

However, very few research has compared N uptake and losses in intercropping versus monocropping (Yin et al., 2019), crop rotation versus continuous cropping systems with and without the application of straw (Limon-Ortega et al., 2008). Additionally, we are not aware of any research comparing N utilization and losses under cooling-warming and freezing-thawing cycling in intercropping and monocropping, as well as in rotational and continuous cropping systems, and the contribution of PSF effects to these processes. It's vital to determine whether and when to apply straw, NI, and N fertiliser in order to achieve the highest NUE and lowest N losses in field situations, under intercrop versus monocrop, crop rotation versus continuous cropping, as well as under cooling-warming and freezing-thawing conditions, respectively.

In conclusion, crop management and soil amendments are additional potential strategies to increase crop NUE and decrease N losses. Numerous studies have shown that intercropping and rotations can increase crop yields; however, the final effects depend on the crop combinations chosen, and illogical intercropping and rotation combinations can lower crop yields. Additionally, the increased labour inputs associated with sophisticated management are elements to be taken into consideration in the agricultural production of rotation and intercropping, as opposed to the simpler management of monocrops and continuous cropping. Similar circumstances apply to soil amendments like NI, straw, charcoal, and sawdust: although sensible management will have a positive effect on crop yields, irrational management will have no effect or even a negative effect. In addition, there are personnel and financial costs associated with applying soil amendments. In conclusion, in order to maximise crop yield and benefit the environment, management practices like intercropping, crop rotation, and the use of soil amendments like NI, straw, biochar, and sawdust should be carefully chosen for adoption based on the unique characteristics of the arable land as well as the costs and labour inputs.

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Subpublications and Records of Achievement

Chapter 1

(1, 2, 3) **Chen, H.**, Rosinger, C., Blagodatsky, S., Reichel, R., Li, B., Kumar, A., Rothardt, S., Luo, J., Brüggemann, N., Kage, H., 2023. Straw amendment and nitrification inhibitor controlling N losses and immobilization in a soil cooling-warming experiment. Science of The Total Environment 870, 162007.

Chapter 2

(1, 2, 3) **Chen, H.**, Rosinger, C., Blagodatsky, S., Reichel, R., Li, B., Kumar, A., Rothardt, S., Luo, J., Brüggemann, N., Kage, H. Straw application and nitrification inhibitor affect soil N losses during cooling-warming and freeze-thaw cycles. Unpublished manuscript

Chapter 3

(4, 5) Kumar, A., Rosinger, C., **Chen, H.**, Protic, S., Bonkowski, M., Temperton, V.M., 2021. Gone and forgotten: facilitative effects of intercropping combinations did not carry over to affect barley performance in a follow-up crop rotation. Plant and Soil 467, 405-419.

(1) The experimental design was planned by author, Prof. Dr. Michael Bonkowski, Prof. Dr. Nicolas Brüggerman and post-doc Christoph Rosinger. Laboratory work was carried out by the author.

(2) Data analysis and statistical was carried out by the author.

(3) The Manuscript was written by the author with guidance from Prof. Dr. Michael Bonkowski and Dr Sergey Blagodatsky.

(4) The author was involved in the writing of the article and refining the final version of the manuscript.

(5) The author contributed in laboratory work and biochemical analysis for the samples.

Erklärung zur Dissertation

Erklärung zur Dissertation

gemäß der Promotionsordnung vom 12. März 2020

Diese Erklärung muss in der Dissertation enthalten sein.
(***This version must be included in the doctoral thesis***)

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Teilpublikationen:

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