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Nitrate leaching from animal manure – Insights from on-farm and greenhouse studies using ^{15}N labelled cattle slurry

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presented by

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Notice how many times
I have said "manure"? It is serious business.
It breaks the farmers' backs. It makes their land.
It is the link eternal, binding man and beast
and earth.

[…] the link
of the manure, that had seemed eternal, is broken.

(From "Marshall Washer" by Hayden Carruth)

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Summary

Animal manures are valuable multi-nutrient fertilizers, but their nitrogen (N) use efficiency (NUE) by crops is often low. Inefficient and untargeted use of animal manure can lead to N losses such as nitrate (NO_3^-) leaching, having adverse effects both on natural ecosystem and on the integrity of drinking water resources. In order to minimize N losses, a better understanding of N turnover dynamics of animal manure in the soil-plant system as well as actual measurements of NO_3^- leaching from animal manure under field conditions are needed. The overarching questions of this thesis were: Does animal manure cause higher NO_3^- leaching than mineral fertilizer? And how could N leaching losses be reduced? These questions were addressed by tracing the fate of ^{15}N labelled cattle slurry in comparison to ^{15}N labelled mineral fertilizer in the soil-plant system during an on-farm field study over 2.5 years. This study was conducted in the Gäu region, Canton Solothurn, Switzerland, which is characterized by elevated NO_3^- levels in the groundwater exceeding the Swiss quality criterion of 25 mg NO_3^- L^{-1}.

To this end, ^{15}N labelled cattle slurry was produced by feeding a heifer with ^{15}N labelled ryegrass (*Lolium multiflorum*) hay. The ^{15}N labelled cattle slurry (Slu) and the ^{15}N labelled mineral fertilizer (Min) were applied to microplots (1.5 m x 2 m) on two neighbouring fields on loamy soil in the Gäu region. Both fields followed the same crop rotation (grass-clover – silage maize – winter wheat), but shifted by one year. The ^{15}N labelled fertilizers were applied in 2018, according to common agricultural practice and at the same rate of mineral N. From 2019, fertilization was done with unlabelled fertilizers by the farmer.

The crops to which the ^{15}N labelled fertilizers had been applied, recovered 45 to 47 % of mineral fertilizer N, but only 19 to 23 % of cattle slurry N. Complementary, recoveries in soil at the end of the first season were greater for Slu (53 to 58 %) than for Min (28 to 32 %), despite greater ammonia emissions from Slu. Fertilizer recovery in the succeeding crops was small (< 4.6 % in the first and < 2.4 % in the second residual year, relative to applied fertilizer amounts) and similar for the two fertilizers.

These results relate well to the finding that 77 to 89 % of residual fertilizer N in soil after the first crop were recovered in the non-microbial organic N pool, irrespective of fertilizer type and of initial differences between them. Depth translocation of fertilizer N was marginal, and at the end of the 2.5 years lasting field study the majority of ^{15}N was still recovered in the top 30 cm. Partly, this might relate to exceptional dry weather conditions during summer 2018, with precipitation between April and October remaining 30 % below the long-term average. However, throughout the whole experimental period, average precipitation still reached approx. 1`000 mm $year^{-1}$ which is about 89 % of the long-term average and, thus, allowed for representative observations. Cumulated amounts of NO_3^- leaching over the three crops reached up to 205 kg NO_3-N ha^{-1}, but less than 5 % of this amount originated from direct leaching of the labelled fertilizers. Although low in absolute values, NO_3^- leaching losses were significantly higher for Slu (4.6 to 7.5 kg NO_3-N ha^{-1}) than for Min (2.3 to 3.7 kg NO_3-N ha^{-1}) cumulated over the whole duration of the study. The highest NO_3^- leaching occurred after termination of grass-clover ley and most of the leached NO_3^- originated from mineralization of soil N. This emphasizes the central role of soil organic N management for mitigating NO_3^- leaching.

In a follow-up experiment under greenhouse conditions, the same ^{15}N labelled fertilizers were applied to soil columns planted with ryegrass (*Lolium multiflorum*). The aim was to test the effect of treating ^{15}N labelled cattle slurry by anaerobic digestion, biochar and/or the nitrification inhibitor DMPP on NUE, NO_3^- leaching and slurry N turnover in soil. All slurry treatments were expected to reduce the NO_3^- leaching potential compared to untreated slurry. Anaerobic digestion increased crop NUE and decreased ^{15}N recovery in soil. However, this did not translate into lower leaching of residual N after 57 days of ryegrass growth compared to undigested slurry. Nevertheless, anaerobic digestion might decrease the long-term leaching potential of slurry, since N accumulation in soil would be lower with repeated applications of digested compared to undigested slurry. Biochar and DMPP had minor effects and are likely less promising strategies to reduce NO_3^- leaching.

The results of this thesis highlight that considering soil organic N turnover is key to understand and mitigate NO_3^- leaching. Crop N uptake originated only to a minor proportion from current fertilizer additions, while most of it came from soil organic N mineralization. In turn, most fertilizer N was rapidly incorporated into the soil organic N pool. This advocates that fertilization should not only target to directly nourish the crops, but also to (re-)fill these soil N reserves that in turn will provide N to the crops. Furthermore, termination of grass-clover was confirmed as the "hot moment" within the crop rotation, increasing soil N mineralization and being responsible for most NO_3^- leaching losses. Better management of these losses might require the introduction of cover crops as well as considering the mineral N release from soil and belowground residues of grass-clover leys, both in terms of amounts and temporal release dynamics, by accordingly reducing N fertilizer input after grass-clover termination.

Zusammenfassung

Hofdünger sind wertvolle Mehrnährstoffdünger, aber ihre Stickstoffausnutzungseffizienz (NUE) durch die Nutzpflanzen ist oft gering. Ineffizienter und ungezielter Einsatz von Hofdüngern kann zu Verlusten von Stickstoff (N) führen, zum Beispiel durch Nitratauswaschung. Nitratauswaschung gefährdet natürliche Ökosysteme und die Integrität von Trinkwasserressourcen. Um diese N-Verluste zu minimieren, sind ein besseres Verständnis der N-Umsatzdynamiken von Hofdüngern im Boden-Pflanze-System sowie eine Quantifizierung der tatsächlichen Nitratauswaschung aus Hofdüngern unter Feldbedingungen notwendig. Die übergeordneten Fragen dieser Dissertation waren: Führen Hofdünger zu höheren Nitratauswaschungsverlusten als Mineraldünger? Und wie können N-Auswaschungsverluste verringert werden? Zur Beantwortung dieser Fragen wurden ^{15}N markierte Rindergülle und ^{15}N markierter Mineraldünger im Boden-Pflanze-System über 2.5 Jahre in einem Feldversuch unter praxisüblicher Bewirtschaftung auf einem Landwirtschaftsbetrieb nachverfolgt. Der Versuch wurde in der Region Gäu, Kanton Solothurn, Schweiz, durchgeführt. Die Gäu-Region ist gekennzeichnet durch hohe Nitratgehalte im Grundwasser, die das Schweizer Qualitätsziel von 25 mg NO_3^- L^{-1} überschreiten.

Um ^{15}N markierte Rindergülle zu produzieren, wurde ein junges Rind mit ^{15}N markiertem Weidelgras-Heu (*Lolium multiflorum*) gefüttert. Die ^{15}N markierte Rindergülle (Slu) und der ^{15}N markierten Mineraldünger (Min) wurden auf Kleinstparzellen (1.5 m x 2 m) auf zwei benachbarten Feldern mit lehmigem Boden in der Region Gäu ausgebracht. Die beiden Felder folgten der gleichen Fruchtfolge (Kleegras – Silomais – Winterweizen), welche zwischen den beiden Feldern um ein Jahr versetzt war. Die ^{15}N markierten Dünger wurden in 2018 praxisüblich und in der gleichen Menge an mineralischem N ausgebracht. Ab 2019 erfolgte die Düngung mit unmarkierten Düngern durch den Landwirt.

Im Ausbringungsjahr der ^{15}N Dünger nahmen die Kulturen 45 bis 47 % des Mineraldünger-N auf, aber nur 19 bis 23 % des Gülle-N. Komplementär dazu war die

^{15}N Wiederfindung im Boden für Slu (53 bis 58 %) grösser als für Min (28 bis 32 %), obwohl die Ammoniakemissionen für Slu höher waren. Die Wiederfindung von Dünger-N in den nachfolgenden Kulturen war gering (< 4.6 % im ersten und < 2.4 % im zweiten nachfolgenden Jahr, relativ zur ausgebrachten N-Menge). Diese Ergebnisse passen gut zur Beobachtung, dass unabhängig vom Düngeverfahren und trotz anfänglicher Unterschiede zwischen Min und Slu, 77 bis 89 % des residuellen Dünger-N im Boden nach der Ernte der ersten Kultur im nicht-mikrobiellen organischen Boden-N-Pool wiedergefunden wurden. Die Tiefenverlagerung des Dünger-N im Boden war gering und zum Ende der 2.5 Jahre andauernden Versuchslaufzeit befand sich immer noch der grösste Teil des Dünger-N in den obersten 30 cm. Dies kann teilweise auf die ausserordentlich trockenen Wetterbedingungen im Sommer 2018 zurückgeführt werden, in welchem die Niederschläge zwischen April und Oktober 30 % tiefer waren als im langjährigen Mittel. Insgesamt lagen die durchschnittlichen Niederschläge während der gesamten Versuchslaufzeit aber bei 1000 mm $Jahr^{-1}$, was 89 % der langjährigen mittleren Niederschläge entspricht, und erlaubten dadurch trotz allem repräsentative Messungen. Die kumulierte Nitratauswaschung über die drei Kulturen lag bei bis zu 205 kg NO_3-N ha^{-1}. Allerdings stammten weniger als 5 % dieser Menge direkt aus den Düngern. Obschon die absoluten Auswaschungsverluste aus den Düngern gering waren, wurde während der gesamten Versuchslaufdauer mehr Gülle-N (4.6 bis 7.5 kg NO_3-N ha^{-1}) als Mineraldünger-N (2.3 bis 3.7 kg NO_3-N ha^{-1}) ausgewaschen. Die höchste Nitratauswaschung trat nach dem Umbruch der Kleegraswiese auf und stammte zum grössten Teil aus der Mineralisierung von Boden-N. Dies unterstreicht die zentrale Rolle des Managements von organischem Boden-N, um Nitratauswaschung zu verringern.

In einem Folgeversuch unter Gewächshausbedingungen wurden die gleichen ^{15}N markierten Dünger auf mit Weidelgras (*Lolium multiflorum*) bepflanzten Bodensäulen ausgebracht. Der Versuch zielte darauf ab, den Effekt von anaerober Vergärung, Pflanzenkohle und dem Nitrifikationshemmstoff DMPP auf NUE, Nitratauswaschung und Gülle-N-Umsätze im Boden zu untersuchen. Es wurde

angenommen, dass alle getesteten Verfahren das Nitratauswaschungspotenzial gegenüber unbehandelter Gülle reduzieren. Anaerobe Vergärung steigerte die Pflanzen-NUE und verringerte die ^{15}N Wiederfindung im Boden. Allerdings resultierte dies nach der Kultivierung von Weidelgras für 57 Tage nicht in einer verringerten Nitratauswaschung des residuellen N verglichen mit unvergorener Gülle. Nichtsdestotrotz ist davon auszugehen, dass anaerobe Vergärung längerfristig das Nitratauswaschungspotenzial von Gülle reduziert, da sich bei wiederholter Ausbringung von anaerob vergorener Gülle weniger N im Boden anreichert als bei unvergorener Gülle. Pflanzenkohle und DMPP hatten nur geringe Effekte und ihr Einsatz zur Reduktion der Nitratauswaschung ist vermutlich weniger vielversprechend.

Die Resultate dieser Dissertation verdeutlichen, dass der Umsatz von organischem Boden-N zentral ist, um Nitratauswaschung zu verstehen und zu verringern. Nur ein kleiner Teil der Pflanzen-N-Aufnahme stammte aus den ausgebrachten Düngern, während der grösste Teil aus der Mineralisierung des organischen Boden-N stammte. Im Gegenzug wurde der meiste Dünger-N rasch in den organischen Boden-N-Pool eingebaut. Diese Ergebnisse sprechen dafür, mit der Düngung nicht nur auf die direkte Pflanzenernährung abzuzielen, sondern auch das Auffüllen der Boden-N-Pools zu berücksichtigen, welche wiederum die Pflanzen mit Nährstoffen versorgen. Der Umbruch der Kleegraswiese wurde als entscheidender Moment innerhalb der Fruchtfolge bestätigt, welcher die Boden-N-Mineralisierung erhöht und verantwortlich ist für den grössten Teil der Nitratauswaschungsverluste. Um diese Verluste besser zu kontrollieren, sind Zwischenfrüchten sowie die Berücksichtigung der N Freisetzung aus dem Boden und der unterirdischen Pflanzenrückstände der Kleegraswiese (sowohl in Bezug auf die Menge als auch auf die zeitlichen Dynamiken der N Freisetzung) durch eine Reduktion nachfolgender Düngergaben notwendig.

List of abbreviations

BEDN	bacterial and endogenous debris N
BNF	biological N fixation
C	carbon
DMPP	3,4-dimethyl-1H-pyrazole monophosphate
DON	dissolved organic nitrogen
K	potassium
MAOM	mineral associated organic matter
N	nitrogen
NDF	neutral detergent fibre
Ndff	nitrogen derived from fertilizer
NH_3	ammonia
NH_4^+	ammonium
Nmin	mineral nitrogen = inorganic nitrogen (NH_4^+-N + NO_3^--N + NO_2^--N)
Nmic	microbial nitrogen
NO_3^-	nitrate
NO_2^-	nitrite
N_2O	nitrous oxide
Norg	non-microbial organic nitrogen
NUE	nitrogen use efficiency
P	phosphorus
POM	particulate organic matter
SOM	soil organic matter
UDN	undigested dietary N
WIN	water-insoluble N
WSN	water-soluble N

Chapter 1 General Introduction

Animal manure has been used by humans for fertilizing soils since millennia. Still, accurately predicting the nitrogen (N) fertilization effect of animal manure remains challenging, resulting in an untargeted use and unintended N losses to the environment. With this work, I aimed at gaining a better understanding of transformations and fluxes of N from cattle slurry within the soil-plant system, which is a widely used type of animal manure, under field conditions. Ultimately, this knowledge will help to improve the recycling of nutrients from animal manure to plants and reduce N losses.

Within this introductory chapter, I will give background information and set the broader scene for my thesis. Coming from a global perspective, the "N dilemma" – resulting from a trade-off between producing enough food and protecting the environment – will be introduced. The underlying drivers for this dilemma require to understand the agricultural N cycle, including its leakiness. Nitrate leaching is one of these N loss pathways and as it is the main focus of my work, I will provide a detailed overview on its causes and consequences. One of the causes for nitrate leaching often is suspected in fertilization with animal manure. I will give an overview on the properties of animal manure and the challenges of efficiently using it as an N fertilizer. Some of the knowledge gaps associated with using animal manure for N fertilization, can be addressed using the stable isotope ^{15}N for which the principles will be shortly explained. Finally, the local situation in Switzerland as well as the study region ("Gäu") are introduced to the reader.

1. 1 Trade-off between food production and environmental protection – the case of nitrogen

Agroecosystems worldwide are expected to feed an increasing world population while minimizing negative environmental impacts. Nitrogen has both aspects: it is an essential plant nutrient and a major pollutant (Kanter et al., 2020a). Mineral or organic N fertilizer inputs are needed to sustain productivity. Nowadays, about half of the global food production relies on mineral fertilizer N inputs, although its accessibility is not uniformly distributed over the globe (Erisman et al., 2008, Ladha

et al., 2005, FAOSTAT, 2019). Since 1960, both synthetic mineral N inputs and also livestock numbers and with it the use of livestock manure have increased tremendously, while the rise was much stronger for synthetic fertilizer (**Fig. 1.1**).

However, productivity did not equally increase, indicating a strong reduction in N use efficiency (NUE). NUE, which is defined as the proportion of fertilizer N taken up by crops, currently reaches only 30 to 50 % of fertilizer N input (Tilman et al., 2002). Nitrogen not taken up by crops is prone to losses. Thus, inefficient N use not only presents a loss in productivity, but threatens the environment as the N losses contribute to global warming, eutrophication and acidification of water bodies, biodiversity decline, as well as air and drinking water pollution (e.g. Cameron et al., 2013, Sutton et al., 2013).

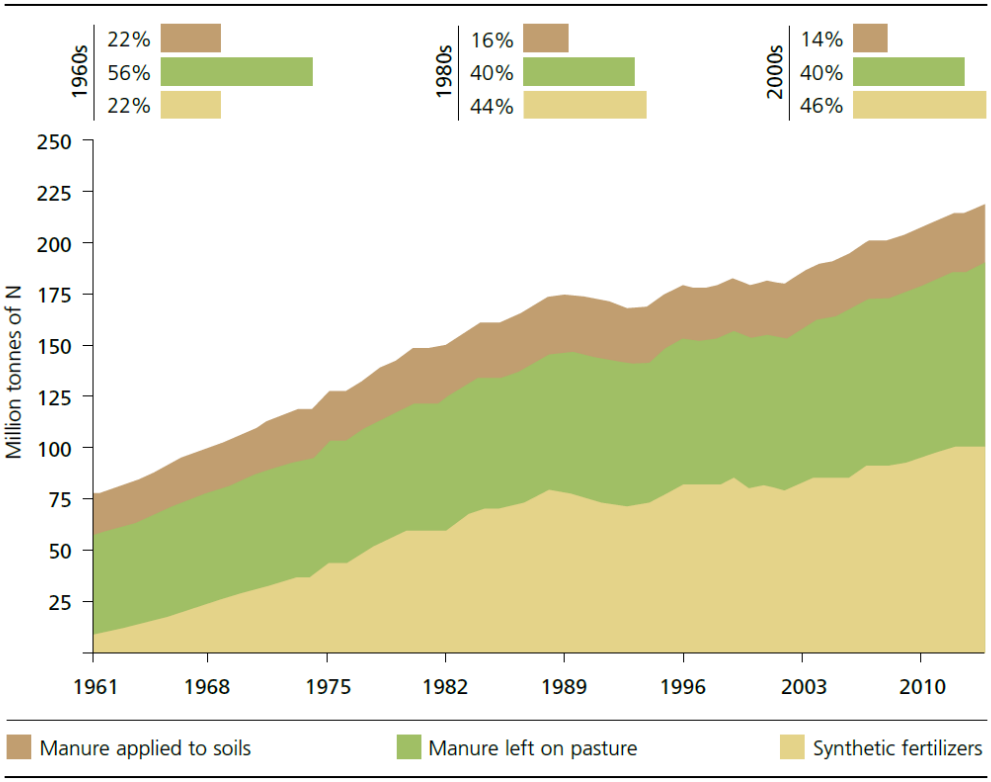

Fig. 1.1: *Cumulative global N input from livestock manure and synthetic fertilizers, 1961 – 2014. Bar charts represent decadal average shares for 1960s, 1980s and 2000s (FAO, 2018)*

Along these lines, decreasing inputs and increasing NUE appears the only feasible way to sustain food production while protecting and conserving environmental quality

(Cui et al., 2014, Sutton et al., 2013). NUE is controlled by the provision of available N, but also depends on crop performance and N uptake, which can be limited by other factors such as water shortage, pests, or deficiencies in other nutrients. Crop N availability is driven by N input amount and type as well as agricultural management and local pedoclimatic conditions that are driving N transformation processes (see **1. 2**). Overall, a refined understanding of these processes and N fluxes from mineral and organic fertilizers in agroecosystems is needed for better synchronizing plant N demand with supply of available N, to improve NUE, and ultimately to reduce N losses to the environment.

1. 2 The agricultural N cycle

Nitrogen is all around us. It is a major element of all living organisms as a part of proteins and DNA. 78 % of our atmosphere is molecular N_2, which is, however, a biologically inert gas and not accessible to most living organisms. Only less than 1 % of the global N reserves are in a so-called "reactive" form, which encompasses both organic and inorganic N molecules (Galloway et al., 2003).

Naturally, reactive N enters the soil system via biological fixation of N_2 by legumes, N deposition, and via organic residues such as plant litter or deposits from herbivores (**Fig. 1.2**). With the invention of the Haber-Bosch process in the early 20th century, humans have added another major pathway that converts inert N_2 into reactive N, entering into soil in the form of synthetic mineral N fertilizer (Erisman et al., 2008). Besides direct input of mineral N, mineralization-immobilization turnover in soil provides plants with available mineral N forms such as ammonium (NH_4^+) or nitrate (NO_3^-) (Jansson and Persson, 1982). It was estimated that the rooting zone of plants holds about 5 to 15 tons of N ha^{-1} (Christensen, 2004). Of this amount 1 to 2 % might be mineralized per year. Mineralization is defined as the transformation of organic N to NH_4^+. Ammonium can be further oxidized to nitrite (NO_2^-) and NO_3^- via the process of nitrification. Immobilization means the transformation of mineral N (NH_4^+, NO_3^-, NO_2^-) to organic N, usually via the assimilation by microbes. In fact, most processes driving N transformation and cycling in soil are largely mediated by microbes

(Robertson and Groffman, 2007). As such, these processes are highly dependent on the microbial community as well as environmental factors such as soil moisture, soil temperature, soil texture, redox conditions, and pH. Especially the addition of organic material rich in available carbon (C) boosts microbial activity and with it the immobilization of inorganic N into microbial biomass, emphasizing the close link between C and N cycling (Gruber and Galloway, 2008). Microbial N can be re-mineralized or enter more recalcitrant soil organic matter (SOM) pools. Likely, microbial necromass is an important pathway for the formation of stable SOM-N (Liang et al., 2019).

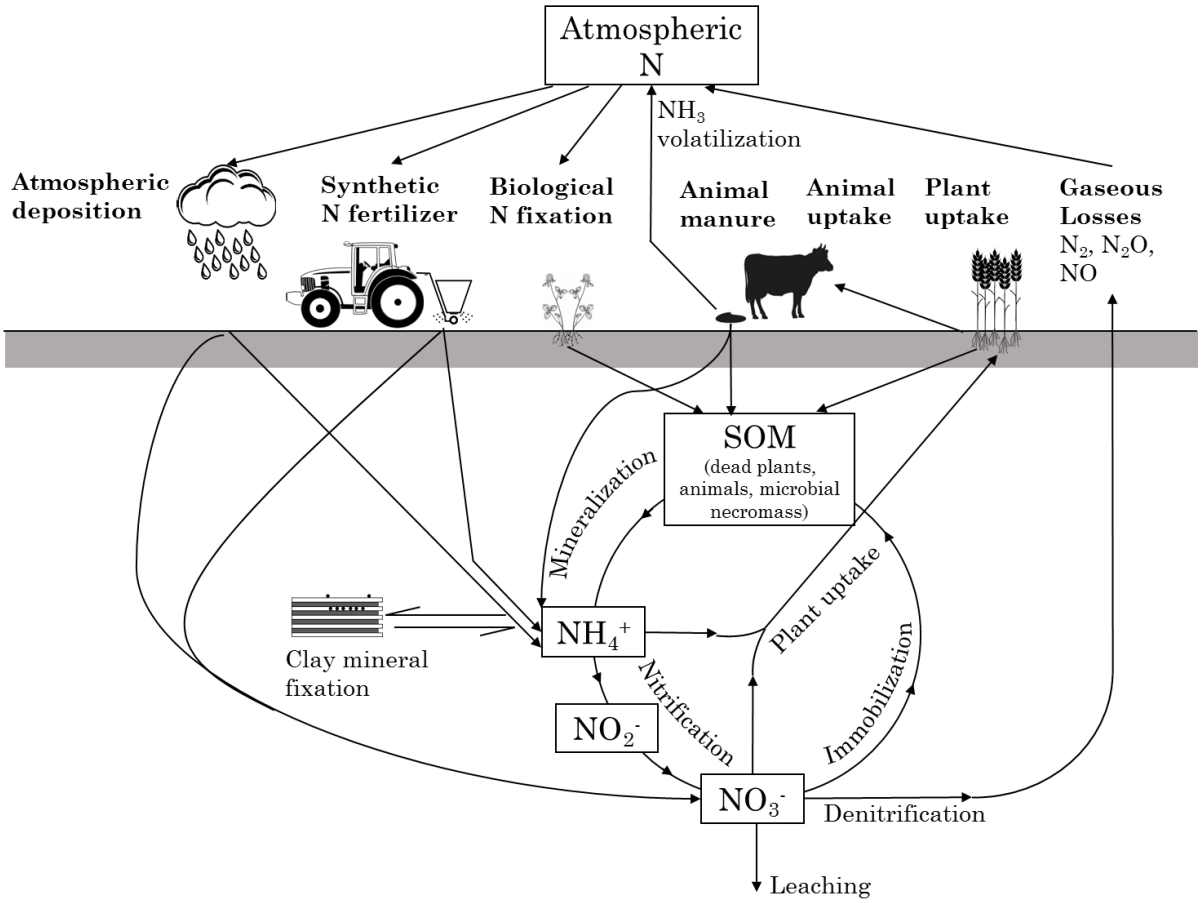

Fig. 1.2*: The agricultural N cycle (SOM = soil organic matter, NH_4^+ = ammonium, NO_2^- = nitrite, NO_3^- = nitrate, NH_3 = ammonia, N_2 = di-nitrogen, N_2O = nitrous oxide, NO = nitric oxide) (adapted after Di and Cameron (2002))*

Especially the process of nitrification makes N prone to losses. In contrast to NH_4^+, NO_3^- ions are negatively charged and, thus, barely get adsorbed to soil particles such

as clay surfaces. Consequently, NO_3^- can be lost via leaching, given sufficient water percolation in the soil. Furthermore, nitrification enhances emissions of nitrous oxide (N_2O) and other nitrogen oxides (NO_x), as these gases are formed both during nitrification and denitrification. Nitrous oxide and NO_x contribute to global warming and production of harmful ozone in the troposphere (IPCC, 2006). Ammonia (NH_3) volatilization constitutes another gaseous loss pathway, usually occurring after spreading of ammonium-rich manures or urea to the soil, causing acidification and eutrophication of natural ecosystems (Guthrie et al., 2018).

In contrast to N limited natural ecosystem, the N cycle in agroecosystems is "open" due to N output with harvested products and the large external inputs for compensating these N exports or losses. Field crops are usually grown in monoculture and have a high N demand, which is restricted to a relatively short time span, demanding targeted supply of N via fertilization. Furthermore, soil tillage influences the rate and dynamics of soil N turnover in soil (Askegaard et al., 2011). Overall, human activities have increased both the size and dynamics of N flows, thereby increasing the potential of losses to the environment (Vitousek et al., 1997).

Besides these alterations, humans also have expanded the spatial scale of N cycling by global trade of agricultural products. While countries such as the USA, Argentina and Brazil are exporting N in the form of animal feed such as soy, Europe or China are importing large amounts of feed N for livestock production (Lassaletta et al., 2014). Due to the low efficiency in animal production, most N will be converted into animal manure for which there is not enough land area in the vicinity of livestock farms to spread in a nutrient efficient way (Oenema and Tamminga, 2005). Local specialization of crop production and animal husbandry has not only happened on a global scale, but occurs also increasingly on a regional level, disintegrating the N cycle even within countries (Garnier et al., 2016, Oenema and Tamminga, 2005, Garrett et al., 2020). Thus, global trade of N-bearing agricultural commodities and especially feed, together with local specialization of farms has added another major component to the N cycle along the biogeochemical processes described above. While manure N

could be a valuable fertilizer and is missing in the crop production areas/countries, in livestock production areas it is often considered as waste and leads to a large structural N surplus enhancing N losses to the environment, e.g. by nitrate leaching.

1. 3 Nitrate leaching – causes and consequences

1.3.1 Causes and indicators for nitrate leaching losses

Nitrate leaching losses arise when nitrate accumulation in soil concurs with high water drainage. Generally speaking, nitrate accumulates in soil when N uptake by plants is not matched with N supply through fertilization or mineralization of soil N. Thereby, nitrate leaching losses have been shown to be linked exponentially to N surplus (Wang et al., 2019).

National N budgets in industrialized countries show a large surplus (Eurostat, 2018), although these budgets are not necessarily comparable between countries due to different assumptions and calculation principles (Klages et al., 2020). The IPCC (2006) estimated that 30 % of N inputs are lost via nitrate leaching. However, this emission factor is very generalized and likely too high under most conditions with less than 500 kg N ha^{-1} $year^{-1}$ input (Wang et al., 2019, Silgram et al., 2001).

Rather, nitrate leaching depends on complex interactions of winter precipitation, soil type, crop rotation, plant growth as well as the type, amount, timing and splitting of fertilizer and manure inputs (Silgram et al., 2001, Gardner and Drinkwater, 2009, Thomsen et al., 1993). Also land use types showed differing nitrate leaching potentials, generally following the order forest < grassland < arable cropping < ploughing of pasture < vegetable production (Di and Cameron, 2002).

In order to reduce leaching losses, synchronization of plant N demand and supply are crucial, which might be achieved by measures such as site-specific fertilization (Argento et al., 2021), improved timing, and splitting of N doses (Gardner and Drinkwater, 2009). For organic fertilizers it is specifically challenging to predict their N availability. Often, they are applied based on their estimated mineral N content in order to meet the short-term N demand of the crop. However, since animal manures,

in contrast to synthetic fertilizer, not only contain mineral N forms, but in addition also a considerable proportion of organic N (30 to 75 % of total N) (Webb et al., 2013), the absolute amount of N applied is usually higher with manure than with mineral fertilizer. It is for this reason that animal manures are suspected to contribute more to nitrate leaching than mineral fertilizers. However, comparative assessments of mineral fertilizer and animal manure in terms of their nitrate leaching potential under realistic agronomic conditions have barely been done.

1.3.2 Consequences of nitrate leaching on human health and natural ecosystems

In many regions, groundwater is a primary source of drinking water to humans which makes elevated nitrate concentrations a human health issue. Enhanced nitrate intake might be linked to several diseases. Methemoglobinemia, an acute disease affecting especially infants younger than six months, has obtained major attention (Ward et al., 2018). It is caused by NO_2^-, forming from NO_3^-, which can interfere with oxygen transport in the blood and lead to a blue skin colour, lethargy, breathing problems and even coma and death. Lately, there is also evidence that long-term nitrate intake can increase the risk of colorectal cancer and other chronic diseases (Ward et al., 2018).

The World Health Organization has set a drinking water threshold of 50 mg NO_3^- L^{-1} (WHO, 2010), while in Switzerland the threshold is set to 40 mg NO_3^- L^{-1} (GSchV, 1998). Besides drinking water, also some vegetables contain considerable nitrate amounts. However, especially under nitrate concentrations close to the legal limit and assuming an average diet, drinking water is the dominating exposure pathway. Thereby, the drinking water threshold was recently criticized as it only considers the acute risk of methemoglobinemia, but not that of chronic diseases after long-term nitrate intake at levels below this threshold (Schullehner et al., 2018, Ward et al., 2018). For Switzerland, a recent report estimated an average daily nitrate intake with drinking water of 5.1 mg $person^{-1}$, however, values reached a maximum daily intake of 86.4 mg $person^{-1}$ (Rohrmann et al., 2021). These values are slightly higher than

from a similar estimation from Denmark, in which a moderate, but significant causal link between nitrate intake and colorectal cancer was found (Schullehner et al., 2018).

Nitrate leaching also has negative effects on natural ecosystems. As groundwater is closely linked to surface waters, nitrate leaching underneath agricultural land can also affect rivers, lakes and marine ecosystems. Disturbing the natural nutrient balances in these water ecosystems leads to out-competition of plant and animal species adapted to low N availabilities and ultimately causes a loss in biodiversity (Grizzetti et al., 2011). In marine ecosystems, which are usually N-limited, excessive N inputs can increase primary production and lead to eutrophication (Le Moal et al., 2019). Eutrophication can be manifested in detrimental algal blooms, which do not only reduce the amount of light entering the water column, but can also produce toxins and lead to oxygen depletion (Ferreira et al., 2011). Global models estimated that N outflows to the sea doubled within the 20th century (from 34 to 64 Tg N year^{-1}) while the relative contribution of agriculture to this outflow increased from 20 to 50 % (Le Moal et al., 2019). In freshwater, usually P is the limiting nutrient, but NO_2^-, forming from NO_3^-, and NH_4^+ can under some circumstances have direct toxic effects on fish and other water organisms.

Besides negative effects on aquatic ecosystems, NO_3^- transfer to the groundwater might also increase denitrification and with it emissions of N_2O, a potent greenhouse gas. This can happen under anoxic conditions both in the subsoil (Clough et al., 2005) and within the aquifer, but the underlying drivers and transport conditions as well as the magnitude of losses remain difficult to predict (Nikolenko et al., 2021).

1. 4 Animal manure – waste or fertilizer?

1.4.1 Properties of animal manures

Ruminants excrete more than 80 % of their N intake as urine or faeces, while for pigs and poultry the share of excreted N is slightly lower with 60 to 70 % of their N intake (Pagliari et al., 2020, Hou et al., 2016). Within this thesis, I will focus on ruminant manure. About 50 to 60 % of the N excreted by ruminants is in urine, mostly consisting

of urea (Haynes and Williams, 1993), while the rest is contained in the faeces. The quality and especially the crude protein content of the feed are strongly affecting the excretion pattern as well as the N distribution in the animal excreta. With high crude protein contents, usually the ratio of urinary-N to faeces-N increases as well as total N and NH_4^+-contents in the excreta (Broderick, 2003, Marini and Van Amburgh, 2005).

Several types of animal manure can be distinguished. Slurry usually refers to a liquid mixture of mostly urine and some faeces, under most animal housing conditions diluted with water for example from rain or cleaning water at the farm. Farmyard manure is composed of a larger portion of faeces and more bedding material and therefore usually contains less water.

Undiluted dairy slurry contains between 2 and 7 kg N m^{-3} fresh slurry, of which 35 to 80 % are in mineral form (Webb et al., 2013). Farmyard manure has N contents ranging between 2 and 8 kg N t^{-1}, but only 10 to 45 % of it are in mineral form and the dry matter content (16 to 43 %) is much higher than for slurry (1.5 to 12 %). These wide ranges indicate how difficult it is to accurately estimate N contents in animal manure as a farmer, even more so since NH_3 losses in the stable and during storage can be considerable (Richner and Sinaj, 2017). Furthermore, it must be noted that analysing manure is not trivial. There are several rapid tests available (Van Kessel and Reeves, 2000). Even in a laboratory, however, standardized procedures are challenged by the high inhomogeneity of animal manure as well as the risk of NH_3 volatilization upon sample preparation or analysis due to an overall high ammonium content and elevated pH.

1.4.2 Animal manure as a fertilizer – recommendations and challenges

Applying animal manure to the field is the oldest way of fertilizing soil. It is also how the nutrient cycle in natural ecosystems works and a way to close nutrient cycles on crop-livestock farms. Furthermore, under organic farming no synthetic fertilizers are permitted. Therefore, animal manure has a central role as a fertilizer in organic farming. Zavattaro et al. (2017) postulated that 4.3 Mio tons of synthetic N fertilizer

could be replaced by bovine manure in Europe alone. However, as outlined above, specialization of farms and local disconnection of animal husbandry and plant production causes some regions to suffer from excess manure while others do not have enough to fulfil the needs of the plants. Furthermore, efficient use of animal manure as a fertilizer requires an in depth understanding of the N availability of animal manure both to meet the N demand of the crop and to avoid losses to the environment.

There can be considerable emissions of NH_3 already in the stable and upon storage, but also upon field application. These losses were estimated to reach up to 38 % of the N amount excreted by the animals (Oenema et al., 2007). In the field, these emissions can be reduced by improved application techniques such as slurry injection or tail hose application as well as by optimized timing of application (not during hot, windy weather). Ammonia losses occurring before application are considered "unavoidable", thus, fertilizer recommendations are usually only based on the estimated N concentration of stored animal manure.

Recommendations for using animal manure as a fertilizer are quite variable throughout Europe (Webb et al., 2013, Klages et al., 2020). Often only the mineral N share (most of it usually is in the form of NH_4^+) of animal manure is considered "available" (Webb et al., 2013). This assumption neglects that considerable parts of the ammonium can get immobilized, while parts of the organic manure N get mineralized, and both processes occur simultaneously (Sørensen, 2004). Net mineralization as the sum of these two contrasting processes often turns negative in the first weeks after manure application, indicating N immobilization (Sørensen and Amato, 2002). Especially the C:N ratio and/or the recalcitrance of the organic C in the manure drive immobilization and mineralization of N (e.g. Chadwick et al., 2000).

Due to the continued (re-)mineralization of organic N, manure has a residual effect beyond the year of application (Gutser et al., 2005). Also mineral fertilizer has a residual effect due to temporally immobilized N (Sebilo et al., 2013). Despite considerable differences in the N availability in the year of application between different manure and fertilizer types, estimates on the residual effect are very similar,

with mineralization rates of 3 to 6 % of total N in the first and 1 to 2 % of total N in the second year (Smith and Chalk, 2018, Webb et al., 2013). However, these effects sum up and should be considered for fertilization recommendations, especially in the case of large contents of organic N in the manure and/or repeated applications (Schröder et al., 2013). If fertilization recommendations are determined on fields which had received regular animal manure inputs, these residual effects are already indirectly counted in. However, appropriate estimates are still debated as they can only be derived from long-term trials or using modelling.

Several approaches to improve the NUE of animal manure have been developed and tested. Some of them aim at increasing the short-term N availability in manure, e.g. anaerobic digestion or ammonium stripping (Möller and Müller, 2012, Nkoa, 2014, Vaneeckhaute et al., 2017). Separating liquid from solid fractions might ease both transportation, application and predictability of N availability from the separated parts (Bosshard et al., 2010). On the other hand, composting or adding C-rich material such as straw might stabilize N in the manure and turn it into a long-term fertilizer improving soil fertility (Hartz et al., 2000, Nicholson et al., 2017). Furthermore, there a several additives discussed such as nitrification inhibitors, effective microorganisms, biochar or acidification, but their effectiveness is not yet fully proved (Fangueiro et al., 2015, Borchard et al., 2019, Alonso-Ayuso et al., 2016). Anaerobic digestion is getting increasingly common. In most cases, animal manure is co-digested with external substrates such as plant material or household wastes in order to optimize biogas yield (El-Mashad and Zhang, 2010). It is therefore difficult to directly conclude on the effect of digestion of animal manure on its NUE.

1.4.3 Animal manure in Switzerland

In Switzerland, animal manure is the largest N fertilizer source (Spiess and Liebisch, 2020). In 2019, livestock numbers amounted to 1.5 Mio cattle, 1.4 Mio pigs and 11.8 Mio poultry (BLW, 2020). Numbers for cattle and pigs have been decreasing in the last years, whereas they are on the rise for poultry. The Swiss fertilizer guidelines assume 15 to 20 % of N excreted by bovine to get lost already in the stable or upon

storage (Richner and Sinaj, 2017). Of the remainder (total N applied), 50 to 70 % get available for plants during the year of application and the following years. On fields with a long-term manuring history, this rate can be considered as the annually available amount of N. Thus, of the excreted amount of N, about 50 % will get plant available, while the fate of the remainder is not well understood. ^{15}N labelling offers possibilities to study the fate of the remaining N (see **1. 5**).

In order to receive subsidies, farmers are obliged to fulfil the "proof of ecological performance" (PEP). The PEP requires a balanced nutrient budget which has to be documented by calculating a Swiss Balance for each farm. In the Swiss Balance, nutrient demand by crops and grassland is balanced against nutrient inputs via animal manure. Additional fertilizer inputs are only admitted as long as N and P inputs with manure do not exceed the crops` demand by more than 10 %. However, as described above, the Swiss Balance assumes gaseous losses in the stable and upon storage (e.g. via NH_3 emissions) to be "unavoidable" and overall considers only 50 % of the excreted N to become plant available while the other half is left out from the N balance (Bosshard et al., 2012, Agridea and BLW, 2020). The Swiss Balance is currently being debated for these assumptions and revisions will be pursued.

1. 5 Stable isotopes – tracing the fate of N inputs and unravelling hidden N transformation pathways

1.5.1 Stable N isotopes as environmental tracers

Nitrogen comes as two stable isotopes, i.e. non-radioactive atoms of the same element with different numbers of neutrons, resulting in a different atomic weight but otherwise identic characteristics. For N, ^{14}N is the prevailing isotope and accounts for 99.6337 % of all atmospheric N_2. ^{15}N, the heavier isotope, is much less frequent with 0.3663 % of all N atoms. The relative abundance of ^{15}N can be expressed as:

$$atom\%^{15}N = \frac{^{15}N}{^{15}N + {}^{14}N} \times 100 \qquad \textbf{Eq. 1.1}$$

Several N transformation processes discriminate against the heavier ^{15}N, leading to natural differences in the isotopic enrichment (Högberg, 1997). Small differences in the ^{15}N abundance caused by biological and/or chemical discrimination can be used to infer about processes and the source of N molecules, for instance biological N fixation by legumes (Shearer and Kohl, 1986) or sources of leached nitrate in a groundwater aquifer (Kendall, 1998). Natural abundance studies are powerful and inexpensive to identify sources of N and to unravel natural processes (Chalk et al., 2019). However, they are not well suited to trace the fate of a specific input such as animal manure N in the soil-plant system.

By using inputs that are artificially enriched in ^{15}N, it is possible to directly follow the fate of these N inputs even in the light of the large soil N pool and when several processes occur simultaneously. The use of ^{15}N enriched inputs offers a multitude of possibilities, however, they are quite expensive (Bedard-Haughn et al., 2003). Therefore, ^{15}N tracer studies are often restricted to lab scale or to rather small microplots when used under field settings.

Whether using the natural abundance method or the ^{15}N enrichment method, the isotopic abundance of the background has to be taken into account by subtracting it from the ^{15}N abundance in the sample in order to calculate its ^{15}N excess. The ratio between ^{15}N excess in a sample and ^{15}N excess in a fertilizer gives the share of N derived from the labelled fertilizer (Ndff) (Barraclough, 1995). This can be converted into absolute amounts by multiplying the Ndff (%) with the N content of a sample. Dividing Ndff by the N input amounts yields the recovery of fertilizer N in a compartment. Ultimately, from this it is possible to calculate ^{15}N fertilizer balances which were found to be more accurate than calculating balances based on total N inputs and outputs (Cusick et al., 2006, Nannen et al., 2011).

Furthermore, ^{15}N labelling allows for directly measuring the residual effect of fertilizers beyond the year of application. It is also possible to trace the applied fertilizer N through different loss pathways, or to assess its integration into soil aggregates or physical soil organic matter fractions (Bosshard et al., 2008, Fuchs et

al., in prep). This requires quite a high ^{15}N enrichment of the originally applied fertilizer.

An important prerequisite for using ^{15}N as a quantitative tracer is that the N input is homogenously labelled (Hauck and Bremner, 1976). This is specifically challenging for organic fertilizers, such as animal manure.

1.5.2 ^{15}N labelling of animal manure – a delicate matter

Using ^{15}N labelling to trace the fate of N from animal manure in the soil-plant-system is demanding as animal manure consists of numerous N-bearing molecules and compounds with differing degrees of recalcitrance (Dittert et al., 1998, Chalk et al., 2020). Animal manure can be mixed with a small amount of highly enriched ^{15}N ammonium or urea, labelling only the ammonium N pool of the manure (e.g. Jensen et al., 2000). Alternatively, ^{15}N labelled urea can be mixed into the feed of the animal. However, both approaches will not label the undigested fibrous feed N (Hoekstra et al., 2011, Powell et al., 2004). Achieving labelling in all fractions is only possible by feeding fully ^{15}N enriched organic feed, such as ^{15}N enriched hay, silage or grains (e.g. Sørensen and Thomsen, 2005, Powell et al., 2005). These feeds can be produced by fertilizing plants with ^{15}N enriched mineral fertilizer. This makes the whole process very complex and costly, but allows to also follow the fate of the more recalcitrant organic N forms contained in faeces. Overall, the preferred method also depends on the research question and the study duration (Powell et al., 2004, Powell et al., 2005).

Besides differing feeding approaches, also the duration of both feeding and collecting excreta is important. Usually, the ^{15}N label appears first in urine and only with a time lag in faeces (e.g. Powell and Wu, 1999, Bosshard et al., 2011, Barros et al., 2017). Reported feeding durations range from pulse feeding over a few hours (e.g. Langmeier et al., 2002) up to several weeks (e.g. Jensen et al., 1999), often after a prior adaptation phase with non-labelled feed of similar composition and quality. Thereby, urine and faeces, and also different faeces N fractions were shown to differ in their ^{15}N labelling. Several studies differentiated undigested dietary N (UDN or NDF-N), which is mainly fibrous parts of feed N, water-soluble N (WSN), and bacterial and endogenous debris

N (BEDN), which consists of rumen abrasions and bacteria from the digestion system of the animal (Langmeier et al., 2002, Bosshard et al., 2011). The ^{15}N labelling in these fractions varies mainly due to dilution with unlabelled N from previous unlabelled feed N still in the digestive system of the animal or due to internal turnover of N within the animal, and follows distinct temporal patterns (Powell et al., 2004). The fractions also differ in their potential plant availability with a decreasing recalcitrance from UDN > BEDN > WSN (Hoekstra et al., 2011).

To circumvent the problem of differing labels in faeces and urine, several studies applied a cross-labelling approach with either ^{15}N labelled urine or ^{15}N labelled faeces, often mixed with the respective non-labelled counterpart (Bosshard et al., 2009, Hoekstra et al., 2011, Thomsen et al., 1997, Jensen et al., 1999). However, this approach adds further treatments to the experimental design and cannot solve the problem of non-homogeneous labelling of faeces fractions.

On the other hand, when the feeding duration with ^{15}N labelled feed was long enough (ideally at least several days), a slurry mixed from urine and faeces portions close to peak ^{15}N enrichments had a sufficiently similar label in the urine and the different faeces fractions to allow for quantitative assessments (Sørensen and Jensen, 1998, Powell and Wu, 1999). However, to assure validity of the approach, homogeneity of labelling should be assessed (Chalk et al., 2020). This could be done by assessing the deviation in labelling between the different faeces fractions (e.g. Langmeier et al., 2002, Bosshard et al., 2011). Another possibility is the assessment of the temporal development of the ^{15}N label in mineralized faeces N in soil incubations (Sørensen et al., 1994, Bosshard et al., 2011) (also see **SI 1.2**).

1. 6 Nitrate leaching in Switzerland and the *Gäu* region

1.6.1 Groundwater nitrate levels and N surplus in Switzerland – current status and abatement measures

Averaged over the whole country, 15 to 20 % of the groundwater measuring points currently exceed the Swiss quality criterion of 25 mg NO_3^- L^{-1} (BAFU, 2019b). Under arable land, the quality criterion is exceeded at 40 % of the measuring points, of which

a fourth even exceed the drinking water threshold of 40 mg NO_3^- L^{-1}. Thereby, nitrate leaching losses are exacerbated by high annual precipitation (> 1`000 mm $year^{-1}$).

While ultimately local field conditions are decisive for nitrate leaching losses, national N balances can give indications for major N flows and identify drivers for potential (im-)balances on a larger scale. The Swiss farm gate N balance, considering all N inputs and outputs from the agricultural sector, shows a continuously high surplus (Spiess, 2011). In 2018, the average surplus amounted to 93 kg N ha^{-1} of agricultural land (Spiess and Liebisch, 2020). A nutrient surplus can indicate both losses and changes in soil N stocks. However, most N surplus must be considered as losses to the environment as changes in soil N stocks are usually only small (Spiess and Liebisch, 2020).

At groundwater wells exceeding the quality criterion of 25 mg NO_3^- L^{-1}, measures must be taken to reduce nitrate contamination. According to article 62a of the Swiss water protection law, local abatement programs can be financially supported (GSchV, 1998, Abs. 62a). These programs are termed nitrate projects, and the currently biggest one in size is located in the Gäu region, Canton Solothurn.

1.6.2 The Gäu region and the research project *NitroGäu*

The Gäu region, located at the Swiss Central Plateau between Olten and Oensingen, is intensively used for agricultural production including both arable farming and vegetable production. Silage maize, winter cereals, canola and grass-clover are the main crops in arable farming. The region is underlain by a sizable groundwater aquifer of 33 km^2 (**Fig. 1.3**). The groundwater table is at a depth of 30 m in the central and western part of the aquifer, while depth is decreasing towards the east, where the groundwater table is at about 6 to 10 m depth (AFU, 2015). Most of the drinking water in this region (88 %) is taken from this aquifer. The groundwater in the Gäu region is known to be specifically vulnerable to nitrate leaching, not only due to agricultural production, but also due to low dilution with water from non-agricultural areas (Gerber et al., 2018). Most groundwater wells in the Gäu region are

characterized by nitrate levels exceeding the Swiss quality criterion of 25 mg NO_3^- L^{-1}, despite abatement measures within the local nitrate project ("Gäu-Olten") being in place since 2000. These measures include regulations on winter cover crops, crop rotations, sowing date, soil tillage as well as partial transformation of agricultural land into extensive grassland (Vetsch, 2000), all of which are part of voluntary contracts with the farmers who get compensation payments for taking these measures. Despite these efforts, nitrate levels in the groundwater in the Gäu region did not decrease, but at least they could be stabilized. The project was recently prolonged for another six years (until 2026) and currently involves an area of almost 1400 ha agricultural land.

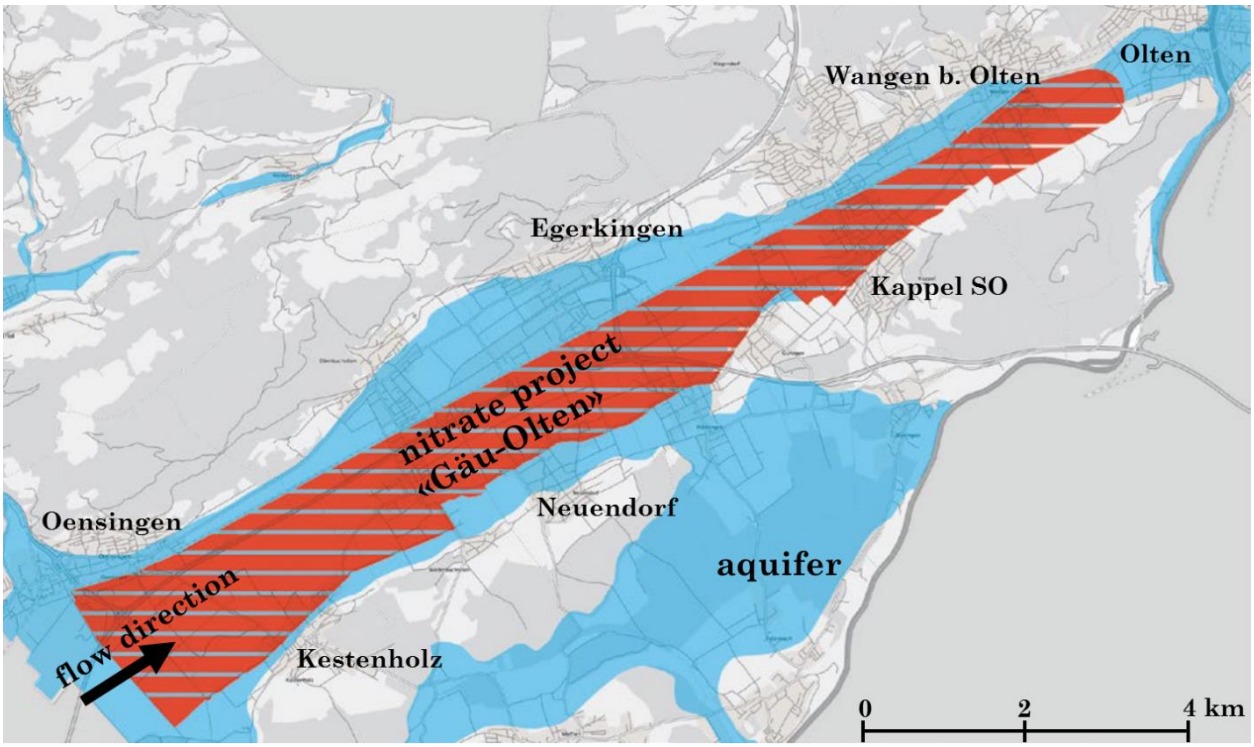

Fig. 1.3*: Groundwater aquifer (blue) and area of the local nitrate project "Gäu-Olten" (red) (adapted after AFU, 2015)*

It is unclear whether the undertaken abatement measures were not effective enough or whether their effect was just not yet visible due to the long lag time in the aquifer, i.e. it takes 6 to 22 years for the precipitation water to reach a groundwater well (Gerber et al., 2018, Hunkeler et al., 2015). Furthermore, the effect of fertilization – including both amount, type and timing – had not been considered in the former

project phases. Fertilization, however, was estimated to be a key component in nitrate leaching management according to the EU Nitrate Directive, which is the nitrate leaching abatement strategy of the European Union (Velthof et al., 2014).

The project *NitroGäu* is a scientific project aiming at 1) assessing the effectiveness of the already introduced measures to reduce nitrate leaching in the nitrate project "Gäu-Olten" and 2) testing and proposing additional abatement measures including aspects concerning fertilization. The project was running between 2017 and 2021 and addressed both arable and vegetable farming. It involved partners from universities, research institutes, authorities, extension agencies and private companies as well as local farmers. This thesis was part of the arable farming section of the *NitroGäu* project and focused on animal manure and its contribution to nitrate leaching.

1. 7 Thesis outline and hypotheses

Using ^{15}N labelling, I aimed at studying nitrate leaching losses and the NUE of cattle slurry under local field conditions. I did this by setting up an on-farm field study in the Gäu region in which I traced N from cattle slurry or mineral fertilizer in the soil-plant system as well as nitrate leaching losses from these fertilizers. Furthermore, I investigated whether NUE of cattle slurry could be increased by anaerobic digestion, biochar or a nitrification inhibitor. The work was split into three experimental chapters (**Chapter 2** to **Chapter 4**), which are briefly introduced here:

In **Chapter 2**, the production and handling of ^{15}N labelled cattle slurry is described. Thereby, the uniform distribution of the ^{15}N label among the different manure fractions – which is an important prerequisite for using ^{15}N as quantitative tracer – was investigated via separating and analysing different manure fractions. A microplot field study on two neighbouring fields was established in order to investigate the differential fate of mineral fertilizer and cattle slurry in the soil-plant system within the year of application under on-farm conditions. Currently, there is a lack of on-farm data for these processes. However, such data is needed as crop NUE tends to be overestimated at research stations (Ladha et al., 2005, Cassman et al., 2002). I expected N availability from cattle slurry during the year of application to be

equal to its NH_4^+ content assuming that mineralization of organic N in slurry would compensate for higher immobilization of slurry mineral N. Thus, I presumed that N derived from fertilizer in plants would be the same for slurry and for mineral fertilizer when the applied dose of mineral N is similar. To this end, I analysed plant N uptake and the recovery of the ^{15}N labelled fertilizers in the soil mineral, microbial and non-microbial organic N pools. In addition, I assessed N losses from the fertilizers via NH_3 volatilization.

Chapter 3 presents the continuation of the field study. To date, actual nitrate leaching losses from mineral fertilizer and cattle slurry have barely been comparatively measured. This chapter aimed at closing this research gap. I hypothesized that cattle slurry has an elevated leaching potential over mineral fertilizer due to a lower recovery in crops and a greater recovery of slurry N in soil. At the same time, I expected a higher residual fertilizer NUE for slurry than for mineral fertilizer. To this end, I assessed nitrate leaching under the microplots using passive samplers containing an ion exchange resin. Tracing the fate of ^{15}N labelled fertilizers in plant uptake over a sequence of three crops allowed to directly quantify the residual fertilizer effect beyond the year of application. Combining ^{15}N recovery in soil, plant uptake and nitrate leaching over 2.5 years I established a full ^{15}N balance and provided valuable information on the fate and turnover rates of mineral fertilizer and cattle slurry under field conditions.

Chapter 4 aimed at assessing the potential of manipulating cattle slurry for increasing its NUE. Under greenhouse conditions, I conducted a microcosm study in which I investigated the effect of anaerobic digestion, biochar addition and the nitrification inhibitor 3,4-dimethyl-1H-pyrazole monophosphate (DMPP) on ^{15}N recovery by ryegrass (*Lolium multiflorum*), soil N transformation processes and the amount of residual N that could be leached after 57 days. Previously, the isolated effect of anaerobic digestion on NUE of cattle slurry has barely been studied as in most studies cattle slurry was co-digested with other feedstock. I tested the hypothesis that increasing NUE, as indicated by higher ^{15}N labelled fertilizer recovery

in plant biomass, would reduce nitrate leaching potential. Furthermore, I provide insights into the potential interaction of the proposed strategies in terms of soil N transformation dynamics.

Finally, in **Chapter 5** the results from the previous chapters are jointly discussed and set into a broader context, aiming to derive recommendations for resource efficient and environmentally friendly fertilization schemes with animal manure. Furthermore, directions and needs for further research are identified.

Chapter 2

Similar distribution of ^{15}N labelled cattle slurry and mineral fertilizer in soil N pools one year after application

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Abstract

Targeted use of animal manures as a nitrogen (N) fertilizer is challenging because of their yet poorly predictable N fertilizer value. An in depth understanding of their N transformation processes in soil under field conditions is necessary to better synchronize N availability and crop N demand. We produced ^{15}N labelled cattle slurry by feeding a heifer with ^{15}N labelled ryegrass hay and used this slurry in an on-farm trial on two neighbouring fields, cropped with maize or grass-clover, in order to assess crop N uptake and N dynamics in the topsoil. Recovery of applied total N in plant biomass was higher for mineral fertilizer (Min) (45 – 48 %) than for slurry (Slu) (17 – 22 %) when applied at the same rate of mineral N. Also N derived from fertilizer in plant biomass was higher for Min than for Slu, due to greater NH_3 emissions from Slu than from Min and greater initial immobilization of slurry N, as indicated by higher fertilizer recoveries in soil microbial N for Slu than for Min. Despite initial differences between Min and Slu regarding the relative distribution of residual fertilizer N in soil N pools, already in the next spring the major share (77 – 89 %) of residual N from both Min and Slu was found in the non-microbial organic N pool. At this time, residual fertilizer N in soil was 18 to 26 % for Min and 32 to 52 % for Slu relative to the applied amounts of total N. Thus, fertilizer N not taken up by the first crop after application will enter the soil organic N pool and has to be re-mineralized before becoming plant available.

Keywords: ^{15}N labelling, on-farm trial, farmer`s practice, N use efficiency, soil N pools

2.1 Introduction

Animal manures such as cattle slurry are widely used as a multi-nutrient fertilizer. However, their targeted use as a nitrogen (N) fertilizer is difficult due to the variable composition of animal manures. A high proportion of total N in animal manures is present as organic N compounds (Pagliari et al., 2020) which have to be mineralized before becoming plant available. Another part of total N in manure present as ammonium is available for uptake by plants, but it is also prone to NH_3 volatilization. In soil, ammonium rapidly gets immobilized by microbes or can be nitrified. If not taken up by plants, there is a high potential for losses via nitrate leaching, especially under high rainfall, and gaseous loss via N_2O or N_2 (Gutser and Dosch, 1996). These losses cause adverse effects on the environment (Erisman et al., 2013, Galloway et al., 2003) and on human health (Ward et al., 2018). At the same time, bovine manure in Europe could replace about 4.3 million tons of mineral fertilizer N (Zavattaro et al., 2017), but to this end, an improved understanding of N transformation processes in soil and dynamics in relation to plant N need under field conditions is necessary.

Under current farming practice in industrialized countries, fertilizer N use efficiency (NUE) of both mineral fertilizer and animal manure is low, with N recoveries in crops in the year of fertilizer application averaging 42 ± 13 % for mineral fertilizer and 26 ± 10 % for animal manure (Smith and Chalk, 2018). This leaves a large share of fertilizer N in the soil, with a consistently low residual fertilizer value in the following years. At the same time, plants take up more than half of their N demand from sources other than current year fertilizer, with major shares assumed to originate from soil organic N mineralization (Yan et al., 2020). It must be noted that NUE tends to be overestimated at research stations and is not necessarily representative for achievable NUE values on farmer`s fields (Ladha et al., 2005, Cassman et al., 2002). Besides a scale effect as researchers` fields commonly are small, this is caused by overly attentive management, often over several years, which is not feasible on the large scale. Therefore, on-farm data obtained from farmers` fields is needed.

NUE of organic fertilizers can be assessed by several means: While non-isotopic approaches such as input-output balances or comparing total N uptake to non-fertilized or synthetically fertilized treatments are relatively easy and cheap to implement, only labelling with the stable isotope ^{15}N allows direct tracing of the fertilizer in the soil-plant system, including direct measurement of its residual effect (Chalk et al., 2020, Dittert et al., 1998). Thereby, ^{15}N labelling represents a sensitive method for assessing fertilizer NUE, but it requires that N in the tested fertilizer is homogeneously labelled (Hauck and Bremner, 1976).

Homogeneous ^{15}N labelling of both inorganic and organic N compounds in animal manures is difficult to achieve (e.g. Bosshard et al., 2011, Chalk et al., 2020, Hoekstra et al., 2011). Therefore, several studies used a cross-labelling approach with either ^{15}N labelled urine or ^{15}N labelled faeces obtained from feeding a ruminant with ^{15}N labelled forages to investigate the N dynamics of animal manure in the soil-plant system (e.g. Hoekstra et al., 2011, Bosshard et al., 2009). Alternatively, Powell and Wu (1999) suggested that feeding ruminants with ^{15}N labelled feed over several days and mixing of excreta portions around peak ^{15}N enrichment would lead to a ^{15}N signature in the slurry that can be used for quantitative tracing of slurry N. In this study, we mixed ^{15}N labelled urine and ^{15}N labelled faeces and estimated the variation in the results due to inhomogeneous labelling by characterizing the ^{15}N distribution in different slurry N fractions (Langmeier et al., 2002, Bosshard et al., 2011).

Tracing N from animal manure into different soil N pools could facilitate the prediction of its NUE, however, field data is scarce. Adding ^{15}N enriched $(NH_4)_2SO_4$ to slurry, Sørensen (2004) and Jensen et al. (2000) found a rapid decline in recovery in both microbial N (Nmic) and mineral N (Nmin) and an increasing share of N recovered in non-microbial organic N (Norg) under field conditions. However, by their labelling approach, only the mineral N but not the organic N in the slurry could be traced. Wachendorf and Joergensen (2011) used urine and faeces from a cow fed with ^{15}N labelled feed to investigate N recovery in soil Nmic under urine or faeces patches. After 27 weeks, they still found high recoveries in Nmic (7 to 21 %), however, N

dynamics under their high application rates (> 1000 kg N ha^{-1}) are likely different from N dynamics under common application rates in arable farming. Others only looked into ^{15}N recovery in Nmin, but not Nmic (Bosshard et al., 2009), or did not provide a direct comparison with mineral fertilizer (Hoekstra et al., 2011). This leaves a knowledge gap regarding the distribution of ^{15}N labelled cattle slurry, produced from mixing ^{15}N labelled urine and faeces, into different soil N pools in comparison with ^{15}N labelled mineral fertilizer under arable field conditions. Furthermore, to our knowledge, none of the previous studies looked into the effect of repeated field applications of labelled fertilizers on the ^{15}N recovery in Nmic and Nmin. However, repeated application of slurry is a common agricultural practice.

We applied ^{15}N labelled cattle slurry under on-farm conditions in order to gain a more realistic view on NUE of cattle slurry under field conditions in Switzerland. While we acknowledge that a microplot design (see 2.1) to some extend contradicts the on-farm setting, placing our experiment on fields managed by a farmer still allows more representative results than experiments on research stations. The Gäu region, where this study was conducted, is located at the Swiss Central Plateau and characterized by intensive agricultural production of vegetables and arable crops. Common arable rotations of mixed crop-livestock farms include silage maize, winter cereals, canola, and grass-clover leys. The groundwater of this region is specifically vulnerable to nitrate leaching, not only due to intensive agricultural production, but also because it is barely diluted with water from non-agricultural land (Gerber et al., 2018). In the Gäu region, thus, ways are searched to improve NUE of both organic and inorganic fertilizers in order to reduce nitrate leaching. We chose silage maize and grass-clover as model crops since these crops are most commonly fertilized with animal manure. Following the official Swiss fertilization recommendations (Richner and Sinaj, 2017), we assumed the NH_4-share of the slurry as proxy for its N *availability* and established a ^{15}N mineral fertilizer treatment with the same rate of mineral N for comparison. Still, plant *available N* in manure comprises not only mineral N, but also organic monomers and easily mineralizable organic N, and it will also depend on N losses (Webb et al., 2011).

Overall, this study aimed at a) assessing NUE of ^{15}N labelled cattle slurry in comparison to ^{15}N labelled synthetic mineral fertilizer in an on-farm field trial under recommended agricultural practice conditions, and b) investigating their N dynamics in topsoil after application of cattle slurry or mineral fertilizer during the season of application. We hypothesized that excreta portions of a heifer fed with ^{15}N labelled hay would not have the same ^{15}N enrichment, but that the ^{15}N signature of a slurry mixed from the portions close to peak ^{15}N enrichment could be used as a source signature for quantitative assessment of its N dynamics in the soil-plant-system. Furthermore, we expected N availability from cattle slurry during the year of application to be equal to its NH_4-content. Thereby, we presumed that mineralization of organic N in slurry would compensate for higher immobilization of slurry mineral N, and that N derived from fertilizer in plants would be the same for slurry and for mineral fertilizer when the applied dose of mineral N is similar. Finally, we assumed fertilizer N recovery in crop biomass to be higher for mineral fertilizer than for slurry, but to be equal for both fertilizers when considering the whole plant-soil system.

2.2 Material and Methods

2.2.1 Field site and experimental design

The field experiment was conducted as an on-farm trial on two neighbouring fields in the Canton Solothurn, Switzerland, between May 2018 and February 2019 (Field A) or May 2019 (Field B). While Field A was cropped with silage maize followed by winter wheat during 2018, Field B was cropped with grass-clover (**Fig. 2.1**). Both fields had been cultivated with sown grass-clover for at least three years before the start of the experiment, and have regularly received animal manure according to common agricultural practice. Fields differed slightly in bulk density and texture, but were overall comparable in basic soil properties in the uppermost 0.15 m (**Table 2.1**). Climatic conditions at the field site are temperate, with a mean annual temperature of 9.0 °C and a yearly precipitation of 1129 mm (1981 – 2010). However, weather conditions in 2018 were exceptionally hot and dry, especially during the summer, with temperatures between April and September about 2.4 °C above average and about

30 % less precipitation (BAFU, 2019a) (**Fig. 2.2**). Overall, potential evapotranspiration strongly exceeded precipitation, with a Standardized Precipitation Evapotranspiration Index (SPEI) for 2018 of < -2 (MeteoSchweiz, 2018).

Three fertilizer treatments were implemented: ^{15}N labelled mineral fertilizer as $^{15}NH_4{}^{15}NO_3$ (Min, 8.00 atom% ^{15}N abundance), ^{15}N labelled cattle slurry (Slu, 7.89 atom% ^{15}N abundance), and a control (Con) treatment not receiving any ^{15}N labelled fertilizers. Each fertilizer treatment was replicated four times, resulting in 12 microplots per field. On both fields, microplots were arranged in a complete randomized block design on a 3 m wide strip, 9 m apart from the fields' edges (**SI 2 Fig. 1**), allowing the farmer to leave out the strip from fertilization or soil tillage performed on the surrounding field. According to the design proposed by Jokela and Randall (1987), non-confined microplots had a size of 1.5 m x 2 m and were located in a way that two maize rows formed the edges of each microplot and one maize row formed the centreline of the plot (0.75 m row spacing) (**SI 2 Fig. 2**). Although Field B was not cropped with maize in 2018, the same dimensions for the microplots were used.

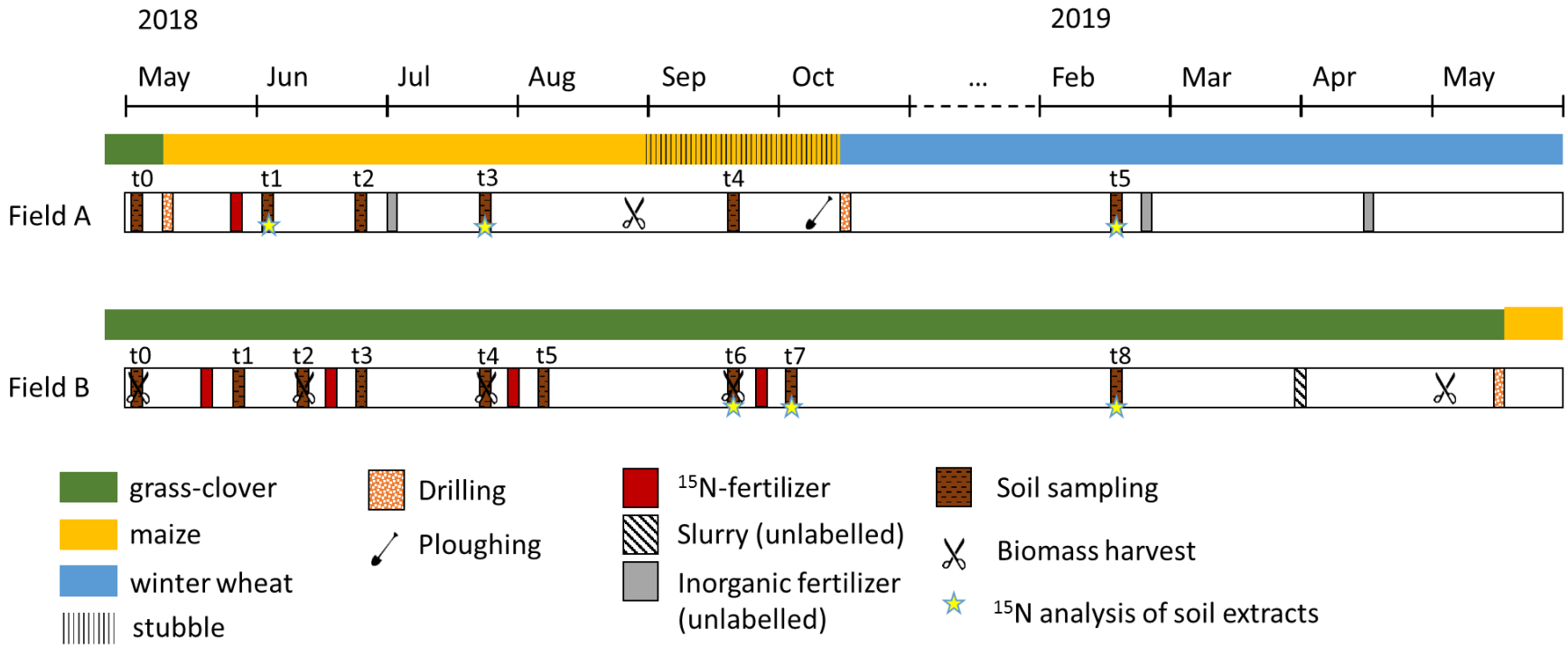

Fig. 2.1: *Overview of the experimental timescale, including crop rotation, cultivation measures, fertilization, and sampling on Field A (upper panel) and Field B (lower panel).*

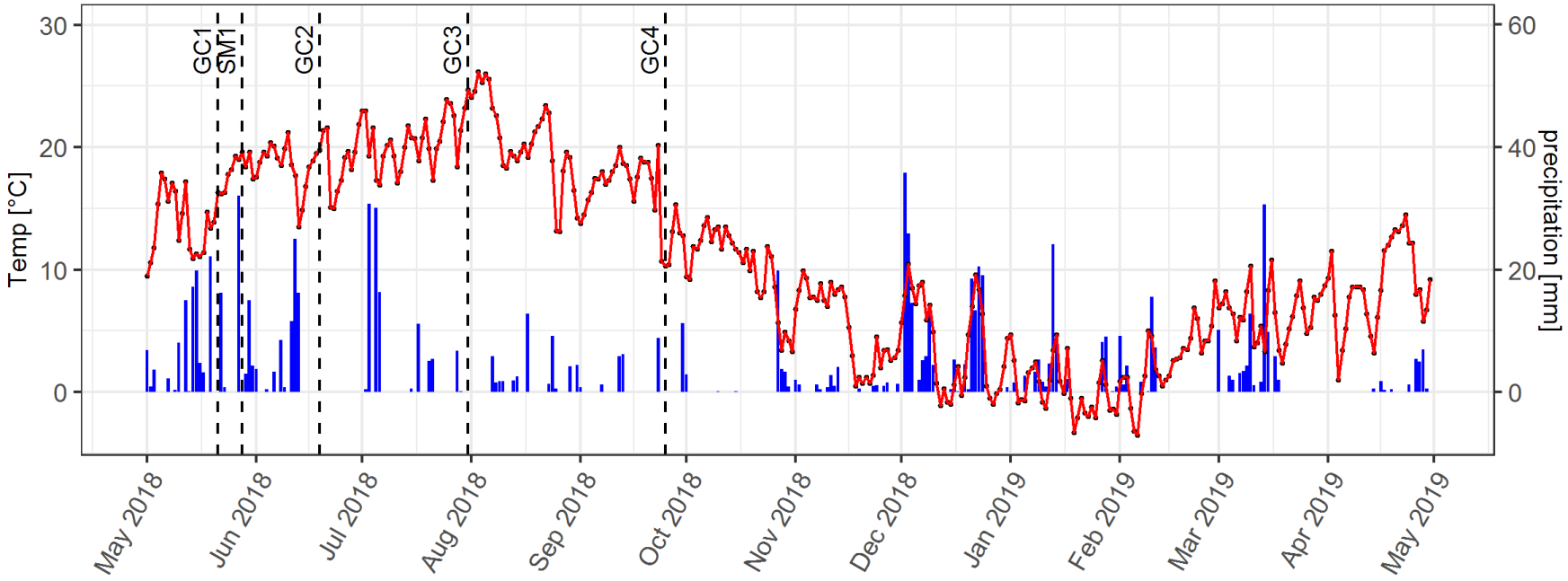

Fig. 2.2: *Daily average temperature and daily precipitation at Wynau (closest meteorological station, 422m a.s.l., 47.255025 / 7.787475) during the timeframe of the experiment. Vertical dashed lines indicate time points for application of ^{15}N labelled fertilizers. (GC = fertilizer application to grass-clover at Field B, SM = fertilizer application to silage maize at Field A)*

Table 2.1: Topsoil properties at the two field sites (0 – 0.3 m) (mean ± standard deviation)

Field	Bulk density[1]	pH ($CaCl_2$, 1:2.5)	C_{org}	Total N	Clay	Silt	Sand
	[t m^{-3}]	[-]	[g kg^{-1} DM]		[%]	[%]	[%]
A	1.34 ± 0.08	5.5 ± 0.2	17.3 ± 0.4	1.9 ± 0.1	22.0 ± 0.8	35.8 ± 1.2	39.6 ± 1.5
B	1.41 ± 0.06	5.7 ± 0.2	17.8 ± 0.6	2.1 ± 0.3	21.6 ± 1.0	42.5 ± 1.5	32.8 ± 0.6

[1]bulk density (0 – 0.15 m) was determined in each microplot with cylinders in 0.05 m increments (0 – 0.05 m, 0.05 – 0.1 m and 0.1 – 0.15 m); for calculations, mean over all microplots per field, as reported here, was used

2.2.2 Production of ^{15}N labelled ryegrass hay and ^{15}N labelled cattle slurry

^{15}N labelled cattle slurry was produced by feeding a female heifer (240 kg live weight) with ^{15}N labelled ryegrass (*Lolium multiflorum* var. Westerwoldicum) hay, partly produced under greenhouse and partly under field conditions. In the greenhouse, ryegrass was grown in container boxes filled with a sand-perlite mix and fertilized with $^{15}NH_4{}^{15}NO_3$ (19.4 atom% abundance). After 32, 53, 74, and 99 days, aboveground biomass was harvested with scissors and dried at 40 °C. In the field, a pure ryegrass stand was fertilized twice with $^{15}NH_4{}^{15}NO_3$ (35 atom% abundance) at a total rate of 40 kg N ha^{-1} in September 2017, cut once in October 2017, and dried at 30 – 40 °C. Hay from the greenhouse and field were mixed for the final feed with an average ^{15}N abundance of 12.6 atom%. The hay contained 167 g kg^{-1} DM crude protein, 231 g kg^{-1} DM crude fibre, and 114 g kg^{-1} DM crude ash.

The heifer was fed with the ^{15}N labelled ryegrass hay for eight days. In a preceding adaptation phase of seven days, feed consisted of non-labelled ryegrass hay, produced under the same conditions as the labelled hay (i.e. also a mixture of greenhouse and field material mixed at the same proportions) in order to allow for the animal to adapt to the feed (Sørensen et al., 1994, Bosshard et al., 2011). Also in the three days after feeding with ^{15}N labelled hay, feed consisted of the same non-labelled ryegrass hay as in the adaptation phase. Feed was offered to the animal three times per day at a daily ratio of 5.6 kg dry weight. From Day 8 (start of feeding with ^{15}N labelled hay) until the end of the feeding period, faeces and urine were collected quantitatively and separately. For this, a urinal was attached to the hindquarter of the animal from

which urine could drain through a tube into a container (Langmeier et al., 2002, Hoekstra et al., 2011). Faeces were collected directly from the rubber mat on which the heifer was bedded. Both portions were frozen in 24h-intervals at -20 °C until later use, which was shown to preserve N forms (Van Kessel et al., 1999). This experiment was approved by the Cantonal Veterinary Office Zurich, Switzerland (licence ZH195/17).

In order to identify the portions with the highest ^{15}N enrichment, subsamples of the individual faeces portions from each feeding day were freeze-dried, pulverized with a ball mill and analysed for ^{15}N (see 2.2.7). Complete faeces and urine portions with the highest N enrichment were then mixed and diluted 1:1 (w/v) with demineralized water, since cattle slurry under common husbandry conditions also usually gets diluted with water at about this ratio. For ^{15}N analysis of the final slurry, a subsample was acidified with concentrated H_2SO_4 prior to freeze-drying in order to minimize N losses upon sample preparation.

2.2.3 Fertilizer application and microplot management

In 2018, ^{15}N labelled fertilizers were applied once to maize on Field A at the three to four leaf stage, and four times (once after each cut of the grass-clover) on Field B (**Fig. 2.1**). ^{15}N slurry was further diluted with demineralized water upon application in order to apply slurry at volume and N content representative for commonly used cattle slurry in Switzerland: Thus, slurry was applied at a rate of 30 m^3 ha^{-1} containing 60 kg N ha^{-1}, equivalent to 36.8 kg NH_4-N ha^{-1}, while the N rate of the mineral fertilizer treatment was equal to the NH_4-N content of the slurry (i.e. 36.8 kg N ha^{-1}). Slurry was applied on the microplot surface using canisters, imitating drag hose application. For the Min treatment, $^{15}NH_4{}^{15}NO_3$ was dissolved in demineralized water and the same volume was applied as for slurry and in the same way. Thereafter, canisters were rinsed with 3 L demineralized water and the rinsing water was evenly applied to the plot surface. On the control plots, the same amount of water as applied with slurry or mineral fertilizer was distributed. To compensate for potassium (K) and phosphorus (P) applied with the cattle slurry, Min and Con plots were additionally

fertilized with 75 kg ha^{-1} K (as potassium sulphate, KaliSop) and 6.7 kg ha^{-1} P (as triple super phosphate) per fertilizer application. Due to hot and dry weather conditions during summer 2018 (**Fig. 2.2**), fertilizer application was mostly performed in the evenings to avoid excessive N losses due to NH_3 volatilization.

Under conventional farming, it is common practice to apply slurry only during an early growth stage of the maize, but using mineral fertilizer for the second fertilizer application. As we aimed to investigate the fate of N from slurry under conditions representative for agricultural practice, five weeks after the "experimental" N dose, non-labelled urea (69 kg N ha^{-1}) was applied to all microplots at Field A. For grass-clover (Field B), farmers usually apply slurry after each cut, resulting in four to five applications per year. Thus, we performed four repeated ^{15}N labelled fertilizer applications always to the same microplots throughout the year. Since in an ongoing experiment, the residual fertilizer value of ^{15}N labelled fertilizers applied during 2018 were to be assessed, in spring 2019, all microplots at Field B received unlabelled cattle slurry by the farmer (95 kg N ha^{-1}).

Herbicide treatment and trichogramma release in silage maize (Field A) were performed by the farmer for the whole field including microplots according to common agricultural practice. Cultivation measures that involved soil movement such as ploughing after harvesting the maize, were conducted manually on the microplots.

2.2.4 Measurement of ammonia volatilization

NH_3 emissions from the microplots were measured according to the Standard Comparison Method (SCM) described by Vandré and Kaupenjohann (1998). In short, passive samplers filled with 20 mL 0.05 M sulfuric acid were installed at about 0.1 m above the soil surface (Field A) or above the recently cut grass-clover canopy (Field B). The acid was exchanged regularly during a 60 hour period after application of the fertilizers and analysed colorimetrically for its ammonium concentration (see 2.2.7). Two reference outgassing systems, emitting NH_3 at known rates, were used for calculating a transfer factor and calibrating the measured ammonium concentration in the solution. Additionally, meteorological measurements (wind speed and direction

at 2 m and 4 m height, air temperature and relative humidity) were recorded. Details can be found in **SI 2.2**.

2.2.5 Biomass and soil sample collection and preparation

Aboveground biomass was harvested from the central area of the microplots, at least 0.375 m away from the plot edges (Jokela and Randall, 1987). Maize plants at Field A were harvested upon maturity, about 0.1 m above ground. Only the centre row on a length of 1.25 m (i.e. 0.375 m away from the plots edge) (**SI 2 Fig. 2**, Sample 5) was used for ^{15}N analysis. The number of maize plants from the central row was counted and the plants were split into stems, leaves, grain, and husk + cobs, dried at 60 °C and weighed. Additionally, the two rows forming the edges of the microplot (**SI 2 Fig. 2**, Samples 3 & 4) and the two adjacent rows outside the microplot (**SI 2 Fig. 2**, Samples 1 & 2) were harvested and fresh weight as well as the number of plants were determined directly in the field. Samples 3 and 4 (edge rows of the microplot) were used for getting a more representative estimate of the dry matter yield compared to only determining the yield based on the central row. A subsample of three plants was taken from each of these rows, processed as Sample 5 and used to check for potential dilution of the ^{15}N label by unlabelled N from outside the microplot. Samples 1 and 2 (outside the microplot) did not have any enrichment in ^{15}N over the control. This indicates that the ^{15}N values from the central row, having the same distance from the plot edge than the outside row, can be considered undiluted from the outside and thus representative for N uptake solely from the area on which ^{15}N labelled fertilizers were applied (see **SI 2 Fig. 2** for illustration of the sampling scheme).

For Field B (grass-clover), aboveground biomass was harvested four times throughout the season with electric scissors from a 0.5 m x 0.5 m frame placed in the middle of each microplot ("inner frame") (**SI 2 Fig. 3**). Biomass was sorted into grass, legumes and other herbs, dried at 40 °C, and the individual botanical groups were weighed before and after drying. To get a representative estimate of the yield, the harvesting area was increased to the whole central area of the microplot (1.25 m x 0.75 m, "outer

frame") and total dry matter yield determined. It was assumed that the relative share of grass, legumes and herbs in the outer frame was the same as in the inner frame.

Dried biomass samples of both maize and grass-clover were homogenized in a cutting mill, and a subsample was pulverized in a ball mill (MM200 Retsch, Haan, Germany) for later analysis of N content and ^{15}N enrichment.

Soil was sampled once before fertilizer application on both fields (t0) and, thereafter, five more times at Field A, and eight more times at Field B, due to repeated fertilizer application (**Fig. 2.1**). Samples were taken as mixed samples from eight cores (0.02 m diameter) per plot to a depth of 0.15 m, with a distance of at least 0.15 m and at maximum 0.375 m from the edge of the microplot, in order to not disturb the central part of the microplot (**SI 2 Fig. 2** and **SI 2 Fig. 3**). Samples were stored in cooling boxes on the field and at 4 °C after reaching the lab. Within 24 hours, soil was sieved at 5 mm and extracted by the chloroform fumigation method in order to determine microbial N (Nmic) (Brookes et al., 1985, Vance et al., 1987). In short, from each sample, two subsamples of 20 g dry weight equivalent each were weighed. One subsample was immediately extracted with 80 mL 0.5 M K_2SO_4, while the other subsample was fumigated with chloroform for 20 to 24 hours and extracted thereafter. Extracts were filtered through folded paper filters (Macherey Nagel Type 615, Ø 185 mm) and stored at -20 °C until analysis on the TOC/TNb-analyser (see 2.2.7). The non-fumigated extracts were analysed for both total dissolved N and mineral N (Nmin) (see 2.2.7). Upon each extraction, a reference soil sample (stored at 4 °C) was included in triplicate and extracted the same way in order to correct for deviations between extraction series.

^{15}N enrichment in different soil N pools was assessed for Sampling T1, T3, and T5 (Field A) and for Sampling T6, T7, and T8 (Field B) (**Fig. 2.1**). For analysis of ^{15}N Nmic, both fumigated and non-fumigated extracts were oxidized by autoclaving extracts with $K_2S_2O_8$ (Cabrera and Beare, 1993) and total N afterwards diffused on acidified filter traps (Whatman QM/A) by adding Devarda's alloy (0.4 g per sample), 4 mL 5 M NaCl, and 0.75 mL 5 M NaOH per 10 mL of extract (Goerges and Dittert,

1998, Mayer et al., 2003). Sample volume was adjusted to contain 25 μg N. Ammonium and nitrate contained in non-fumigated extracts were diffused in order to determine ^{15}N Nmin following a similar procedure on non-oxidized extracts, but by adding only 0.2 g MgO and 0.4 g Devarda`s alloy.

2.2.6 Slurry fractionation for testing homogeneity of slurry label

The fractionation followed the method as described by Mason (1969) and simplified by Kreuzer and Kirchgessner (1985). According to their definition, total faeces N (Ntot) can be divided into i) water soluble N (WSN), ii) undigested dietary N (UDN), consisting mainly of (hemi-)cellulose and some denatured proteins from plant cell walls of the animal`s feed, and iii) bacterial and endogenous debris N (BEDN), which includes N compounds derived from microbial cells of the rumen and hindgut or from abrasions from the digestive tract tissue. Thereby, BEDN and UDN together make up the water insoluble N fraction (WIN). While concentrations of N and ^{15}N in UDN and WIN were determined directly, corresponding values in BEDN were calculated as the difference between WIN and UDN.

We applied this procedure to the faeces (mixed from Day 11 until Day 16 of the feeding period) and to the whole slurry used in the field experiment. The WSN and the WIN fractions of the slurry or faeces mix were separated by centrifugation of a 30 mL subsample at 2650 *g* at 4 °C for 30 min, followed by a washing step with milliQ water. The pellet (WIN) was lyophilized, pulverized and analysed for N and ^{15}N. We determined N and ^{15}N in WSN on the filtered supernatant by TOC/TNb-analysis and direct analysis in the liquid mode of the mass spectrometer (see below). However, the latter was only done for the slurry, but not for the faeces as the N concentration of the WSN fraction of the faeces was too low for direct ^{15}N measurement. Thus, ^{15}N WSN of faeces had to be calculated based on a mass balance (Bosshard et al., 2011, Langmeier et al., 2002).

UDN was determined for the slurry by boiling a dried and milled subsample with a neutral detergent fibre solution (Van Soest et al., 1991) in an automatic Fibretherm FT12 system (Gerhardt Analytical Systems, Germany). Thus, UDN was defined as

the amount of N in the neutral detergent fibre (NDF) fraction of the slurry. After boiling, the samples were dried at 130 °C and analysed for their total N content. Ash content was determined by ashing a separate subsample at 600 °C. For faeces, UDN was not measured, but calculated with a mass balance approach assuming that all UDN in slurry would originate from faeces.

2.2.7 Laboratory analysis of slurry, soil and biomass samples

Total N, NH_4-N, P and K content of the slurry were analysed on the fresh slurry at the laboratory for soil and environmental analysis (LBU, Eric Schweizer AG, Steffisburg, Switzerland).

Total N in fumigated and non-fumigated soil extracts was measured with a TOC/TNb-analyser (Shimadzu, TOC-L (Model CPH), Japan). Microbial N (Nmic) was calculated as the difference between fumigated and non-fumigated extracts using a conversion factor of k_{EN} = 0.54 (Joergensen and Mueller, 1996). Non-fumigated extracts were additionally analysed colorimetrically for nitrate and ammonium. Nitrate content of the extracts was determined according to Keeney and Nelson (1982), while ammonium, both in soil extracts and acid traps for ammonia measurements, was determined using the modified indophenol blue reaction (Krom, 1980).

All Ntot and ^{15}N analyses (cattle slurry, soil, biomass, diffusion filters) were performed on an elemental analyser coupled with a continuous flow isotope ratio mass spectrometer (Pyro cube + isoprime100, Elementar, Germany). Urine samples and WSN fractions of the slurry fractionation were analysed for total N and ^{15}N enrichment by using a liquid autosampler mounted on the elemental analyser. Amount or volume of samples were adjusted to contain 20 to 30 µg N. International standards (IAEA-N1, IAEA-N2) and internal references were included as quality check in each analysis run.

2.2.8 Calculations

For all ^{15}N data, isotopic excess was calculated by subtracting the mean ^{15}N abundance (i.e. proportion of ^{15}N relative to total N) of non-labelled reference samples

from the measured ^{15}N abundance: For the mineral fertilizer, the natural abundance of ^{15}N in air was subtracted as a reference (i.e. 0.3663 atom%), while for slurry the weighted mean ^{15}N abundance of the non-labelled faeces and urine samples was used as non-labelled reference (0.386 atom%). For plant biomass, soil or soil extracts, the mean of the control treatment (Con) at the corresponding sampling time in the corresponding sample type (soil, plant, extracts) was used as a reference.

The ^{15}N excess was used to calculate the share of N derived from fertilizer (Ndff) in the corresponding compartment (Hauck and Bremner, 1976):

$$Ndff_{rel}\,[\%] = \frac{atom\%\ \ ^{15}N excess\ sample}{atom\%\ \ ^{15}N excess\ fertilizer} \times 100 \qquad \textbf{Eq. 2.1}$$

where atom% ^{15}Nexcess sample is the ^{15}N enrichment of the considered compartment (i.e. soil, extracts or different plant groups or parts) and atom% ^{15}Nexcess fertilizer refers to N enrichment of either mineral fertilizer or slurry.

The amount of N derived from the fertilizer was calculated as:

$$Ndff\ [kg\ ha^{-1}] = \frac{Ndff_{rel}\,[\%]}{100} \times TN_i \qquad \textbf{Eq. 2.2}$$

where TN_i is the total amount of N in the considered compartment expressed in kg N ha^{-1}. TN_i was calculated from the N concentration in the compartment multiplied with its dry weight in kg ha^{-1}. The mass of the topsoil (0 – 0.15 m) was determined by multiplying its volume with the bulk density (**Table 2.1**).

For Field B (cropped with grass-clover) also biological nitrogen fixation (BNF) was determined. For the fertilized treatments (Min and Slu), N from BNF of clover (Nfix) was determined by the ^{15}N enriched dilution method (McAuliffe et al., 1958), while for the control treatment, Nfix was obtained by the natural abundance method (Shearer and Kohl, 1986) (see **SI 2.3 for details**).

The remaining part of N uptake by crops, N derived from other sources (Ndfo) such as soil, deposition or unlabelled fertilizer N, was estimated via the difference between total N uptake and Ndff for Field A, or via the difference between total N uptake and Ndff and Nfix for Field B.

The ^{15}N enrichment in the Nmic pool was calculated according to Mayer et al. (2003):

$$^{15}N_{mic}\,[atom\%] = \frac{total\ N_{fum} \times atom\% \quad ^{15}Nexcess_{fum} - total\ N_{nonfum} \times atom\% \quad ^{15}Nexcess_{nonfum}}{total\ N_{fum} - total\ N_{nonfum}} \qquad \textbf{Eq. 2.3}$$

where "fum" indicates fumigated samples while "nonfum" indicates non-fumigated samples. Total N concentrations determined by TOC/TNb were used for both total N_{fum} and total N_{nonfum}.

The recovery of the applied fertilizer in the different compartments was then calculated as:

$$Recovery[\%] = \frac{Ndff\ [kg\ ha^{-1}]}{N\ applied\ [kg\ ha^{-1}]} \times 100 \qquad \textbf{Eq. 2.4}$$

where N applied is the total amount of N applied with the labelled fertilizer. For the repeated fertilizer applications and cuts of the grass-clover at Field B, recovery was calculated cumulatively over the applications and cuts.

Accordingly, recovery of the mineral N was calculated relative to the amount of mineral N applied with the fertilizers which was the same for Min and Slu.

2.2.9 Statistical analysis

Data preparation and statistical analysis were performed using R (Version 3.5.3) (R Core Team, 2019). Throughout, a significance level of $p < 0.05$ was applied.

For Field A, differences between treatments for dry matter yield, total N uptake, Ndff and recovery were analysed using analysis of variance (ANOVA) including both treatment and block as factors. Due to repeated harvests of the biomass at Field B, linear mixed-effects models (*lmer* within lme4-package) were used for analysing data on grass-clover yield, TN uptake, Ndff, cumulative recovery of Ntot, cumulative recovery of mineral N and biological N fixation (Nfix), including *treatment*, *block* and *cut* as well as the interaction between *treatment:cut* as fixed effects and *microplots* as a random effect. Model validation was performed by qq-plotting and Shapiro-Wilk normality test. Due to non-normal distribution of residuals, statistical analysis for

Nfix was done on square-root transformed data, while log-transformation was used for cumulative recovery of Ntot and of mineral N.

For N in different soil N pools (Nmin, Nmic), data for Field A and Field B were analysed separately. Again, due to repeated measurements, linear mixed-effects models were used, including *treatment*, *block* and *sampling time* as well as the interaction between *treatment:sampling time* as fixed effects and *microplots* as a random effect. For NO_3-N and NH_4-N at both fields, statistical analysis were performed on log-transformed data due to non-normal distribution of residuals.

Contrast-function within the *emmeans*-package was used for deriving p-values for pairwise comparisons. p-value adjustment for multiple comparisons was performed according to the Holm-Bonferroni-method (Holm, 1979). Spearman`s rank correlation coefficient was used for analysing the relationship between soil moisture content and both Nmic and Nmin.

2.3 Results

2.3.1 Slurry characterization and homogeneity of slurry labelling

The highest ^{15}N enrichment in both urine and faeces was reached between three to eight days after start of feeding with ^{15}N labelled feed (i.e. from Day 11 until Day 16 of the feeding period) (**Fig. 2.3**). In faeces N, a maximum of 9.48 atom% ^{15}N was achieved while urine N reached up to 7.64 atom% ^{15}N. During these days, the animal had an average daily N intake of 142 ± 4.6 g, of which about 23 % were excreted as faeces and 37 % as urine. Based on ^{15}N calculations, about 16 % of N intake were recovered in faeces and 21 % in urine.

For the final slurry used in the field experiment, the complete urine (56 kg) and faeces (48 kg) portions collected between Day 11 and Day 16 were mixed and diluted 1:1 with demineralized water (see 2.2). Thereby, of the total slurry N, 61 % originated from urine and 39 % from faeces, with weighted averages over the sampling days of 7.25 atom% ^{15}N for urine and 8.92 atom% ^{15}N for faeces (**Fig. 2.3**).

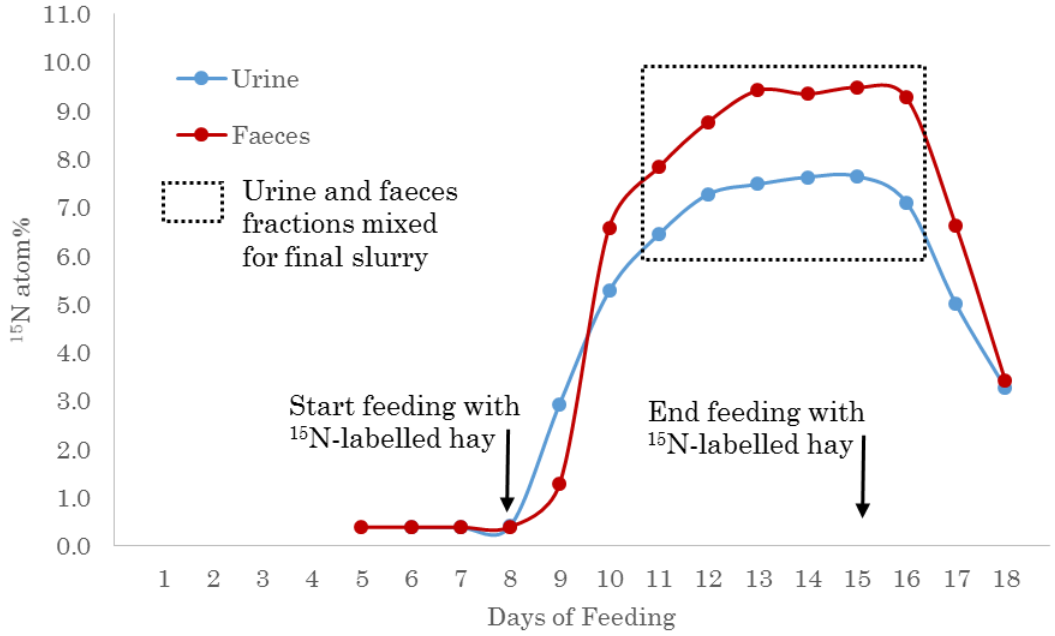

Fig. 2.3: *^{15}N label in urine and faeces portions over feeding period (n = 3 for faeces, n = 1 for urine)*

The fractionation revealed that about 75 % of total slurry N was WSN, compared to about 25 % for faeces N (**Table 2.2**); 62 % of slurry-N was in the form of ammonium (**Table 2.3**). UDN in slurry was about 4.6 % of total slurry N. UDN of the faeces mix was not determined, but since about 39 % of slurry N originate from faeces and presuming that UDN was only derived from faeces, about 12.1 % of faecal N was UDN.

Overall, the enrichment in the different slurry fractions ranged from 7.39 to 8.78 atom% ^{15}N and followed the order of WSN < total slurry N < UDN < BEDN (**Table 2.2**). Total slurry N had an enrichment of 7.89 atom% ^{15}N. Thus, the enrichment of the individual fractions deviated from that of total slurry N by -6 %, +3 % and +11 % for WSN, UDN and BEDN.

Table 2.2: *Fractionation of slurry (urine + faeces from Day 11 until Day 16 of the feeding period) and mixed faeces samples (faeces mixed from Day 11 until Day 16 of the feeding period); Ntot = total N, N_{sum} = total N as sum of WSN and WIN, WSN = water-soluble N, WIN = water-insoluble N, UDN = undigested dietary N (= NDF-N), BEDN = bacterial and endogenous debris N; nd = not determined*

	Slurry		**Faeces mix**	
	g N kg^{-1} DM (Share of Ntot (%))	^{15}N atom%	g N kg^{-1} DM (Share of Ntot (%))	^{15}N atom%
Ntot (measured)	67.8 (100)	7.89	37.9 (100)	8.99
N_{sum} (calculated)	70.0 (103.2)	8.01	35.3 (93.1)	[-]
WIN [1]	19.3 (28.5)	8.67	26.0 (68.6)	8.86
UDN	3.2 (4.7)	8.12	nd	nd
BEDN[2]	16.1 (23.7)	8.78	nd	nd
WSN [1]	50.7 (74.9)	7.39	9.3 (24.5)	9.36[3]

[1]fractionation of slurry into WIN and WSN was done on n = 6 samples: five samples originated from the five slurry applications in the field, and one sample from the storage
[2]BEDN was calculated based on difference between WIN (n = 6) and UDN (n = 2); ^{15}N BEDN was calculated based on mass balance between ^{15}N WIN and ^{15}N UDN
[3]calculated based on mass balance for ^{15}N WIN and ^{15}N Ntot

Table 2.3: *General slurry characteristics; NDF = neutral-detergent fibre; FM = fresh matter; DM = dry matter*

Sample	**pH**	**DM**	**C_{org}**	**P**	**K**	**Ca**	**Mg**	**S**	**Ntot**	**NH_4-N**	**NDF**
	[-]	[g kg^{-1} FM]					[g kg^{-1} DM]				
Slurry	8.3	34	393	7.5	83.5	11.5	5.0	4.9	67.8	42.2	268

Note: Slurry analysis was performed on fresh slurry in which excreta were already diluted with demineralized water.

2.3.2 Yield and source of N uptake in the crop

For silage maize on Field A, dry matter yield was 164 to 179 dt ha^{-1}, while N uptake ranged between 137 and 150 kg N ha^{-1}. N uptake was slightly higher for Slu than for both Min and Con (by 7 to 9 %), while dry matter yield was similar in all treatments (**Table 2.4**). For Field B, only treatment differences upon Cut 3 and Cut 4 were statistically significant, with grass-clover dry matter yield being 26 to 34 % greater for the fertilized treatments than for the non-fertilized control (**Table 2.5**). On

average, N uptake did not differ between treatments ($p = 0.13$), but was 28 to 31 % lower for Con than for Min or Slu upon Cut 4.

Crops clearly differed in their source of N uptake (**Table 2.4** and **Table 2.5**): The amount of N derived from the labelled fertilizers, Ndff [kg N ha^{-1}], was markedly higher for Min than for Slu on both Field A ($p < 0.001$) and Field B ($p < 0.01$), for the latter referring to cumulated values over the four cuts. There was a significant interaction between treatment and cut for grass-clover at Field B ($p = 0.03$), with Ndff higher for Min than for Slu for the first three cuts, which took place during summer and autumn 2018. For the cut in spring 2019, the trend was reversed with a higher Ndff value for Slu than for Min. However, this effect was not significant.

Since Field B was cropped with a grass-clover mixture, also biological nitrogen fixation (BNF) by clover added to the sources of N uptake for the biomass. For Con, cumulated Nfix over all four cuts reached up to 40 kg N ha^{-1}, while it was 19 kg N ha^{-1} for Slu and 14 kg N ha^{-1} for Min. Nfix was significantly affected by the interaction between treatment and cut ($p = 0.02$). While the effect of cut was highly significant ($p < 0.001$), differences between the non-fertilized control and the two fertilized treatments were not statistically significant ($p = 0.24$).

Table 2.4: ^{15}N fertilizer input, dry matter yield, N uptake and N sources for silage maize (Field A); recovery of total N (Ntot) as well as recovery of mineral N refer to harvested aboveground biomass.

Ndff = N derived from labelled fertilizer; Ndfo = N derived from other sources (soil, deposition, unlabelled fertilizer)

	Date of harvest	Ntot input [kg N ha^{-1}]	Dry matter yield [dt ha^{-1}]	Total N uptake [kg N ha^{-1}]	Ndff [kg N ha^{-1}]	Ndfo [kg N ha^{-1}]	Recovery of Ntot [%]	Recovery of mineral N [%]
Con	2018-08-28	[-]	164.0 ± 6.0^{ns}	137.3 ± 9.9^{a}	[-]	137.3 ± 9.9^{a}	[-]	[-]
Min	2018-08-28	36.8	179.1 ± 9.2^{ns}	140.3 ± 4.5^{a}	16.4 ± 0.9^{a}	123.9 ± 5.1^{b}	44.7 ± 2.6^{a}	44.7 ± 2.6^{a}
Slu	2018-08-28	60.0	176.1 ± 10.3^{ns}	149.7 ± 4.9^{b}	11.5 ± 1.0^{b}	138.2 ± 4.8^{a}	19.2 ± 1.7^{b}	31.3 ± 2.7^{b}

mean ± standard deviation; n = 4
numbers followed by different letters within each column are significantly different ($p < 0.05$); ns = not significant

Table 2.5: ^{15}N fertilizer input, dry matter yield, N uptake and N sources for grass-clover (**Field B**); cumulative recovery of total N (Ntot) as well as cumulative recovery of mineral N refer to harvested aboveground biomass.
Ndff = N derived from labelled fertilizer; Nfix = N from biological N fixation by legumes; Ndfo = N derived from other sources (soil, deposition, unlabelled fertilizer)

Cut	Treat-ment	Date of harvest	Cumulative Ntot input[1]	Dry matter yield	Total N uptake	Ndff	Nfix[3]	Ndfo	Cumulative recovery of Ntot[1,4]	Cumulative recovery of mineral N[1,4]
			[kg N ha^{-1}]	[dt ha^{-1}]	[kg N ha^{-1}]	[kg N ha^{-1}]	[kg N ha^{-1}]	[kg N ha^{-1}]	[%]	[%]
1	Con	2018-06-14	0.0	26.0 ± 3.1^{ns}	63.2 ± 14.9^{ns}	[-]	9.8 ± 6.6^{ns}	53.4 ± 8.7^{ns}	[-]	[-]
1	Min	2018-06-14	36.8	29.2 ± 3.7^{ns}	68.7 ± 5.8^{ns}	16.7 ± 4.7^{a}	9.4 ± 6.9^{ns}	42.5 ± 8.1^{ns}	45.4 ± 12.8^{a}	45.4 ± 12.8^{a}
1	Slu	2018-06-14	60.0	26.3 ± 4.0^{ns}	62.8 ± 12.2^{ns}	9.9 ± 1.5^{b}	6.5 ± 3.6^{ns}	46.3 ± 8.1^{ns}	16.5 ± 2.4^{b}	26.9 ± 4.0^{b}
2	Con	2018-07-23	0.0	24.7 ± 1.3^{ns}	66.6 ± 11.5^{ns}	[-]	15.6 ± 10.4^{ns}	51.1 ± 2.1^{ns}	[-]	[-]
2	Min	2018-07-23	73.6	29.7 ± 3.8^{ns}	72.1 ± 9.8^{ns}	18.7 ± 2.5^{a}	7.3 ± 6.0^{ns}	46.1 ± 3.2^{ns}	48.1 ± 5.3^{a}	48.1 ± 5.3^{a}
2	Slu	2018-07-23	120.0	28.8 ± 1.5^{ns}	68.3 ± 10.2^{ns}	13.6 ± 1.2^{b}	5.6 ± 4.7^{ns}	49.1 ± 9.9^{ns}	19.6 ± 1.7^{b}	32.0 ± 2.7^{b}
3	Con	2018-09-18	0.0	14.5 ± 2.0^{a}	39.2 ± 8.2^{ns}	[-]	10.4 ± 7.7^{ns}	28.8 ± 1.6^{ns}	[-]	[-]
3	Min	2018-09-18	110.4	21.2 ± 2.6^{b}	56.0 ± 7.2^{ns}	17.7 ± 2.4^{a}	1.7 ± 2.0^{ns}	36.6 ± 3.9^{ns}	48.1 ± 3.1^{a}	48.1 ± 3.1^{a}
3	Slu	2018-09-18	180.0	19.5 ± 1.6^{ab}	50.0 ± 4.7^{ns}	12.3 ± 2.5^{b}	1.9 ± 2.5^{ns}	35.8 ± 7.0^{ns}	19.9 ± 0.9^{b}	32.5 ± 1.5^{b}
4	Con	2019-04-23	0.0	21.2 ± 3.6^{a}	58.7 ± 11.0^{a}	[-]	5.4 ± 4.6^{ns}	53.3 ± 6.7^{a}	[-]	[-]
4	Min	2019-04-23	147.1	29.7 ± 5.9^{b}	81.3 ± 22.1^{b}	13.9 ± 5.0^{ns}	0.4 ± 0.7^{ns}	66.9 ± 18.2^{b}	45.5 ± 5.0^{a}	45.5 ± 5.0^{a}
4	Slu	2019-04-23	240.0	32.8 ± 3.7^{b}	84.7 ± 7.2^{b}	16.0 ± 1.1^{ns}	0.1 ± 0.2^{ns}	68.6 ± 6.2^{b}	21.6 ± 0.6^{b}	35.2 ± 0.9^{b}
ANOVA[2]										
Treatment				**	ns	**	ns	ns	***	**
Cut				***	***	ns	***	***	*	*
Treatment:Cut				x	x	*	*	**	x	x

*mean ± standard deviation; n = 4; x, *, **, *** significant at p < 0.10, 0.05, 0.01 and 0.001 probability level; ns not significant*
Different letters indicate significant differences between fertilizer treatments within each cut (p < 0.05); ns = not significant
[1]Amount of Ntot applied and ^{15}N fertilizer recovery cumulated over repeated fertilizer applications and repeated harvests.
[2] Analysis of variance (ANOVA) on mixed effect model including *Treatment, Cut, Block* as well as the interaction of *Treatment:Cut* as fixed effects and *Microplots* as random effect. Although the interaction between treatment and cut did not show a significant effect on neither dry matter yield (p = 0.06), N uptake (p = 0.07), recovery of Ntot (p = 0.07) nor recovery of mineral N (p = 0.07), it was kept in the model because as due to repeated fertilizer applications after each harvest, these two factors are highly interlinked and also because the interaction showed p values close to the significance level of α = 0.05.
[3] Statistical analysis performed on square-root transformed data due to non-normal distribution of residuals.
[4]Statistical analysis performed on log-transformed data due to non-normal distribution of residuals.

Ndfo was significantly lower for Min than for the other treatments at Field A ($p < 0.01$). While the same trend was observed at Field B, at least for the first two cuts, these differences were not statistically significant. Instead, there was a significant interaction of cut and treatment with regard to Ndfo ($p = 0.008$).

On average, less than 25 % of N taken up by grass-clover at Field B derived from mineral fertilizer, while for Slu Ndff in grass-clover was less than 20 %. Silage maize on Field A took up less than half of these shares. On Field B, Nfix added about 18 % of total N uptake for Con and about 7 % and 5 % for Min and Slu, respectively. Thus, for both fields, 69 to 92 % of N uptake originated from other sources than ^{15}N labelled fertilizer application or BNF, probably mainly from soil N and non-labelled mineral fertilizer (Field A) or slurry N (Field B). Noteworthy, for the latter non-labelled slurry was only applied before the last cut, thus, for the first three cuts, Ndfo presumably equals N derived from soil.

2.3.3 ^{15}N fertilizer recovery in the soil-plant system

Fertilizer recovery in aboveground biomass was on both fields significantly higher for Min than for Slu ($p < 0.001$), ranging between 45 to 46 % for Min and 19 to 22 % for Slu (for Field B cumulated over all four cuts of the grass-clover and all ^{15}N labelled fertilizer applications). Although the same amount of mineral N was applied, also recovery of mineral N was significantly higher for Min than for Slu ($p < 0.01$), for which it reached up to 35 % (**Table 2.4** and **Table 2.5**).

Fertilizer recoveries in topsoil were markedly higher for Slu than for Min at all sampling times, except for the first sampling at one week after fertilizer application at Field A (silage maize) (**Fig. 2.4**). At this time point, fertilizer recovery for both Min and Slu reached approximately 100 %. Interestingly, this was not the case in grass-clover at Field B, where only 29 ± 4 % and 61 ± 3 % of ^{15}N applied with Min or Slu were recovered in topsoil one week after fertilizer application. At Field A, fertilizer recovery in topsoil decreased significantly over the time course of the experiment ($p < 0.001$), while for Field B, recovery tended to increase at the time points one week after fertilizer application (t3, t5 and t7).

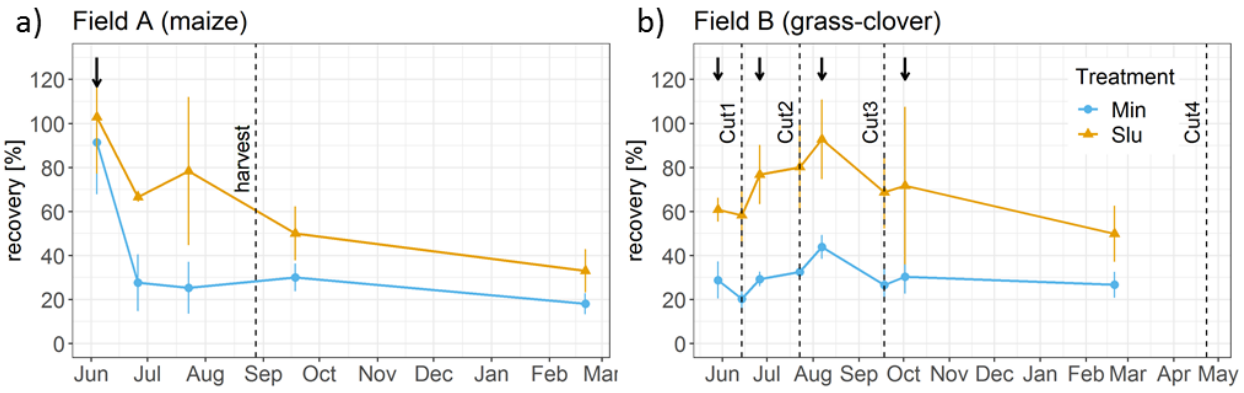

Fig. 2.4: *Temporal development of ^{15}N recovery in total soil N in topsoil (0 – 0.15 m) for Field A (Silage maize) (a) and for Field B (Grass-clover) (b), (mean ± standard deviation, n = 4). Arrows indicate samplings at one week after fertilizer application.*

For Field A, considering recovery in topsoil three weeks after harvest plus the recovery in the harvested biomass, total recovery in the topsoil-plant system summed up to 75 % for Min and 69 % for Slu. On Field B, topsoil was sampled at the same time points as the grass-clover harvests, at least for Cut 1, Cut 2 and Cut 3. At these three cuts, cumulative recovery in the harvested aboveground biomass and recovery in topsoil at the respective sampling times summed up to 66 %, 81 % and 75 % for Min and to 77 %, 100 % and 89 % for Slu. The last soil sampling took place in February 2019 in order to sample before the spring application of non-labelled cattle slurry by the farmer. This last sampling point of topsoil cannot be directly linked to fertilizer recovery in aboveground biomass at cut 4, which took place in April 2019. The sum of fertilizer N recovered in soil in February and in aboveground biomass at cut 4 was about 72 % for both Min and Slu.

Cumulated NH_3-emissions at Field B were markedly higher for Slu (24 kg N ha^{-1}), than for Min (5 kg N ha^{-1}) (**SI 2 Fig. 4**). For the single fertilizer application at Field A, about 4 kg N ha^{-1} were lost via NH_3 volatilization from Slu, while for Min no emissions were detected. Noteworthy, for NH_3 emissions only absolute N amounts but not the ^{15}N label could be measured, since ammonium concentrations in the acid traps were too low for reliable ^{15}N measurements. However, it can be assumed that emitted ammonia originated only from the applied fertilizers.

2.3.4 N dynamics in top-soil and ^{15}N recovery in different soil N pools

Nmin and especially NO_3-N responded strongly to fertilizer addition, except for the first and the second fertilizer application at Field B, where no differences in Nmin between fertilized treatments and Con were detected (**Fig. 2.5**). NH_4-N followed a similar pattern, but differences were less clear than for NO_3-N, mostly due to the high data variability for Slu.

Nmic was less directly affected by fertilizer addition than Nmin. Likely, Nmic was more controlled by weather conditions and/or soil moisture than by fertilizer applications. Indeed, averaged over both fields, Nmic, but also NH_4-N and NO_3-N were significantly correlated with the gravimetric moisture content of the soil upon sampling (Spearman`s rank correlation coefficient r = 0.24 for Nmic, r = -0.40 for NH_4-N, and r = -0.37 for NO_3-N). Under grass-clover at Field B, there was a significant interaction between sampling time and treatment (p = 0.002), with differences between treatments becoming clearer over time, resulting in Nmic in the order Slu > Con > Min (**Fig. 2.5**).

In order to trace the fertilizer also into different soil N pools, extracts from selected time points were analysed for their ^{15}N abundance. For Field A, shortly after fertilizer application, most N from Min was found in the mineral N pool, but already some of it was assimilated into Nmic (**Fig. 2.6**). For slurry N, one week after fertilizer application the biggest share was found in the Norg pool. With time, the share of Ndff in Nmin decreased drastically, while the share in the Norg pool increased.

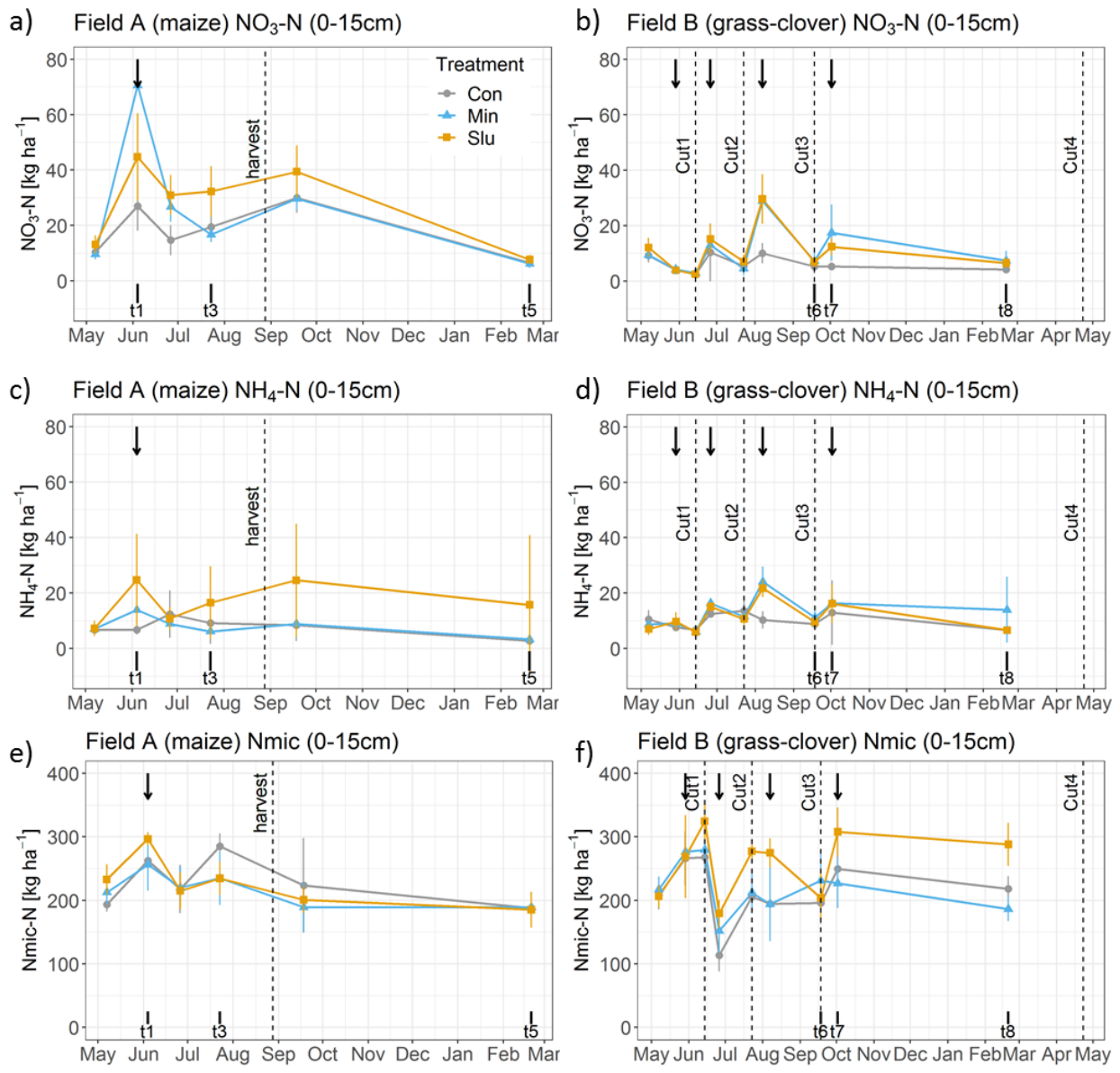

Fig. 2.5: *Temporal development of a-b) NO_3-N, c-d) NH_4-N, e-f) Nmic-N (mean ± standard deviation, n = 4 , except Field B Nmic sampling t1 control n = 2). Black arrows indicate sampling(s) at one week after fertilizer application, dashed lines indicate aboveground biomass harvest. Sampling time points for which ^{15}N abundance in the Nmic and Nmin pool were determined, are marked with black bars at the bottom.*

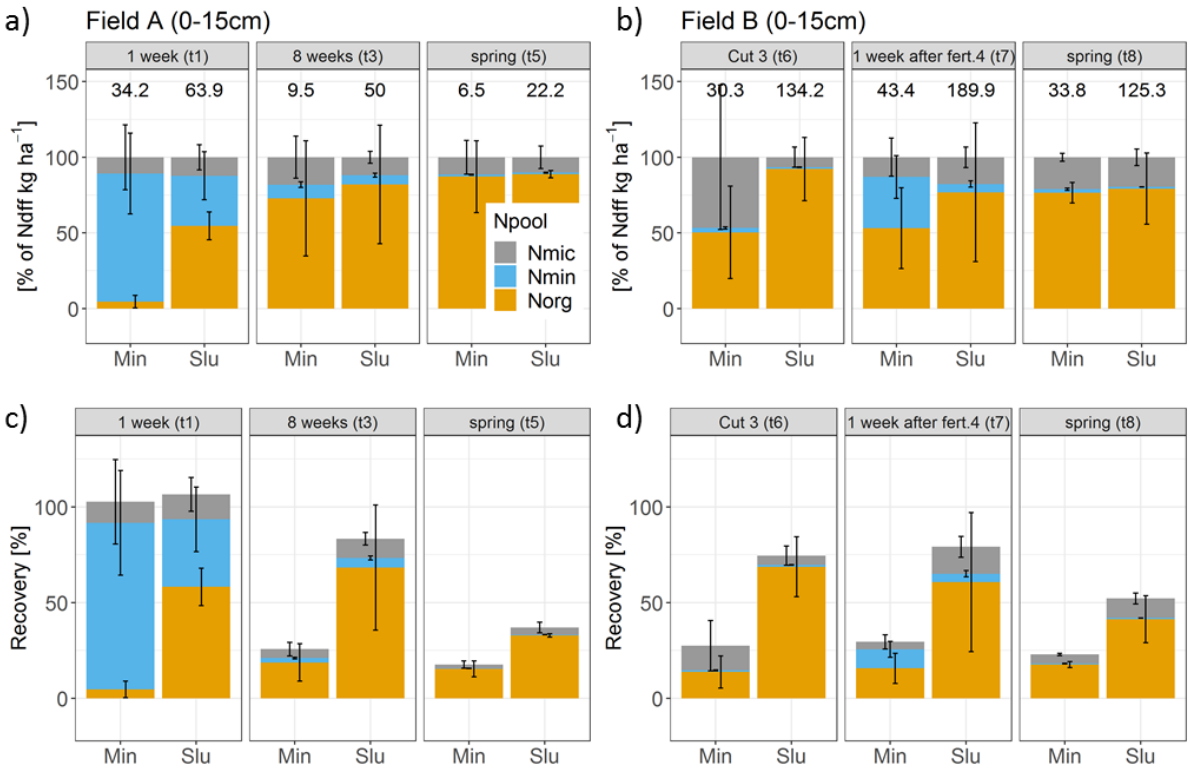

Fig. 2.6: *a, b) Relative distribution of residual fertilizer N in different soil N pools; numbers on top indicate absolute values for residual N derived from fertilizer (Ndff) [kg N ha^{-1}]; c, d) ^{15}N recovery in different soil N pools relative to applied amounts of ^{15}N; Nmic = microbial N, Nmin = mineral N, Norg = non-microbial organic N; (mean ± standard deviation, n = 4)*

For Field B, a clear effect of the repeated applications of ^{15}N labelled fertilizer to grass-clover was observed: One week after the third fertilizer application (t7), an increase in the share of Nmin, especially for the Min treatment was observed, similar to Field A. By that time point, however, there was already quite a big share of fertilizer N from both fertilizers found in the Norg pool, originating from previous ^{15}N labelled fertilizer applications.

With time, differences regarding the distribution of fertilizer N in soil between treatments and between fields declined and reached a similar distribution upon sampling in spring. More than 77 % of residual fertilizer N in soil, defined here as the amount of fertilizer N not taken up by the crop by a certain time point, were found in the Norg pool and only minor shares found as Nmin. Differences persisted for the share of fertilizer N found in Nmic, which was higher for Field B (20 – 21 %) than for Field A (10 – 12 %). Since total recovery of fertilizers in the total soil N pool was higher

for Slu than for Min, this translated into a higher amount of slurry N recovered in the Nmic pool. Thus, in spring of the year after fertilizer application, more slurry N was still in a relatively dynamic pool of Nmic as compared to N from mineral fertilizer.

2.4 Discussion

2.4.1 ^{15}N signature of slurry mixed from ^{15}N labelled faces and urine allows for quantitative tracing of N

For our study, we needed to produce ^{15}N labelled cattle slurry and to assess whether the ^{15}N enrichment in the slurry, mixed from ^{15}N labelled faeces and ^{15}N labelled urine, could be used as source signature for quantitative tracing of its N transformation and uptake processes in an on-farm field experiment.

The cattle slurry used in our experiment had high contents of total N (67.7 g kg^{-1} DM) and NH_4-N (42.2 g kg^{-1} DM) (**Table 2.3**) compared to values given for cattle slurry in the Swiss fertilizer guidelines (43 g total N kg^{-1} DM, 23.3 g soluble N kg^{-1} DM) (Richner and Sinaj, 2017). However, our values correspond well with those reported by Hoekstra et al. (2011) for total N (81 to 91 g N kg^{-1} DM) and NH_4-N (39 to 56 g N kg^{-1} DM) in cattle slurry. In the Swiss fertilizer guidelines, N losses due to ammonia volatilization occurring under common husbandry conditions are already accounted for and are assumed to amount to 20 % of excreted N. Such losses were kept minimal in our experimental setting by immediate freezing of the excreta after collection and overall cold surrounding temperatures during the production of the slurry. In addition, the hay fed to the animal had a rather high crude protein content (on average 16.7 %) and was highly digestible. Combined with the low estimated energy content (5.9 MJ kg DM^{-1}), this could explain the high total N and NH_4-N contents and the high share of urinary-N to faeces-N excreted by the animal, as the ratio of urine-N to faeces-N usually increases with crude protein intake (Broderick, 2003, Marini and Van Amburgh, 2005).

Based on absolute amounts of N intake and N excretion, the animal excreted about two thirds of its N intake, which was lower than values reported by some authors who

found that on average about 80 % of N intake were excreted (Pagliari et al., 2020, Haynes and Williams, 1993). Also, ^{15}N from feed recovered in urine and faeces, summing up to 40 % in our experiment, was lower than the 51 to 64 % found by others (Powell and Wu, 1999). The deviations from previous studies possibly occurred because the heifer was only about six months old, having a higher N retention than an adult animal used in other studies. The pronounced differences in N excretion estimates based on total N or ^{15}N indicate that within the digestive system of the heifer, labelled feed N was assimilated while non-labelled body N was excreted.

The temporal development and overall ^{15}N enrichment of faeces and urine (**Fig. 2.3**) followed an already well-documented pattern for feeding with ^{15}N labelled feed over several days, with the ^{15}N label first appearing in the urine, rapidly being exceeded by the ^{15}N label in the faeces (e.g. Barros et al., 2017, Powell et al., 2004, Bosshard et al., 2011).

Fractionation into WSN, UDN and BEDN was done in order to assess the ^{15}N distribution in soluble and more recalcitrant N pools, and to compare it with the total ^{15}N enrichment in the slurry. In previous publications, fractionation into different N fractions was usually done for faeces only (Bosshard et al., 2011, Langmeier et al., 2002). In our study, we were interested in the N distribution and corresponding ^{15}N labelling in the whole slurry and, therefore, additionally applied the fractionation procedure to the whole slurry. Haynes and Williams (1993) found that on average 20 – 25 % of faecal N of dairy cows was water-soluble, 15 – 25 % was in the UDN fraction, while the remainder was considered as BEDN. Compared to that, faeces in our slurry ranged at the higher end for WSN (**Table 2.2**) and were even twice as high as WSN reported for cow faeces (Langmeier et al., 2002) or sheep faeces (Bosshard et al., 2011). This might partially be explained by a leaking urine pipe during day 12 and day 14 of the feeding period, adding WSN to faeces. At the same time, UDN content was at the lower end of values reported, but similar to results by Langmeier et al. (2002) and Hoekstra et al. (2011). The dominance of N in the WSN fraction rather than in the recalcitrant UDN fraction suggests that a high share of N in the

slurry used in this experiment was easily accessible for plants and microbes. This was also indicated by a narrow ratio of $C:NH_4$ which was considered a good predictor for dairy manure N availability (Griffin et al., 2005). As expected, slurry fractions differed in their ^{15}N enrichment, with differences in a similar range and order as reported by Bosshard et al. (2011).

We assumed that within the time scale of one year considered in this experiment, only BEDN and WSN will become plant available due to the stable nature of UDN (Bosshard et al., 2011, Kreuzer and Kirchgessner, 1985, Sørensen et al., 1994). Thus, for the slurry label used for calculating plant uptake, only the enrichment of BEDN and WSN might be relevant. With their relative share, their combination results in a label of 7.72 atom% ^{15}N, which is slightly lower than the 7.89 atom% ^{15}N label of total slurry N (**Table 2.2**). Furthermore, cross-labelling experiments with similar faeces-N to urine-N ratios found $Ndff_{rel}$ values from urine in the range of 20 to 26 % and for faeces in the range of 3 to 7.7 % (Langmeier et al., 2002, Bosshard et al., 2009, Jensen et al., 1999). This indicates that inhomogeneous labelling within the faeces fractions is of less importance compared to the usually more homogeneous urine label. Based on these insights, we concluded that differing enrichments in slurry fractions would only have minor implications for the conclusions drawn from comparing mineral fertilizer and cattle slurry in our experiment.

2.4.2 Lower fertilizer value of cattle slurry than mineral fertilizer under on-farm conditions

In accordance with the assumptions underlying the Swiss fertilization guidelines, and since equal rates of mineral N were applied with slurry and mineral fertilizer, we expected no differences in dry matter yield, N uptake or Ndff between Min and Slu, as we assumed that organic N mineralization would compensate for expected higher immobilization of inorganic slurry N compared to Min.

Indeed, there were no differences in dry matter yield between Min and Slu on either of the fields (**Table 2.4** and **Table 2.5**). Also total N uptake was similar for all

treatments throughout the experiment. Even the Con treatment reached the same yield level except for the last two grass-clover cuts at Field B. Yield levels on both fields stayed rather at the lower end of yields expected for Switzerland (Richner and Sinaj, 2017), likely due to dry and hot weather conditions during summer 2018. Weather conditions also impaired the identification of clear differences in N uptake between Min and Slu, as water limitation was probably a more limiting factor than N. Furthermore, both fields had been under grass-clover previously and had received regular N inputs with three to four applications of cattle slurry per year by the farmer.

Contrary to our expectations, both Ndff and recovery of mineral N in aboveground biomass were higher for Min than for Slu at both fields and all sampling points, except for the last cut at Field B (**Table 2.4** and **Table 2.5**). Lower Ndff and recoveries of mineral N for Slu than for Min were also observed by others (Paul and Beauchamp, 1995). It could possibly be explained by either high immobilization or volatilization losses of inorganic slurry N, or low mineralization of organic slurry N, or a combination of all. Mineralization of organic slurry N could not be directly assessed within our study. Higher N immobilization in soil for Slu than for Min, induced by the additional C input with Slu (**Table 2.3**), seems likely and is supported by greater amounts of residual fertilizer N in Nmic for Slu than for Min (see section **2.4.3** and **Fig. 2.6**) and was also found in several other studies (Griffin et al., 2005, Paul and Beauchamp, 1995, Gutser and Dosch, 1996). NH_3-emissions were lower for Min (3.4 % of applied N) than for Slu, where losses reached up to 6.6 or 10 % of total applied N for Field A and Field B, respectively, which is equivalent to 10.8 or 16.4 % of applied mineral N (**SI 2 Fig. 4**), leaving less mineral N in the soil. Higher Ndff values for Slu than for Min upon the last cut of the grass-clover could be indicative for a higher residual fertilizer value for Slu than for Min, as already reported by others (Webb et al., 2013). It agrees both with an increasing cumulative recovery of mineral N in harvested biomass for Slu with time and a greater recovery of slurry N than of mineral N in soil Nmic in spring 2019 (**Fig. 2.6**).

As Ndfo represents the counterpart to Ndff, higher Ndff values for Min than for Slu could have pointed towards an opposite trend for Ndfo. However, this was only the case for silage maize at Field A, but not for grass-clover at Field B. A possible explanation for the observed effect at Field A could be that Slu induced soil N mineralization more than Min (priming effect), as for example observed by Nannen et al. (2011) and supported by an overall higher N uptake for Slu than for Min (**Table 2.4**). Ultimately, with our practice oriented experimental approach it was not possible to differentiate between N uptake from non-labelled fertilizer, which especially at Field A represented a major share of added N, and from soil N preventing conclusions on the effect of fertilizer addition on mobilization of soil N.

Recovery of total fertilizer N in plant biomass was markedly lower for Slu than for Min, which fits the expectations, as applied amounts of total N were higher with Slu than with Min (about 1.6 times). At the same time, we presumed that the additional organic N applied with slurry would be preserved and recovered in soil, thus, the sum of fertilizer N recovered in soil and biomass should be equal for Slu and Min. For Field A, upon harvest of the maize, total recovery in aboveground biomass and topsoil reached similar levels for Min and Slu and, thus, fulfilled our expectation (**Table 2.4** and **Fig. 2.4**). This was not the case for Field B, where throughout Cut 1 to Cut 3, higher total recoveries in the topsoil-biomass-system were reached for Slu compared to Min (**Table 2.5** and **Fig. 2.4**). It must be noted, though, that N recovered in stubble and roots was not accounted for, as no destructive sampling took place. Since recovery in aboveground biomass was higher in Min than in Slu throughout these cuts, we assumed the recovery in roots and stubble to follow the same pattern, thus to be higher for Min than for Slu. This could explain the observed differences between total recoveries in the topsoil-biomass-system, respectively the higher proportion of N that remained unaccounted for in Min.

2.4.3 Similar fate of residual N from cattle slurry or mineral fertilizer in topsoil

To complement the assessment of sources of plant N uptake, we also assessed N dynamics in the topsoil in order to gain insights into the fate of fertilizer N not taken up by the crop.

^{15}N recovery dynamics in topsoil differed between a single fertilizer application under silage maize (Field A) and repeated fertilizer applications under grass-clover (Field B) (**Fig. 2.4**). At Field A, one week after fertilizer application, approximately 100 % of applied fertilizer N were recovered in topsoil, both for Slu and Min, and recovery decreased thereafter. It can probably be explained by increasing N uptake of the maize plants and/or translocation into deeper soil layers over time (Hoekstra et al., 2011). In contrast, at Field B, markedly less than 100 % of fertilizer N were recovered in topsoil one week after the first fertilizer application. This could be explained by rapid and more efficient N uptake by grass-clover compared to young maize plants. It fits well with the recovery of 17 % and 45 % of Slu and Min in the biomass of the first cut which took place only 24 days after the fertilizer application, and 17 days after the first soil sampling. With repeated fertilizer applications at Field B, cumulative ^{15}N recovery in total soil N increased. Besides increasing amounts of residual fertilizer N in soil, also internal re-cycling and mineralization of roots could explain the observed temporal pattern. Furthermore, with time also more organic residues from the slurry applied on the soil surface might have gotten incorporated into soil, for example by earthworms (Hoekstra et al., 2011).

While the temporal development of Nmin seemed to be mostly driven by fertilizer addition and the counteracting uptake by plants and microbes (**Fig. 2.5**), for Nmic no clear pattern with regard to the fertilizers was observed. However, Nmic was positively correlated with soil water content (**SI 2 Table 1**), indicating that besides fertilizer addition, also dry and hot weather conditions during summer 2018 (**Fig. 2.2**) probably influenced soil N accumulation and transformation processes. Previous studies found increased NH_4 concentrations in soil during drought periods, mostly

due to reduced uptake by plants and microbes and the missing hydrological connectivity between microsites in soil, disconnecting nitrifying microbes from N sources (Parker and Schimel, 2011). In our study, also water was added with the fertilizers, potentially increasing microbial activity and re-assuring hydrological connectivity, thereby, inducing net nitrification (Joergensen et al., 1994, Van Gestel et al., 1992, Fierer and Schimel, 2002). This could be seen by increased NO_3 values at the time points one week after fertilizer addition in Con, which only received water, but no N (**Fig. 2.5**).

Overall, we expected fertilizer N immobilization to be higher for Slu than for Min due to additional C input with slurry (Sørensen, 2004). Indeed, we observed an increased immobilization for slurry N, as both fertilizer recovery in Nmic, but also absolute Ndff in Nmic was always markedly higher for Slu than for Min, except for sampling t6 at Field B (**Fig. 2.6**), supporting our hypothesis. Our measured values for ^{15}N recovery in Nmic ranged between 2 and 14 % and compared well to values reported by others (Jensen et al., 2000, Hoekstra et al., 2011).

Despite initial differences, relative distribution of N recovered from the fertilizers in different soil N pools reached similar levels between Min and Slu upon sampling in spring of the next year at both fields (**Fig. 2.6**). Thus, averaged over both treatments, at Field A 88.0, 10.9, and 1.1 % of residual fertilizer N in soil could be found in Norg, Nmic and Nmin, while at Field B it was 77.9, 20.4 and 1.7 %, which is supported by results found by others (Jensen et al., 2000, Sørensen, 2004, Douxchamps et al., 2011). Since total recovery in soil was much higher for Slu than for Min (**Fig. 2.4**), these results indicate that in absolute terms, more N derived from fertilizer was still in a rather dynamic and plant available form in Slu compared to Min. This could explain also the higher Ndff values in biomass upon Cut 4 and indicate a higher residual fertilizer value of Slu compared to Min (Schröder et al., 2013, Webb et al., 2013). Norg from fertilizers was previously found to re-mineralize only very slowly and remain in soil for a long time (up to decades) (Sebilo et al., 2013, Sørensen, 2004).

By providing a comprehensive overview not only focusing on the plant part but also including soil N processes, our results, showed that the relative distribution of ^{15}N from mineral fertilizer and slurry in different soil N pools were already similar in the next spring after application and only very little N was recovered in Nmin. This supports the similar and low residual plant uptake values reported in the literature. It contradicts Smith and Chalk (2018), who concluded from consistently low residual fertilizer values that ^{15}N labelling approaches were inappropriate for assessing residual fertilizer effects due to dilution in the large soil N pool. They see the method suitable, though, to trace fertilizer N into different soil N pools.

2.5 Conclusion

In this study, ^{15}N labelling was successfully used under on-farm conditions to assess fertilizer value of both cattle slurry and mineral fertilizer in the year of application, and to trace fertilizers into different soil N pools. The overall ^{15}N enrichment in the slurry allowed for quantitative results, with only minor uncertainties due to inhomogeneous labelling of faeces fractions or differing ^{15}N enrichments in urine and faeces. Contrary to the assumptions of the Swiss fertilizer guidelines, the fertilizer value of slurry was lower than its ammonium content, probably due to increased NH_3 emissions, increased immobilization, or lower than expected mineralization of organic slurry N. Despite these differences in the year of application, residual N of both fertilizers was found mostly in the organic N pool in the next spring, indicating that most inputs not taken up within the first season will end up in the non-microbial organic N pool, potentially providing N for plant uptake over a very long timeframe, but at a slow rate.

Chapter 3

Leached nitrate under loamy soil originates mostly from soil organic N with minor contributions from recent fertilizer additions

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Abstract

Animal manures are suspected to be a major source of nitrate leaching due to their low nitrogen use efficiency (NUE) by crops. However, actual measurements of nitrate leaching from animal manure under field conditions are scarce. In an on-farm field trial over 2.5 years, we used ^{15}N labelling to trace the fate of N from cattle slurry in the soil-plant system and to test whether more nitrate was leached from slurry than from mineral fertilizer. The experiment was conducted on two neighbouring fields with loamy soil in the Gäu region, Switzerland – a region with persistently high nitrate levels in the groundwater. Both fields followed the same crop rotation (silage maize – winter wheat – grass-clover), but shifted by one year. We compared three fertilizer treatments: Control (Con), ^{15}N mineral fertilizer (Min), ^{15}N cattle slurry (Slu). In order to provide a comprehensive fertilizer N balance over several years, we traced the labelled fertilizers into crop biomass, soil, and nitrate leaching. The crop to which we applied the ^{15}N labelled fertilizer recovered 45 to 47 % of mineral fertilizer, but only 19 to 23 % of cattle slurry N. Complementary, recoveries in soil were greater for Slu than for Min, despite greater NH_3 emissions from Slu. Fertilizer recovery in the succeeding crops was small (< 4.6 % in the first and < 2.4 % of the originally applied fertilizer N in the second residual year) and similar for the two fertilizers. Depth translocation of fertilizer N was marginal and after 2.5 years, the majority of ^{15}N was still recovered in the top 30 cm. Along with higher recoveries in soil for Slu, we found significantly more slurry N than mineral fertilizer N lost through leaching. However, although cumulated amounts of nitrate leaching over the three crops reached up to 205 kg NO_3-N ha^{-1}, less than 5 % of this amount originated from direct leaching of the labelled fertilizers. Likely, most nitrate leaching originated from mineralization of soil N, indicating that this needs to be taken into account in planning of fertilization and crop rotation.

Keywords: nitrate leaching, ^{15}N labelling, on-farm trial, cattle slurry, residual N use efficiency

3.1 Introduction

In many regions across Europe, nitrate levels in groundwater exceed quality criteria or even legal threshold values for use as drinking water (Grizzetti et al., 2011). In Switzerland, 15 to 20 % of the groundwater measuring points exceed the Swiss quality criterion of 25 mg NO_3 L^{-1}. Under arable land, the quality criterion is exceeded at 40 % of the measuring points (BAFU, 2019b). Since nitrate is harmful both to human health (Ward et al., 2018) and natural ecosystems (Galloway et al., 2003, Erisman et al., 2013), mitigation of nitrate leaching is crucial. At the same time, nitrogen (N) is usually the limiting factor for plant growth. Thus, crop productivity depends on N input with either mineral or organic fertilizers, such as animal manure. Optimization of N use efficiency (NUE) in agriculture, which here is defined as fertilizer recovery in crops, is, thus, needed for agronomic as well as environmental reasons.

Animal manures are suspected to be a major source of nitrate leaching. In areas with high animal densities, there is frequently a nutrient surplus (e.g. Dalgaard et al., 2012, Oenema and Tamminga, 2005). Thereby, nitrate leaching losses often increase exponentially with N surplus (Wang et al., 2019, Zhao et al., 2016). But even when applied according to current legislation, animal manures have a consistently low NUE mostly due to NH_3 losses or temporal immobilization of NH_4 and only about 26 ± 10 % of N in animal manures are taken up by the crop in the year of application (Smith and Chalk, 2018). Organic N in manure is not directly available to plants and might get mineralized at times when plants are not readily taking it up, increasing the potential for nitrate leaching compared to mineral fertilizer (e.g. Sørensen and Jensen, 2013, Bergström and Kirchmann, 2006, Thomsen et al., 1997). Overall, the fate of N from animal manure not taken up by crops remains poorly understood, and its actual contribution to nitrate loads in the groundwater is debated.

Following ^{15}N labelled fertilizer inputs throughout the soil-plant-atmosphere system over several years can enhance our understanding of N uptake and of loss dynamics and pathways, helping to improve management strategies. Reviewing numerous ^{15}N labelling studies, Gardner and Drinkwater (2009) found that refined timing, splitting

and placing of synthetic fertilizers can increase N uptake into crops by up to 43 % and thereby reduce potential for nitrate leaching losses. However, they also found that despite lower N recovery of organic fertilizers such as animal manure in crop biomass, combined recovery in crop biomass and soil was greater for organic fertilizers as compared to mineral fertilizer. Thus, recoupling carbon (C) and N in agroecosystems could contribute to reducing nitrate leaching and, thus, leaching losses might not necessarily be greater for animal manure than for mineral fertilizer, but experimental verification is missing.

So far, only few studies actually measured nitrate leaching from ^{15}N labelled animal manure and most of these studies were conducted using lysimeters and based on pasture systems (Chalk et al., 2020). In a field experiment, Jayasundara et al. (2010) measured nitrate leaching from ^{15}N labelled swine manure under corn using suction cups. We are unaware of any study directly measuring nitrate leaching losses from ^{15}N labelled cattle slurry under field conditions during an arable crop sequence. In addition, previous field studies with ^{15}N labelled animal manure focused on either gaseous or leaching losses, but did not provide a complete ^{15}N balance with all potential N uptake and loss pathways measured. ^{15}N not recovered in biomass, soil or the measured loss pathway was then assumed to have been lost via NO_3 leaching and/or as NH_3 or N_2O gaseous emissions. However, estimates remain somewhat vague by this indirect approach. Clough et al. (1998) represent an exception, having measured fertilizer recovery in crop, nitrate leaching, N_2O emissions, NH_3 volatilization and soil over 406 days after application of ^{15}N labelled urine to lysimeters. Despite their efforts, the fate of 20 to 30 % of added urine N remained unresolved, calling for further investigations.

Improved management of animal manures involves adequately considering their residual effect beyond the year of application, arising both from organic N that has not yet been mineralized and from mineral N that was temporarily immobilized (Schröder et al., 2013). Recovery of different types of animal manure in subsequent crops generally is low, ranging between 3 and 6 % in the second year and between 1

and 2.5 % of total N applied in the third year (Webb et al., 2013). With repeated manure applications, residual manure in soil sums up, not only increasing total N and mineral N stocks in soil, but also N mineralization rate (Glendining et al., 1996, Schröder et al., 2013, Schröder et al., 2005, Webb et al., 2013). If the mineralization of accumulated N is not considered in current fertilization, the risk for nitrate leaching is increased (Edmeades, 2003). Nevertheless, mineralizable organic N in soil cannot be readily measured and further depends, amongst others, on the nature of the manure itself, soil (texture) and climatic conditions (Schröder et al., 2013, Bhogal et al., 2016). Thus, recommendations for farmers on how to consider the residual fertilizer effect of (repeated) animal manure applications can only be based on models. Data for informing such models can be obtained by several means, with ^{15}N labelling being the method with least variability (Cusick et al., 2006, Berntsen et al., 2007).

The main objective of this study was to assess the NUE of animal manure and mineral fertilizer both in the year of application and during the following crops, the N retention in soil as well as N losses via nitrate leaching. To this end, we conducted a microplot study over three vegetation periods in which we used ^{15}N labelled mineral fertilizer (Min) and ^{15}N labelled cattle slurry (Slu). The study was located in the Swiss *Gäu* region, in which groundwater nitrate levels persistently exceed the Swiss quality criterion (Gerber et al., 2018). The region is characterized by intensive arable and vegetable production and has high annual rainfall (> 1000 mm $year^{-1}$), which increases the potential of nitrate leaching. Overall, we aimed at providing insights into the fate of N from cattle slurry in comparison to mineral fertilizer in the soil-plant system over several years, helping to develop strategies to optimize its NUE and to reduce nitrate leaching. We hypothesized that i) recovery of applied fertilizer N in plants was greater for mineral fertilizer than for cattle slurry, ii) a greater proportion of cattle slurry than mineral fertilizer N would remain in soil and that therefore iii) cattle slurry has an elevated leaching potential over mineral fertilizer. At the same time, we expected iv) a higher residual fertilizer NUE for Slu than for Min.

3.2 Material and Methods

3.2.1 Field site and experimental design

The field experiment was conducted as an on-farm trial on two field sites in the *Gäu* region, Canton Solothurn, Switzerland, between May 2018 and April 2020 (Field A) or July 2020 (Field B). It presents the continuation of Frick et al. (in revision) and further details can be found therein. In brief, the two fields followed a shifted crop rotation with silage maize – winter wheat – grass-clover (Field A) and grass-clover – silage maize – winter wheat (Field B) (**Fig. 3.1**). Both fields had been cultivated with sown grass-clover at least during three years before start of the experiment, receiving animal manure three to four times per year according to common agricultural practice. Fields differed slightly in texture, but were overall comparable in basic soil properties (**Table 3.1**). Bulk density, determined by cylinders in 5 cm increments, was similar on both fields. However, especially Field A had a considerable stone content below 30 cm. At Field B, stone content below 30 cm increased from east to west. Climatic conditions at the field site are temperate, with a mean annual temperature of 9.0 °C and a yearly precipitation of 1129 mm (1981 – 2010). However, weather conditions during 2018 and 2019, were characterized by abnormally hot and dry summer conditions (**Fig. 3.2**).

***Table 3.1**: Soil properties at the two field sites*

		Field A			Field B		
		0-30 cm	**30-60 cm**	**60-90 cm**	**0-30 cm**	**30-60 cm**	**60-90 cm**
Bulk density	[g cm^{-3}]	1.40 ± 0.06	1.55 ± nd	nd	1.45 ± 0.04	1.57 ± 0.03	1.63 ± 0.02
Stone content[a]	[Vol%]	5	30	40	0	0/20	10/50
pH[b]	[-]	5.5 ± 0.2	5.6 ± nd	5.6 ± nd	5.7 ± 0.2	5.8 ± 0.1	5.8 ± 0.1
C_{org}	g kg^{-1} DM	17.3 ± 0.4	10.1 ± 0.8	4.6 ± 0.4	17.8 ± 0.6	9.2 ± 1.5	5.4 ± 0.4
Total N	g kg^{-1} DM	1.9 ± 0.1	1.2 ± 0.1	0.6 ± 0.0	2.1 ± 0.3	1.0 ± 0.1	0.7 ± 0.1
Clay	[mass%]	22.0 ± 0.8	24.3 ± 0.9	26.3 ± 2.1	21.6 ± 1.0	22.3 ± 1.1	25.5 ± 0.5
Silt	[mass%]	35.8 ± 1.2	33.3 ± 1.8	23.9 ± 1.9	42.5 ± 1.5	42.9 ± 2.3	35.5 ± 4.3
Sand	[mass%]	39.6 ± 1.5	40.2 ± 1.8	49.0 ± 2.9	32.8 ± 0.6	33.2 ± 1.3	38.2 ± 3.9

[a] stone content was estimated visually in the field; at Field B, there was gradient in the stone content below 30 cm
[b]pH measured in CaCl2, 1:2.5

Three fertilizer treatments were implemented: ^{15}N labelled mineral fertilizer as $^{15}NH_4{}^{15}NO_3$ (Min, 8.00 atom% ^{15}N abundance), ^{15}N labelled cattle slurry (Slu, 7.89 atom% ^{15}N abundance), and a control treatment not receiving any ^{15}N labelled fertilizer (Con). Each fertilizer treatment was replicated four times, resulting in 12 microplots per field. On both fields, microplots were arranged in a complete randomized block design on a 3 m wide strip, 9 m apart from the fields' edges. According to the design proposed by Jokela and Randall (1987), the unconfined microplots had a size of 1.5 m x 2 m and were located in a way that two maize rows formed the edges of each microplot and one maize row formed the centreline of the plot (0.75 m row spacing).

^{15}N labelled cattle slurry was produced by feeding a young heifer with ^{15}N labelled ryegrass for 8 days. Faeces and urine were sampled separately and frozen daily. Later, faeces and urine fractions with the highest ^{15}N label were recombined and diluted 1:1 with demineralized H_2O in order to achieve a representative slurry. Details on the production and characterization of ^{15}N labelled slurry can be found in Frick et al. (in revision).

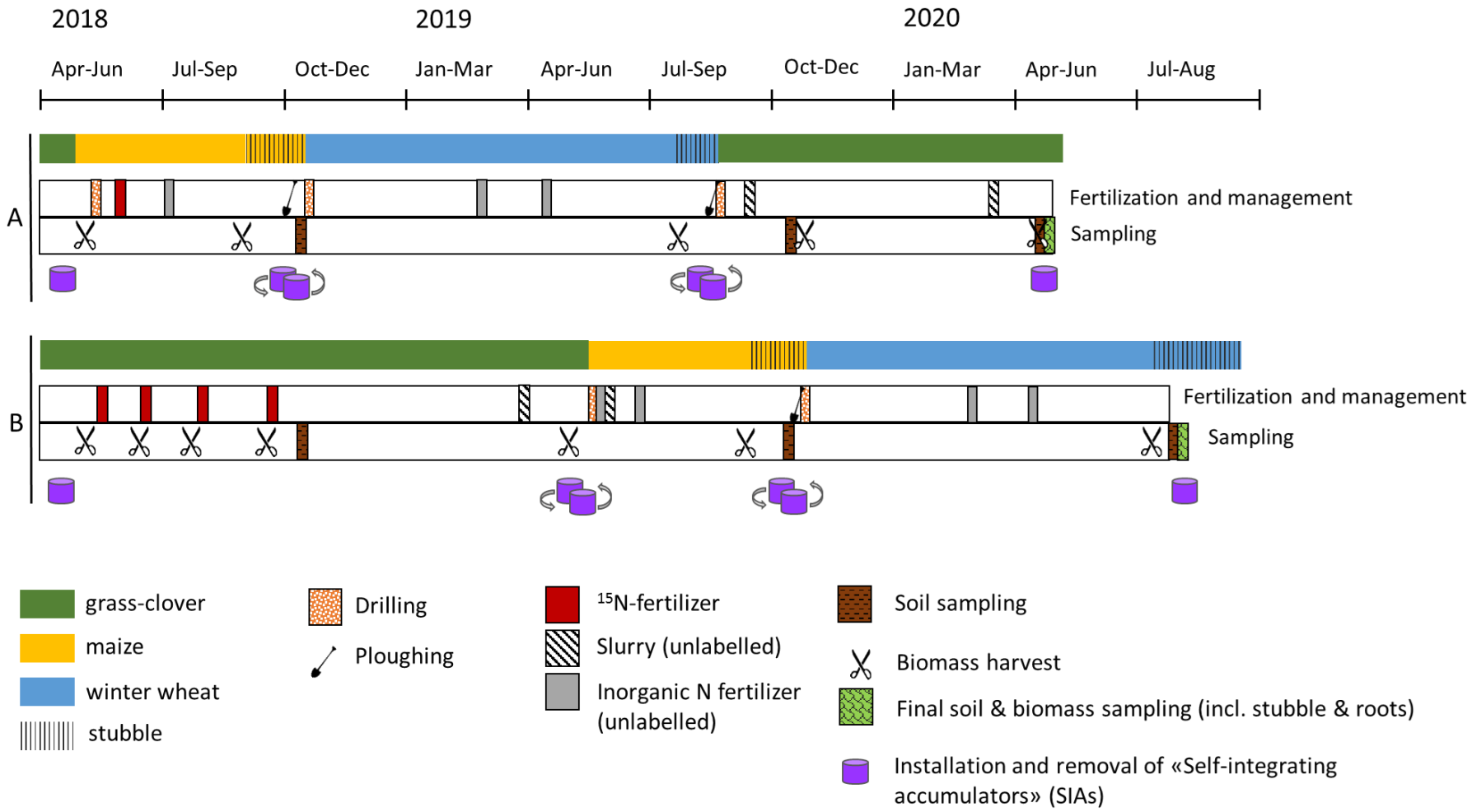

Fig. 3.1: *Crop rotation, fertilization and management as well as sampling scheme at the two fields A and B*

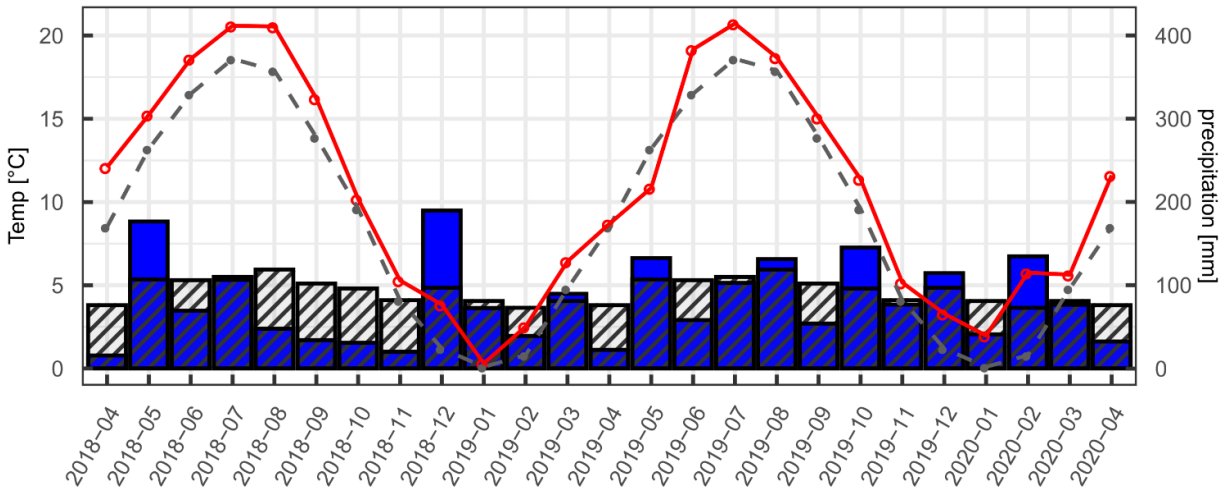

Fig. 3.2: *Weather conditions at Wynau (closest meteorological station) during the time frame of the experiment. Monthly mean temperature [°C] is indicated in red (open circles), monthly sum of precipitation [mm] is indicated with blue bars. Grey dotted line and shaded bars show long-term average values (1981 – 2010)*

3.2.2 Fertilizer application and microplot management

In 2018, both fields were fertilized with ^{15}N labelled fertilizers in amount and timing according to recommended agricultural practice.

On Field A, a single ^{15}N labelled fertilizer application was performed in the three to four leaf stage of the silage maize. Slurry was applied to contain 60 kg N ha^{-1}, equivalent to 36.8 kg NH_4-N ha^{-1}, while ^{15}N mineral fertilizer solution was applied at a rate equal to the NH_4-N-content of the slurry (i.e. 36.8 kg N ha^{-1}). Application was performed using canisters imitating drag hose application. On Con and Min microplots, additionally, phosphorus (P) (6.7 kg ha^{-1} P as triple super phosphate) and potassium (K) (75 kg ha^{-1} K as potassium sulphate, KaliSOP) were applied to compensate for the amounts of these elements contained in the slurry. Also, the amount of water added with the slurry was compensated in the other treatments. During a later growth stage, non-labelled urea was applied to the whole field including all microplots (69 kg N ha^{-1}). Application of animal manure to an early growth stage of the maize, but applying later N doses in form of urea, is a common

practice in the region. Furthermore, this way we were able to follow the fate of a single ^{15}N labelled fertilizer application.

On Field B, in total four repeated ^{15}N labelled fertilizer applications took place after each cut of the grass-clover during 2018. Upon each application, the same amounts and procedure as indicated for Field A were followed.

Weed and pest control were performed by the farmer for the whole field including microplots. From 2019, also fertilization was done by the farmer with non-labelled fertilizers (**Fig. 3.1**, **Table 3.2**). Cultivation measures that involved soil movement such as ploughing after harvesting the maize or winter wheat were conducted manually on the microplots.

Table 3.2*: N inputs over the duration of the experiment (details on management operations and other nutrient inputs can be found in* ***SI 3 Table 1)***

Field	Crop (Year)	Input type	N input amount
			kg N ha^{-1}
Field A	Maize (2018)	^{15}N fertilizer	0/36.8/60 for Con/Min/Slu
		Urea	69
	Winter wheat (2019)	Nitrophos	60
		Urea	92
	Grass-clover (2019/2020)	Cattle slurry	95 (of which 55 NH_4-N)
		Cattle slurry	95 (of which 55 NH_4-N)
Field B	Grass-clover (2018/2019)	^{15}N fertilizer (1st application)	0/36.8/60 for Con/Min/Slu
		^{15}N fertilizer (2nd application)	0/36.8/60 for Con/Min/Slu
		^{15}N fertilizer (3rd application)	0/36.8/60 for Con/Min/Slu
		^{15}N fertilizer (4th application)	0/36.8/60 for Con/Min/Slu
		Cattle slurry (spring)	95 (of which 55 NH_4-N)
	Maize (2019)	NPK	30
		Cattle slurry	76 (of which 44 NH_4-N)
		Urea	92
	Winter wheat (2020)	Nitrophos	40
		Urea	69

3.2.3 Biomass and soil sample collection and preparation

For maize and wheat, aboveground biomass was harvested from the central area of the microplots, at least 0.375 m away from the microplot edges. Plants were harvested upon maturity, about 10 cm above ground. Only the centre row on a length of 1.25 m (i.e. 0.75 m away from the plots edge) was used for ^{15}N analysis. The number of plants

(maize) or the number of ears (winter wheat) from the central row were counted. Maize plants were split into stems, leaves, grain, and husk + cobs, while wheat plants were split into stems, grains and husk. All plant parts were dried at 60 °C and weighed. Additionally, the two adjacent rows were harvested and fresh weight as well as the number of plants (maize) or ears (winter wheat) were determined directly in the field and used for getting a more representative estimate of the dry matter yield compared to only determining the yield based on the central row. Furthermore, the rows at 0.75 m distance outside the microplots were sampled and processed as the central row. These samples were used to check for potential dilution of the ^{15}N label by unlabelled N from outside the microplot. Since these samples did not have any ^{15}N enrichment above the level in Con, we assumed that the ^{15}N values from the central row, having the same distance from the plot edge as the outside row, can be considered undiluted from the outside and, thus, representative for N uptake solely from the area on which ^{15}N labelled fertilizers were applied.

For grass-clover, aboveground biomass was harvested with electric scissors from a 0.5 m x 0.5 m frame placed in the middle of each microplot ("inner frame"). Biomass was sorted into grass, legumes and other herbs, and dried at 40 °C. To get a more representative estimate of the yield, the harvesting area was increased to the whole central area of the microplot (1.25 m x 0.75 m, "outer frame") and total dry matter yield determined. It was assumed that the relative share of grass, legumes and herbs in the outer frame was the same as in the inner frame.

Upon the end of the experiment, final sampling included sampling of stubble and roots: For Field A, grass-clover stubbles were cut in the same 0.5 m x 0.5 m inner frame as the shoot biomass. Within this frame, soil was excavated in a 0.3 m x 0.2 m x 0.3 m cuboid, weighed and sieved through a 12 mm mesh in the field in order to quantify the amount of stones. Roots remaining on the sieve were collected and later washed under running tap water in the laboratory. From the sieved soil, a subsample of approx. 1 kg was brought to the laboratory, where it was washed through a 1 mm sieve in order to quantify the amount of roots in the sieved soil. For this, the roots in

the sieve residue were separated from mineral debris and exogenous organic material by combined decantation and manual sorting with tweezers (Hirte et al., 2017). Roots were dried in the oven at 60 °C. On a separate soil subsample, water content was determined by weighing before and after drying at 105 °C. For Field B (winter wheat), a similar procedure was followed, with cutting and collecting the stubble from the three central rows of the microplot on a length of 1.25 m. Afterwards, the soil from a 0.3 m x 0.2 m area in the centre of each microplot was excavated to a depth of 0.3 m. Again, separate soil subsamples were taken for root washing and determination of water content in soil, after sieving the soil through a 12 mm mesh in the field.

Dried biomass samples were homogenized in a cutting mill, and a subsample was pulverized in a ball mill (MM200 Retsch, Haan, Germany) for later analysis of N-content and ^{15}N enrichment.

Soil was sampled to a depth of 90 cm, divided into 30 cm increments, at the end of the vegetation periods in mid-October in 2018 and 2019. In 2020, soil sampling was performed upon finalizing the experiment, i.e. in April 2020 after harvest of grass-clover (Field A) and in July 2020 after harvest of winter wheat (Field B). Samples were taken as mixed samples from three cores (2 cm diameter) per plot with a distance of at least 37.5 cm from the edge of the microplot. Samples were stored in cooling boxes on the field and at 4 °C after reaching the lab. Within 24 hours, soil was sieved at 5 mm, and a subsample extracted with 0.5 M K_2SO_4, filtered through folded paper filters (Macherey Nagel Type 615, Ø 185 mm) and stored at -20 °C until analysis for ammonium and nitrate. The remaining sieved soil was air-dried and pulverized for analysis of ^{15}N in the total N pool.

3.2.4 Measuring nitrate leaching with self-integrating accumulators

Nitrate leaching was assessed cumulatively per growing-season using so-called self-integrating accumulators (SIAs) (Bischoff, 2007, Grunwald et al., 2020, Wey et al., 2022). In short, SIAs are patented passive samplers, consisting of PVC-tubes (diameter = height = 10 cm) filled with an ion-exchange-resin-sand mixture collecting leached nitrate and ammonium (TerrAquat Consultants; patent no. 197 26 813).

Three SIAs per microplot were installed at 1 m depth in horizontal access tunnels in order to place the SIAs underneath the undisturbed soil profile. SIAs were regularly exchanged after harvest of each crop (**Fig. 3.1**). After removing the SIAs, the sand-resin mix was split into three layers and the material was well mixed within each layer. Both, the uppermost layer (0 to 5 cm) and the middle layer (5 to 6 cm) were extracted with 1M NaCl solution for analysis of ammonium and nitrate. Thereby, the uppermost layer is supposed to hold all leached nitrate and ammonium, while the middle layer is used to check for the validity of this assumption. For analysing ^{15}N enrichment in nitrate, extracts were diffused on acidified glass fibre discs. First 200 mg MgO were added for the diffusion of ammonium during 72 hours shaking and afterwards 400 mg Devarda`s alloy were added to the same sample and again shaken for 72 hours for the sequential diffusion of nitrate on a separate filter disc (Goerges and Dittert, 1998).

3.2.5 Laboratory analysis of slurry, soil and biomass samples

Total N, NH_4-N, P and K content of the slurry were analysed on the fresh slurry at the laboratory for soil and environmental analysis (LBU, Eric Schweizer AG, Steffisburg, Switzerland).

Soil and SIA extracts were analysed colorimetrically for nitrate and ammonium: Nitrate content of the extracts was determined according to Keeney and Nelson (1982), while ammonium, both in soil and SIA extracts, was determined using the modified indophenol blue reaction (Krom, 1980). Both analyses were performed on an automated discrete analyser (Smartchem 450 Discrete Analyser, AMS Alliance).

All Ntot and ^{15}N analyses (soil, biomass, diffusion filters) were performed on an elemental analyser coupled with a continuous flow isotope ratio mass spectrometer (Pyro cube + isoprime100, Elementar, Germany). The amount of sample was adjusted to contain 20 to 30 µg N. International standards (IAEA-N1, IAEA-N2) and internal references were included as quality check in each analysis run.

3.2.6 Calculations

For all ^{15}N data, isotopic excess was calculated by subtracting the mean ^{15}N abundance (i.e. percentage of ^{15}N relative to total N) of non-labelled reference samples from the measured ^{15}N abundance. For the mineral fertilizer, the natural abundance of ^{15}N in air was subtracted as a reference (i.e. 0.3663 atom%), while for slurry the weighted mean ^{15}N abundance of non-labelled faeces and urine samples from the same animal was used as non-labelled reference (0.386 atom%) (Frick et al., in revision). For plant biomass or SIA extracts, the mean of the control treatment (Con) at the corresponding sampling time in the corresponding sample type (plant, soil, extracts) was used as a reference.

The ^{15}N excess was used to calculate the proportion of N derived from fertilizer (Ndff) in the samples (Hauck and Bremner, 1976):

$$Ndff_{rel}\,[\%] = \frac{atom\%\ \ ^{15}Nexcess\ sample}{atom\%\ \ ^{15}Nexcess\ fertilizer} \times 100 \qquad \textbf{Eq. 3.1}$$

where atom% ^{15}Nexcess sample is the ^{15}N enrichment of the considered compartment (i.e. plant (part), soil, extracts) and atom% ^{15}Nexcess fertilizer refers to N enrichment of either mineral fertilizer or slurry.

The amount of N derived from the fertilizer was calculated as:

$$Ndff\ [kg\ ha^{-1}] = \frac{Ndff_{rel}\,[\%]}{100} \times TN_i \qquad \textbf{Eq. 3.2}$$

where TN_i is the total amount of N in the considered compartment expressed in kg N ha^{-1}. TN_i was calculated from the N-concentration in the compartment multiplied with its dry weight in kg ha^{-1}. The mass of the soil per layer was determined by multiplying its volume with the bulk density (**Table 3.1**).

N derived from other sources (Ndfo) such as soil, unlabelled fertilizer or deposition was determined as difference between total N uptake and Ndff, or when grass-clover was grown, as difference between N uptake and the sum of Ndff and N from biological N fixation by clover (Nfix). Thereby, Nfix was calculated by the ^{15}N enriched dilution method for Slu and Min (McAuliffe et al., 1958), while for Con, Nfix was calculated by

the natural abundance method (Shearer and Kohl, 1986). Further details can be found in (Frick et al., in revision).

The recovery of the applied fertilizer in the different compartments was calculated as:

$$recovery[\%] = \frac{Ndff}{N_{applied}} \times 100 \quad \textbf{Eq. 3.3}$$

where $N_{applied}$ is the total amount of N applied with the labelled fertilizer.

Leached nitrate collected in SIAs was calculated as follows:

$$NO_3\ [kg\ N\ ha^{-1}] = \frac{NO_{3resin} \times weight_{resin}}{area_{SIA}} \quad \textbf{Eq. 3.4}$$

where NO_{3_resin} is the NO_3-N amount in the resin [kg N kg^{-1} resin], $weight_{resin}$ is the total weight of the adsorber resin material in the SIA [kg], and $area_{SIA}$ is the surface area of the SIA [ha].

For statistical analysis and data visualization, mean values of the three replicate SIAs per microplot were used. Since nitrate concentration was too low for reliable ^{15}N determination in eleven SIAs and one SIA was lost upon excavation, in five out of 72 cases mean values were only based on two replicates, in two cases on one replicate and for one microplot in year 2018 no reliable data could be obtained. However, since nitrate leaching losses in most of these cases were minimal, overall results are hardly affected.

Cumulative recovery in harvested biomass, cumulative nitrate leaching, as well as recovery in soil, roots and stubble upon the final sampling were summed up in order to assess the fate of the labelled fertilizers in the soil-plant-system over the duration of the experiment. To complement the balance, NH_3 emission upon application of the fertilizers in the first year were included (Frick et al., in revision).

In order to assess the availability of the residual fertilizer N left in the system after harvest of the pre-crop(s), for 2019 and 2020 residual recovery was calculated by two different approaches: In the first approach, residual recovery was calculated relative

to the measured amount of ^{15}N labelled fertilizer left in soil (compare method 3 in Smith and Chalk, 2018):

$$residual\ recovery_{soil}[\%] = \frac{Ndff_{crop}}{Ndff_{soil}} \times 100 \qquad \textbf{Eq. 3.5}$$

with $Ndff_{crop}$ denoting Ndff in crop in the residual years and $Ndff_{soil}$ denoting Ndff in soil (0 – 90 cm) in October of the preceding year, thus, residual fertilizer N in soil. Both are given in kg N ha^{-1}.

With this approach, however, ^{15}N in stubbles and roots of the pre-crop, which might get mineralized and become plant available later, is not taken into account. Thus, we applied an additional approach as:

$$residual\ recovery_{output}[\%] = \frac{Ndff_{crop}}{N_{applied} - Ndff_{precrop(s)} - Ndff_{leaching} - NH_3} \times 100 \qquad \textbf{Eq. 3.6}$$

where $Ndff_{crop}$ is Ndff in crop, $N_{applied}$ is the amount of labelled N applied in 2018, $Ndff_{precrop(s)}$ is Ndff amount harvested with aboveground biomass of the preceding crop(s), $Ndff_{leaching}$ is Ndff collected in leached nitrate of the preceding year(s) and NH_3 is the mean amount of NH_3-N volatilized upon application of the fertilizers in 2018 (all numbers in kg N ha^{-1}).

3.2.7 Statistical analysis

Data preparation and statistical analysis were performed using R (Version 3.5.3) (R Core Team, 2019). Throughout, a significance level of $p < 0.05$ was applied. Statistical analysis were performed separately for the two fields using mixed effect linear models (lmer within package *lme4*). Throughout, model validation was performed by qq-plotting. *emmeans*-package was used for pairwise comparisons. p-value adjustment for multiple comparisons was performed according to the Tukey-method.

For biomass yield, TN uptake, Ndff and recovery, analyses were performed separately for the years and included *treatment* as fixed effect and *block* as random effect. For recovery, log-transformed data were used.

For assessing statistically significant difference between the fertilizers in terms of depth translocation in soil over the years, separate mixed effect linear models were fitted for recovery and Ndff in soil with *depth, year* and *treatment* as well as their two-way interactions as fixed effects. To account for repeated measurements with time and the non-independent structure of the different depth layers, *microplot, year:microplot* and *block* were introduced as random factors. Analysis were performed on log-transformed data. Since measurements in the 60 – 90 cm depth layer in 2018 were close to the quantification limit, and thus, turned negative, these values had to be excluded from statistical analysis (concerned n = 6 for Field A, n = 2 for Field B). However, for calculations of the residual $recovery_{soil}$ and within figures, negative values were replaced by 0.

For nitrate leaching, including Ndff and recovery in leached nitrate, *microplot* as well as *block* were used as random effects to account for repeated measurements. *Treatment* and *year* as well as their interaction were included as fixed effects in the mixed effect linear models. In addition, cumulated values for nitrate leaching, Ndff in nitrate and recovery over the three sampling periods were compared between the fields considering *treatment, field* and their interaction as fixed effects and *block* as random effect. Log-transformed data were used, except for cumulated nitrate leaching and Ndff, where non-transformed data could be used.

Usually missing values were excluded from statistical analysis. This concerned two missing values upon biomass sampling at Field A in 2019, caused by game damage, and one missing value for nitrate leaching, because concentration was too low for ^{15}N determination (see 3.2.6). To calculate the overall balance and *residual* $recovery_{output}$, however, we replaced missing values by the mean of the other replicates per treatment and field.

3.3 Results

3.3.1 N use efficiency in crops

Overall, dry matter yield and N uptake were similar for all fertilizer treatments throughout the experiment (**Table 3.3**). There were two exceptions with grass-clover yield at Field A in 2019/20 being significantly greater for Slu than for the other treatments, and grass-clover yield at Field B in 2018/19 being lower in Con than in the fertilized treatments. N uptake was slightly but significantly greater for Slu than for the other treatments in maize (2018) and in grass-clover (2019/20) at Field A (**Table 3.3**). Dry matter yield levels for the different crops were similar between the two fields. However, N uptake for both maize (2018, Field A) and wheat (2019, Field A) was lower than values obtained in the succeeding year at Field B.

In contrast to total N uptake and dry matter yield, Ndff was about 1.3-times higher for Min than for Slu in 2018, both at Field A ($p = 0.004$) and Field B ($p = 0.028$) (**Table 3.3**). In the year of application, plants took up 11.7 % (Field A) or 23.3 % (Field B) of their N-demand from ^{15}N labelled mineral fertilizer. For Slu, these shares amounted to 7.7 % at Field A and 19.3 % at Field B. In the two following years, $Ndff_{rel}$ declined to less than 1.5 % (Field A) or less than 5 % (Field B) of plant N uptake. Thereby, differences in absolute amounts of Ndff were always statistically significant between the two fertilizer treatments, but in contrast to the first year, Ndff for Slu was higher than Ndff for Min (**Table 3.3**).

***Table 3.3**: Total dry matter yield, N uptake and source of N uptake for the three crops at the two fields during 2018-2020; mean ± standard deviation; n = 4 (except Field B Maize 2019 Min and Slu n = 3); Ndff = N derived from fertilizer, Nfix = N from biological nitrogen fixation by clover; Ndfo = N derived from other sources such as soil N, deposition, unlabelled fertilizer N*
Different letters indicate statistically significant difference between the treatments within the same crop at $p < 0.05$.

Field	Crop (Year)	Treatment	Yield	N uptake	Ndff	Nfix[3]	Ndfo
			dt ha^{-1}	kg N ha^{-1}			
Field A	Maize (2018)	Con	164.0 ± 6.0^{ns}	137.3 ± 9.9^{a}	-	-	137.3 ± 9.9^{a}
		Min	179.1 ± 9.2^{ns}	140.3 ± 4.5^{a}	16.4 ± 0.9^{a}	-	123.9 ± 5.1^{b}
		Slu	176.1 ± 10.3^{ns}	149.7 ± 4.9^{b}	11.5 ± 1.0^{b}	-	138.2 ± 4.8^{a}
	Winter wheat (2019)	Con	129.5 ± 10.8^{ns}	192.2 ± 17.5^{ns}	-	-	192.2 ± 17.5^{ns}
		Min	125.5 ± 13.6^{ns}	182.8 ± 18.1^{ns}	1.3 ± 0.1^{a}	-	181.5 ± 18.0^{ns}
		Slu	122.8 ± 3.8^{ns}	192.9 ± 14.3^{ns}	2.5 ± 0.1^{b}	-	190.3 ± 14.3^{ns}
	Grass-clover (2019/2020) [1]	Con	45.6 ± 2.0^{a}	129.7 ± 10.3^{ab}	-	27.9 ± 13.8^{ns}	101.7 ± 7.0^{a}
		Min	44.1 ± 1.0^{a}	117.8 ± 1.9^{a}	0.6 ± 0.1^{a}	14.3 ± 4.9^{ns}	102.9 ± 6.1^{ab}
		Slu	51.4 ± 4.0^{b}	143.5 ± 10.5^{b}	1.5 ± 0.3^{b}	17.1 ± 15.8^{ns}	125.0 ± 17.4^{b}
Field B	Grass-clover (2018/2019) [2]	Con	96.0 ± 5.8^{a}	254.2 ± 36.0^{ns}	-	43.1 ± 28.5^{ns}	211.1 ± 9.5^{ns}
		Min	115.8 ± 5.2^{b}	297.3 ± 21.9^{ns}	69.4 ± 7.4^{a}	19.0 ± 15.6^{ns}	208.9 ± 23.1^{ns}
		Slu	114.4 ± 7.8^{b}	287.4 ± 27.9^{ns}	55.4 ± 1.4^{b}	14.2 ± 10.2^{ns}	217.8 ± 26.3^{ns}
	Maize (2019)	Con	184.3 ± 16.0^{ns}	194.9 ± 17.5^{ns}	-	-	194.9 ± 17.5^{ns}
		Min	184.8 ± 5.1^{ns}	192.6 ± 1.5^{ns}	5.7 ± 0.1^{a}	-	186.9 ± 1.5^{ns}
		Slu	197.8 ± 7.9^{ns}	211.0 ± 23.8^{ns}	11.1 ± 1.3^{b}	-	200.0 ± 22.7^{ns}
	Winter wheat (2020)	Con	123.8 ± 6.2^{ns}	220.5 ± 19.7^{ns}	-	-	220.5 ± 19.7^{ns}
		Min	123.1 ± 8.8^{ns}	222.8 ± 16.9^{ns}	2.4 ± 0.3^{a}	-	220.4 ± 16.6^{ns}
		Slu	124.0 ± 10.6^{ns}	221.0 ± 21.5^{ns}	4.6 ± 0.6^{b}	-	216.3 ± 21.2^{ns}

[1]Grass-clover data refers to cumulated values over two cuts between Sep 2019 and Apr 2020
[2]Grass-clover data refers to cumulated values over four cuts between Jun 2018 and Apr 2019
[3]For Min and Slu, Nfix was calculated by the ^{15}N enriched dilution method (McAuliffe et al., 1958), while Nfix of Con was calculated by the natural abundance method (Shearer and Kohl, 1986)

Relative to the total amounts of ^{15}N labelled mineral fertilizer applied in 2018, harvested plant biomass recovered 44.7 to 47.1 % in 2018, 3.6 to 3.9 % in 2019 and 1.6 to 1.7 % in 2020. For Slu, recoveries in biomass amounted to 19.2 to 23.1 % in 2018, 4.2 to 4.6 % in 2019 and 1.9 to 2.4 % in 2020 (**Table 3.4**, **Fig. 3.3**). Thereby, differences between Min and Slu were small, but statistically significant except for Field A 2019.

Upon finalizing the experiment, also root and stubble biomass and ^{15}N contents were assessed. For Field A, shoot biomass of the final grass-clover cut in April 2020 (25 to 29 dt ha^{-1}), stubble and root each yielded about the same amount of dry matter (**SI 3 Table 2**). Recovery of originally applied fertilizer N was < 0.8 % in roots and < 0.6 % in stubble. For Field B, combined root plus stubble biomass amounted to about 17 % of harvested aboveground biomass of wheat, and ^{15}N fertilizer amounts recovered in stubble and roots were negligible (< 0.5 %) (**Fig. 3.3**).

***Table 3.4**: Recovery and residual fertilizer recovery in crop biomass.* Recovery *is expressed relative to the originally applied amount of ^{15}N fertilizer.* Residual $recovery_{soil}$ *refers to the recovery based on residual ^{15}N amount measured in soil (0 – 90 cm) in October of the preceding year.* Residual $recovery_{output}$ *refers to the recovery of calculated residual ^{15}N left in system after considering N-uptake by pre-crop(s) and losses via NO_3 leaching and NH_3: mean ± standard deviation; n = 4 (except Field B Maize 2019 Min and Slu n = 3). Different letters indicate statistically significant differences between the treatments within the same crop at p < 0.05.*

Field	Crop (Year)	Treatment	Recovery	Residual $recovery_{soil}$ %	Residual $recovery_{output}$
Field A	Maize	Min	44.7 ± 2.6^{a}	-	-
	(2018)	Slu	19.2 ± 1.7^{b}	-	-
	Winter wheat	Min	3.6 ± 0.4^{ns}	13.0 ± 2.6^{a}	6.7 ± 1.1^{ns}
	(2019)	Slu	4.2 ± 0.2^{ns}	7.6 ± 2.0^{b}	5.8 ± 0.2^{ns}
	Grass-clover	Min	1.7 ± 0.3^{a}	3.8 ± 1.1^{ns}	3.7 ± 1.2^{ns}
	(2019/2020)[1]	Slu	2.4 ± 0.5^{b}	5.0 ± 1.1^{ns}	3.9 ± 0.6^{ns}
Field B	Grass-clover	Min	47.1 ± 5.0^{a}	-	-
	(2018/2019[2]	Slu	23.1 ± 0.6^{b}	-	-
	Maize	Min	3.9 ± 0.0^{a}	$10.8^{3} \pm 2.6^{ns}$	8.0 ± 0.9^{ns}
	(2019)	Slu	4.6 ± 0.5^{b}	$8.1^{3} \pm 2.4^{ns}$	7.1 ± 0.7^{ns}
	Winter wheat	Min	1.6 ± 0.2^{a}	4.2 ± 0.7^{ns}	3.6 ± 0.4^{ns}
	(2020)	Slu	1.9 ± 0.2^{b}	3.0 ± 0.4^{ns}	3.2 ± 0.4^{ns}

[1]Grass-clover data refers to cumulated values over two cuts between Sep 2019 and Apr 2020
[2]Grass-clover data refers to cumulated values over four cuts between Jun 2018 and Apr 2019
[3]Note: With grass-clover as pre-crop, residual $recovery_{soil}$ might be underestimated as soil samples were always taken in October of the preceding year, neglecting the overwintering

grass-clover. If ^{15}N taken up by overwintering grass-clover is considered, residual recovery$_{soil}$ in maize 2019 would increase from 10.8 to 12.2 % for Min and from 8.1 to 8.8 % for Slu.

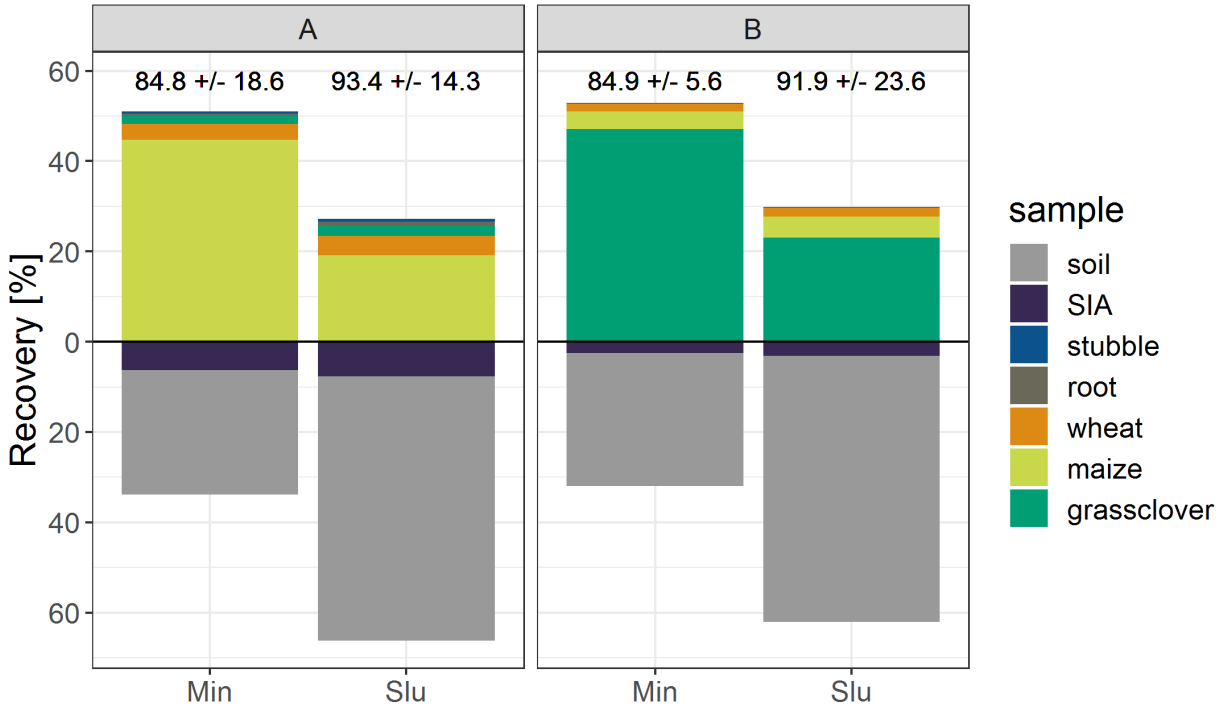

Fig. 3.3: *Recovery of ^{15}N labelled fertilizers over the years 2018, 2019 and 2020 at Field A (left) and Field B (right). For aboveground biomass data is shown separately for the individual crops. For nitrate leaching collected in self-integrating accumulators (SIA) data is shown cumulative over the three years. For stubble, roots and soil (0 – 90 cm), data from the final sampling in 2020 were used. Numbers on top indicate cumulated recovery in all measured compartments (mean ± standard deviation, n = 4)*
Note: Ammonia emission were recorded within Frick et al. (in revision) and amounted at Field A to ~0 % for Min and 6.6 % for Slu and at Field B to 3.4 % for Min and 10.1 % for Slu relative to the applied amounts.

3.3.2 Fertilizer recovery in soil

Throughout the experiment, fertilizer recovery in different soil depth layers showed a similar distribution for both fields and over time (**Fig. 3.4**): Most fertilizer N was recovered in the top 30 cm for both fertilizers and both fields. Thereby, recovery tended to be greater for Slu than for Min, but differences were only statistically significant in 2018 and 2020 (Field A) or in 2020 (Field B). Even upon the final sampling in 2020, still 44 to 52 % of applied slurry N was recovered in topsoil while for mineral fertilizer this share amounted to 20 to 23 % of the mineral fertilizer applied in 2018.

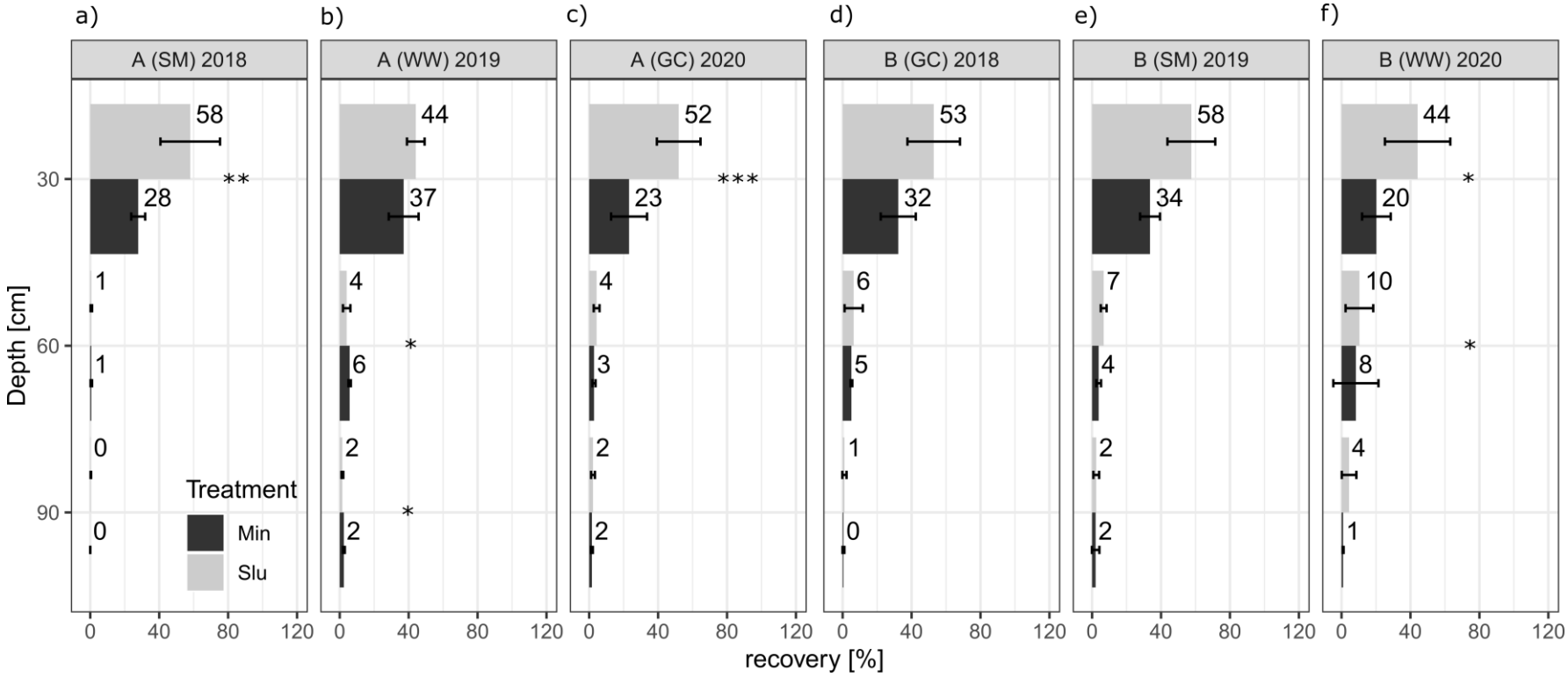

Fig. 3.4: *^{15}N recovery in soil relative to originally applied fertilizer for Field A (a – c) and for Field B (d – f). Samples in 2018 and 2019 were taken at the end of the vegetation period in mid-October, while sampling in 2020 took place upon harvest of the grass-clover in April (Field A) or upon harvest of winter wheat in July (Field B)*
SM = silage maize, WW = winter wheat, GC = grass-clover
*mean ± standard deviation, n = 4, with *($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$)*

Only minor shares of fertilizer or slurry N were translocated into deeper soil layers during the 2.5 years duration of this experiment. At Field A, recovery of labelled N in deeper soil layers was negligible in 2018, but increased slightly in 2019 ($p < 0.0001$). At Field B, already in the first year, 5 to 6 % of both Slu and Min was translocated to the 30 to 60 cm layer, but except a significant increase in 90 cm from 2018 to 2019 in Slu, further increases over the years were not statistically significant and similar between the two treatments. However, a slightly higher share of Slu compared to Min was recovered in the 30 to 60 cm layer in 2020 ($p = 0.03$).

In contrast to recovery, which gives relative results based on the originally applied fertilizer N, absolute amounts of residual fertilizer N in soil showed clear differences between the fertilizer treatments, with Ndff for Slu up to two to three times higher than for Min (**SI 3 Fig. 5**). Again, depth translocation was minor and on average less than 2 kg labelled N (Field A) or less than 10 kg labelled N (Field B) were found below 60 cm depth in 2020. Differences between the years were small, but at Field A, the increase from 2018 to 2019 in Ndff in the soil layers below 30 cm was highly significant for both Min and Slu ($p < 0.001$). For Field B, only the increase from 2018 to 2019 in the 90 cm depth layer was significant for Slu ($p = 0.04$).

3.3.3 Nitrate leaching from ^{15}N labelled fertilizers

Nitrate leaching did not differ between treatments, but there was a highly significant effect of the leaching period ($p < 0.001$). Thereby, the highest leaching under both fields was found under winter wheat, with values ranging between 73 and 106 kg NO_3-N ha^{-1} at Field A (2019) and between 128 and 194 kg NO_3-N ha^{-1} at Field B (2020) (**Fig. 3.5**). High nitrate leaching coincided with high nitrate levels in soil in October of the preceding year (**SI 3 Fig. 4**).

The amount of nitrate leached from ^{15}N labelled fertilizers was low. At both fields, Ndff in leached nitrate underneath winter wheat was significantly higher than underneath the other crops. During this time, more slurry N (for Field A in 2019: 3.5 kg N ha^{-1}, for Field B in 2020: 6.9 kg N ha^{-1}) than mineral fertilizer N (for Field A in

2019: 1.5 kg N ha^{-1}, for Field B in 2020: 3.5 kg N ha^{-1}) was leached, but these differences were not statistically significant (**Fig. 3.5**).

There were no differences between Min and Slu for the recovery of labelled fertilizers in leached nitrate. Total nitrate leaching, Ndff, and recovery were highest underneath winter wheat with no differences between the other crops (**Fig. 3.3**, **Fig. 3.5**).

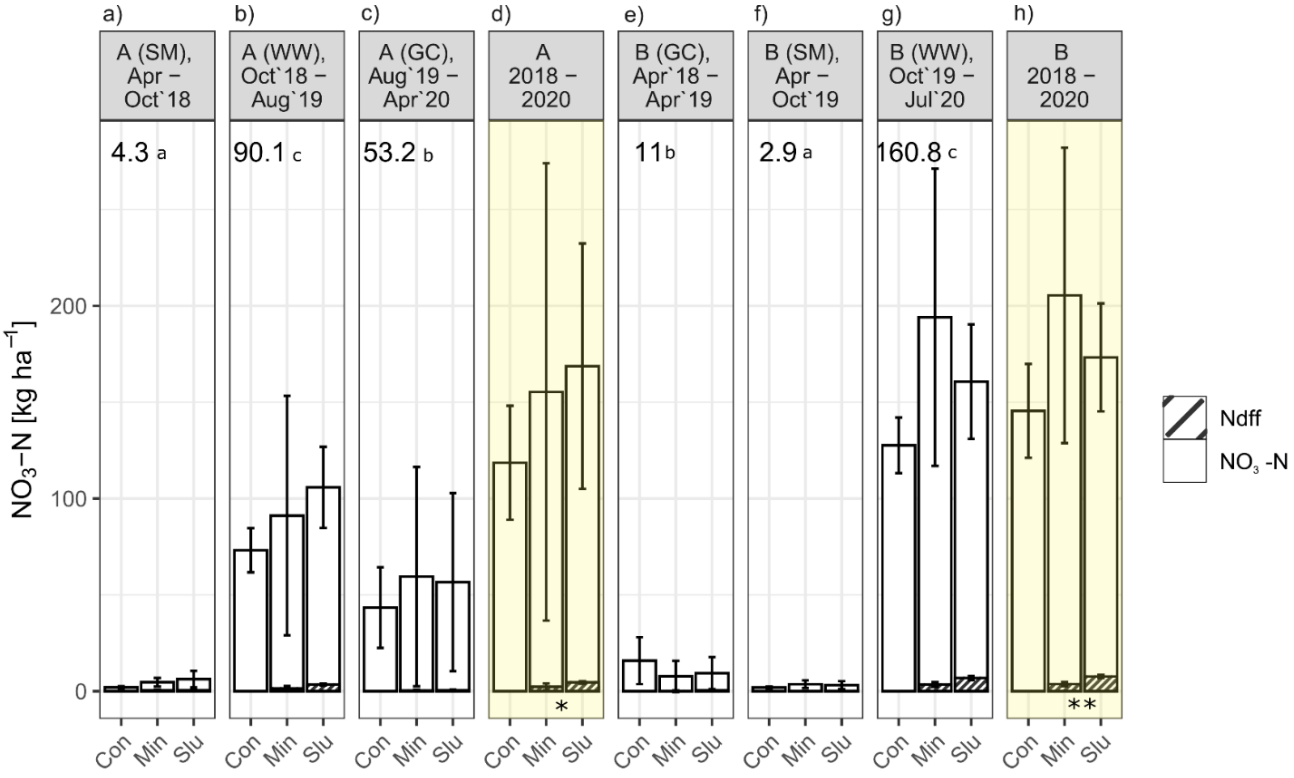

Fig. 3.5: *Nitrate leaching measured with self-integrating accumulators (SIAs) at Field A (a – c) and at Field B (e – g) during three consecutive leaching periods. Cumulated values over the whole time frame are indicated in d) and h); n = 4, mean ± standard deviation; SM = silage maize, WW = winter wheat, GC = grass-clover; Ndff = N derived from fertilizer; numbers on top show average nitrate leaching at the individual leaching periods. Within each field, numbers followed by different letters are significantly different at p < 0.05.*
*Statistically significant differences between Min and Slu in Ndff are indicated with *(p < 0.05) and ** (p < 0.01). For total NO_3-N leaching there were no statistically significant differences.*

Cumulated over the three vegetation periods, NO_3 leaching did not differ between the two fields nor between the three fertilizer treatments and ranged on average between 119 and 205 kg NO_3-N ha^{-1} (**Fig. 3.5**). Of this amount, 2 to 8 kg NO_3-N ha^{-1} originated from the ^{15}N labelled fertilizers. Thereby, the absolute amount of fertilizer N lost via nitrate leaching was significantly different between treatments ($p < 0.001$) and fields

($p = 0.004$), with Ndff (kg N ha^{-1}) for Slu higher than for Min and for Field B higher than for Field A. Thus, summarized over both fields, for Slu > 95 % and for Min > 98 % of leached nitrate did not originate from ^{15}N labelled fertilizers applied in 2018. Recovery of ^{15}N labelled fertilizers in leached nitrate was significantly higher ($p = 0.003$) for the single ^{15}N labelled fertilizer application to maize at Field A (6.3 to 7.7 %) than for the repeated applications to grass-clover at Field B (2.5 to 3.2 % of applied ^{15}N labelled fertilizer), but there were no differences between Min and Slu.

3.3.4 Residual fertilizer value of cattle slurry and mineral fertilizer

Higher proportions of applied ^{15}N slurry than ^{15}N mineral fertilizer were recovered in crop biomass in the two residual years (compare 3.3.1). However, when considering only the amounts of ^{15}N fertilizers left in soil or the soil-plant system after harvest of the preceding crop(s), residual recovery of mineral fertilizer and slurry showed no differences (**Table 3.4**). In the first residual year (2019), residual recovery ranged between 5.8 to 13 % of the remaining fertilizer in soil, while in the second residual year (2020), values ranged between 3 to 5 %. It must be noted, that estimates based on the ^{15}N measured in soil in October of the preceding year, tended to be slightly higher and more variable than values based on the calculated residual fertilizer amount considering losses via nitrate leaching, ammonia volatilization and plant uptake.

Summing up the recovery of residual fertilizer N in plant uptake, leaching and soil ideally should yield values close to ±100 %. For $residual\ recovery_{output}$, this was roughly achieved, however, especially for $residual\ recovery_{soil}$, some values clearly exceeded 100 % (**SI 3 Fig. 2**).

3.3.5 ^{15}N soil-system balance over three cropping seasons

Similarly, upon the end of the experiment, cumulative recovery of originally applied fertilizer N in plants, leached nitrate, soil, stubble and roots should, together with NH_3 emissions, sum up to approximately 100 %. For Min, we recovered about 85 % of applied fertilizer N in the measured compartments, with NH_3 losses adding up to 3 %

of the applied amounts (**Fig. 3.3**). For Slu, these values were slightly higher and reached 92 to 93 %, with NH_3 emissions adding 7 to 10 %. Cumulative recoveries obtained on the two fields showed a high accordance. Despite overall similar cumulative recoveries, distribution between aboveground and belowground recoveries differed between Min and Slu, with higher recovery of Min in plants, but higher recoveries of Slu in soil.

3.4 Discussion

3.4.1 Nitrogen use efficiency in crops greater for mineral fertilizer than for cattle slurry

^{15}N mineral fertilizer recovery in aboveground biomass of the first crop was about double the recovery of slurry N at both fields (**Table 3.4**). This is in accordance with our hypothesis and was also reported by others (Bosshard et al., 2009, Thomsen et al., 1997). Partly, this can be explained by the fact that the applied amount of total N was about 1.6 times higher for Slu than for Min since the same amount of mineral N was applied with both fertilizers. Recovery, as a percentage of total applied N, thus, is lower for Slu than for Min (**Eq. 3.3**). However, also Ndff was significantly higher for Min than for Slu in the first year (**Table 3.3**). This contradicts results by Bosshard et al. (2009), but it indicates that mineral N within slurry was less available for plants, as also found by others (Sørensen, 2004). Likely, it results from a combination of processes such as higher NH_3 volatilization from Slu than from Min and a higher microbial immobilization of ammonium N from slurry in soil due to simultaneous addition of organic material with the slurry (Frick et al., in revision, Gutser and Dosch, 1996).

Differences in biomass yield and N uptake between the treatments were small or absent (**Table 3.3**). This can be explained by the overall small differences in N inputs between the treatments and by the non-labelled fertilizers applied by the farmer (**Table 3.2**). Furthermore, both fields had been cultivated with grass-clover for at least three years before commencement of the experiment, receiving three to four

applications of cattle slurry per year. Thus, soils presumably had a high mineralization potential of accumulated N.

Recoveries in aboveground biomass in the subsequent years were similar between the fertilizer treatments (**Table 3.4**) and fell in the range of values reported in the literature (e.g. Smith and Chalk, 2018). Despite considerable biomass production by roots and stubble, fertilizer recovery in these plant parts at the end of the experiment was low and, thus, only contributed marginally to the cumulative recovery of fertilizer N in the soil-plant-system (**SI 3 Table 2**).

3.4.2 Persistently high fertilizer recoveries in soil

Complementary to greater fertilizer recovery in biomass for Min than for Slu, we anticipated that more cattle slurry N than mineral fertilizer N would remain in soil. This was indeed the case, although differences were not statistically significant at most time points (**Fig. 3.4**). Overall, ^{15}N amounts recovered in 0 to 30 cm depth (for Min ranging between 20 to 37 %, for Slu ranging between 44 to 58 %) were comparable to results obtained by others (Sørensen, 2004, Muñoz et al., 2003). We did not observe major changes over time in the ^{15}N recovered from mineral fertilizer or cattle slurry in the different depth layers (**Fig. 3.4**). Similarly, in a 3-year field study, Muñoz et al. (2003) found recovery of animal manure in the 0 to 30 cm depth layer to persist at > 82 % of total ^{15}N recovered in soil up to 90 cm depth. Changes could have been expected due to a) plant N uptake, b) losses via nitrate leaching, or c) losses as N_2O or N_2 emissions from nitrification or denitrification. In our study, both plant N uptake of residual fertilizer N and nitrate leaching only happened to a minor extent (**Table 3.4** and **Fig. 3.5**). Denitrification losses, despite their relevance for climate change, only concern about 1 % of applied fertilizer N (IPCC, 2006) and are, thus, only a minor loss pathway. Overall, these observations fit well with the continued high recovery of fertilizer N in soil. Fluctuations, especially in topsoil, were likely due to mineralization of ^{15}N that had been previously incorporated in plant roots or stubble

(Hoekstra et al., 2011), but these plant parts were not sampled except at the final sampling.

Observing that about a fifth to a quarter of mineral fertilizer N and about half of cattle slurry N remained in soil even after the third vegetation period (**Fig. 3.4**) could hint towards N accumulation in soil, especially considering that arable fields are usually regularly fertilized with both mineral fertilizer and manures. However, with increased N stocks under continuous inputs, also mineralization-immobilization turnover and potential nitrification rates were shown to be increased (Luxhøi et al., 2004, Luxhøi et al., 2007). We found that plants took up most of their N demand from sources other than the labelled fertilizers, even in the first year (**Table 3.3**). It can be assumed that most of it originated from mineralization of soil N. Thereby, plants under Min had higher Ndff values in the first year than plants fertilized with Slu, indicating that plants fertilized with slurry needed to take up more N from soil to reach the same levels of total N uptake. The observed higher amounts of slurry-N remaining in soil, thus, indicate an enhanced refilling of these soil N reserves and a potential long-term supply over the level of mineral fertilizer. Indeed, we could confirm the latter as we observed higher Ndff values from Slu than for Min in the two residual years (**Table 3.3**).

Nevertheless, in remains challenging to predict the long-term development of soil N levels under continuous fertilization and there is no consensus on the differential effect of repeatedly applied animal manure versus mineral fertilizer. Mulvaney et al. (2009) argue that mineral N fertilizers deplete soil N by increased mineralization due to a lowered C:N ratio, but this was queried by others (Powlson et al., 2010, Glendining et al., 1996). Edmeades (2003) reported increased organic matter with long term manure application. At the same time, there is concern about declining soil organic matter (SOM) and declining soil N stocks under cultivated land and it was shown that manure application could just barely compensate for it (Ladha et al., 2011, Bosshard, 2007). It is also emphasized by the fact that total N outputs (plant N uptake + nitrate leaching losses) exceeded N inputs with fertilizers (both labelled and

unlabelled) when summarized over the whole experimental crop rotation (**Table 3.2**, **Table 3.3**, **Fig. 3.5**).

Also, protection of N inputs in aggregates and different SOM fractions plays an important role in understanding the fate of residual mineral fertilizer and slurry. Bosshard et al. (2008) found most ^{15}N in soil recovered in the mineral associated organic matter fraction (MAOM), irrespective whether it originated from ^{15}N labelled mineral fertilizer or ^{15}N labelled sheep faeces. Thereby, MAOM was assumed to have a low turn-over rate, potentially explaining the observed low residual fertilizer effect (**Table 3.4**). However, recent evidence suggests that also N from MAOM might get plant available (Daly et al., 2021, Jilling et al., 2018). Furthermore, Bosshard et al. (2008) showed that upon experimental fractionation, a substantial amount of N was lost, confirming the importance of aggregates to protect SOM and highlighting the potential effect of soil tillage on re-mineralization and potential loss of stabilized fertilizer N in soil. We could confirm this observation as we found the highest mineral N release (both in terms of nitrate levels in soil as well as in terms of nitrate leaching) after termination of grass-clover (compare 3.4.4, **SI 3 Fig. 4**, **Fig. 3.5**).

3.4.3 Minor nitrate leaching from recently added fertilizers

We hypothesized that cattle slurry had a higher leaching potential than mineral fertilizer due to a greater total N input combined with a larger proportion of cattle slurry N remaining in soil and a potentially increased mineralization-immobilization turnover rate in soil fertilized with cattle slurry. In terms of total nitrate leaching, we did not observe differences between the fertilized treatments nor to the unfertilized control (**Fig. 3.5**). This can be related to the small differences in N inputs between the treatments and is in accordance with the insignificant differences in total N uptake by plants. In agreement with our hypothesis, cumulated nitrate leaching from slurry N was indeed higher than from mineral fertilizer N. It supports the suggestions by others (e.g. Sørensen, 2004, Thomsen et al., 1997, Gutser and Dosch, 1996) that increased accumulation in soil under animal manure would increase leaching. The amounts of labelled fertilizer N leached were small for both Min and Slu, though. This

is in accordance with several other studies finding that newly added fertilizer N gets barely leached (Glendining et al., 1996, Glendining et al., 2001, Thomsen et al., 1997, Jayasundara et al., 2010, Macdonald et al., 1989). Reported shares of N leaching from cattle slurry or mineral fertilizer during two to three years after fertilizer addition range between 3 to 10 % of applied N, but they also depend on soil type and climatic conditions. Using suction cups, Jayasundara et al. (2010) found that under a corn-corn rotation on silt loam 4.5 to 6.9 kg of ^{15}N labelled swine manure N were leached over two years, which is in the same range as the values we found. However, on sandy soil they found higher values, ranging between 12.8 to 21.5 kg manure N ha^{-1}, equivalent to a relative Ndff share of up to 25 % of leached nitrate originating from swine manure. It must be noted, though, that total amounts of mineral N leaching found by Jayasundara et al. (2010) were considerably lower than in our experiment (annual mineral N leaching losses were less than 65 kg N ha^{-1} in the first and less than 30 kg N ha^{-1} in the second year after addition of 150 kg N ha^{-1}). These differences might relate to the measurement method: In our study, we used SIAs for measuring nitrate leaching and as shown by Wey et al. (2022), this method usually yields higher values than suction cups as the latter cannot fully account for preferential flow through macropores. Especially within the first measurement period underneath maize between April and September 2018, we observed high $Ndff_{rel}$ values, ranging between 2.3 and 12.1 % of leached nitrate (**SI 3 Fig. 1**). Overall, leaching amounts in this rather dry period with high evapotranspiration and low precipitation were small. We, thus, assumed that the leached nitrate originated from preferential flow through desiccation cracks in the soil, which likely was fostered by several heavy thunderstorms during summer 2018.

We observed the highest nitrate leaching under winter wheat (**Fig. 3.5**). Termination of grass-clover ley within a crop rotation is considered a "hot moment" in N cycling and associated with increased losses from nitrate leaching and N_2O emissions due to exacerbated mineralization of accumulated soil N (Buchen et al., 2017, Velthof et al., 2010, Wagner-Riddle et al., 2020). In the *Gäu* region, but also in other areas with temperate climate, farmers are therefore usually advised to avoid grass-clover

termination in autumn (Velthof et al., 2010). However, our results indicated that termination of grass-clover leys in spring followed by maize and a winter cereal just shifts the leaching to the next winter, which was also found by Wey et al. (2022) for fields in the same study region. In our study, maize was drilled by rotary band seeding after killing the grass-clover with a broadband herbicide. After maize, fields were ploughed which might explain the delayed mineralization. However, Helfrich et al. (2020) also observed elevated soil Nmin levels even two years after ley termination independent whether ley termination was done by ploughing or purely chemically without any soil tillage. Shifting the time point for ley termination from autumn to spring, thus, is not enough, and further measures might be necessary. These measures might include undersown cover crops for the next winter (Sørensen, 2004, Eriksen et al., 2004, Wachendorf et al., 2006, De Notaris et al., 2018), changes in the crop rotation (e.g. replacing winter wheat by winter barley due to its higher N uptake in fall or shifting to a summer cereal), or including plants with biological nitrification inhibition capacity into the grass-clover mixture (Coskun et al., 2017).

3.4.4 Low residual fertilizer value of both cattle slurry and mineral fertilizer

We expected that with greater recoveries of slurry N in soil, also the residual fertilizer NUE would be larger for Slu than for Min. Following the discussion in Smith and Chalk (2018), we compared different calculation approaches for assessing the residual recovery of labelled fertilizer N. Calculating N recoveries relative to initially applied amounts neglects N already taken up by the pre-crop or lost in the first year and, thus, cannot fully assess the *availability* of residual fertilizer N. Therefore, residual recovery should rather be expressed relative to the amount of ^{15}N labelled fertilizer left in soil after harvest of the pre-crop(s) (Smith and Chalk, 2018). However, assessing the residual recovery based on measured soil ^{15}N recoveries after biomass harvest (*residual recovery*$_{soil}$) can be biased by the difficulty of accurately assessing ^{15}N recoveries in soil. These difficulties arise from the dependency on an accurate assessment of soil weight, which in turn depends on bulk density. The observed large variation in total N stocks indicates limited accuracy (**SI 3 Fig. 3**). Furthermore, ^{15}N

gets diluted in a large soil N pool and reliable results can only be obtained when the ^{15}N enrichment clearly exceeds natural abundance. In our study, ^{15}N abundances in top soil still exceeded 0.38 atom% at the final sampling (for Min at Field A, all other > 0.40 atom %) which is four times natural abundance level in delta notation. Besides difficulties in accurately assessing the ^{15}N recoveries in soil also the proportion of ^{15}N in roots and stubble, which might get re-mineralized later, blurs estimates of *residual recovery*$_{soil}$. Therefore, we tested an additional approach by measuring plant N uptake and all potential losses of ^{15}N labelled fertilizers and accounted for them in calculating the residual recovery (*residual recovery*$_{output}$).

Relative to the originally applied amount of N, residual recoveries in biomass were greater for Slu than for Min (see 3.3.1), which is in agreement with our hypothesis. However, we detected no differences between Min and Slu neither in *residual recovery*$_{soil}$ (**Eq. 3.5**) nor *residual recovery*$_{output}$ (**Eq. 3.6**) (**Table 3.4**). Both estimates were in a similar range, but values tended to be slightly higher and more variable for *residual recovery*$_{soil}$. In our set-up, where roots and stubble could not be sampled during the ongoing experiment, *residual recovery*$_{soil}$ probably was slightly overestimated as it did not consider remaining fertilizer N in these plant parts. The greater variability in *residual recovery*$_{soil}$ estimates might be further linked to a comparably high uncertainty when assessing total N stocks in soil (**SI 3 Fig. 3**). This uncertainty also affected calculations of the cumulated residual recovery in nitrate leaching, plant uptake and soil in succeeding year, which tended to reach values > 100 % (**SI 3 Fig. 2**).

The low recoveries of residual fertilizer N in succeeding crops are in good agreement with previous studies (e.g. Sørensen, 2004, Glendining et al., 2001, Jensen et al., 1999). The generally low residual recoveries indicate that availability of residual fertilizer N remaining in the soil was low. It goes along well with Frick et al. (in revision) finding that most residual fertilizer N for both Min and Slu was recovered in the non-microbial organic soil N pool already in the next spring after application.

This experiment has been conducted on soils with rather high N levels, due to long-term N input, but also caused by their alluvial origin. High soil N levels combined with a high mineralization rate contribute to both, high nitrate leaching and low residual fertilizer N recoveries. On the other hand, there is evidence that SOM levels are not a major influencing factor on the residual value of fertilizers (Berntsen et al., 2007, Langmeier et al., 2002, Glendining et al., 2001), contradicting that high N stocks in soil might be responsible for low residual fertilizer recoveries. Rather, mineralization rate of the remaining fertilizer N might be decisive, which in turn is closely coupled to C cycling. Sørensen (2004) found that 17 to 35 % of applied slurry ^{15}N-NH_4 was immobilized due to organic matter addition with slurry and not re-mineralized within the following two to three years. Webb et al. (2013) indicated that mineralization of residual N from animal manure might continue over decades, but its agronomic relevance would vanish during ten years. In contrast to our results, Sørensen (2004) found lower release rates for residual slurry N than mineral fertilizer N and attributed it to the ongoing immobilizing effect of organic material added with the slurry. Sørensen and Amato (2002) reported release rates of organic N to be dependent on soil texture and found less mineralization of organic N from fertilizer in clayey soils. With both our fields having clay contents of about 22 % in the top soil, this could explain the absent differences between Min and Slu as mineralization might have been lowered by the high clay content.

3.4.5 ^{15}N soil-system balance for mineral fertilizer and cattle slurry

We measured crop N uptake and all major loss pathways from both ^{15}N labelled cattle slurry and ^{15}N labelled mineral fertilizer in the field. Slightly larger cumulative recoveries (compare 3.2.6 and **Fig. 3.3**), combined with a larger standard deviation, found for Slu than for Min are in accordance with others (Sørensen, 2004) and hint to a tendency for slightly less accurate estimates for Slu due to less homogenous distribution in soil (Bosshard et al., 2009). Overall, with all measured pools within this study summing up to 85 to 94 % of applied N and complemented with NH_3 emission to sum up to approximately 100 %, gives a good indication that we could

obtained reliable data (**Fig. 3.3**). The remainder could be attributed to dissolved organic N (DON) leaching or N_2, NO or N_2O emission. Based on assumptions derived from Van Kessel et al. (2009), DON leaching in our study might account for 1 to 2.5 % of applied fertilizer N (compare 3.4.3). Emissions of N_2O and N_2 are usually estimated to account for less than 1 % of applied N (IPCC, 2006). However, since we minimized N losses upon slurry production in the stable, slightly higher values could be expected as also indicated by Oenema et al. (2007) who found that up to 7 % of excreted N could get lost via denitrification. Furthermore, upon grass-clover termination usually elevated N_2O emission from mineralization of incorporated stubbles and roots can be expected (Krauss et al., 2017). Since ^{15}N recovery in crops and presumably also in stubble and roots was higher in Min than in Slu in the first year, this could hint to higher N_2O losses from Min than from Slu and could explain the slightly lower overall recovery for Min than for Slu, especially at Field B.

3.5 Conclusion

We used ^{15}N labelled cattle slurry and mineral fertilizer in a field experiment in order to assess differences in nitrate leaching between these two fertilizer types under on-farm conditions. Following the fate of fertilizer N throughout three cropping seasons, we found only minor shares of the added slurry or mineral fertilizer N leached, but significantly greater values for Slu than for Min. Overall, the major share of nitrate leaching derived from mineralization of soil N. This indicates that an improved understanding on soil N dynamics, also considering C and other nutrients involved in SOM cycling, is needed in order to reduce leaching losses. Further studies should focus on the effect of reduced N inputs combined with the role of crop rotations and soil tillage as well as balanced C and N inputs in order to reduce leaching losses while at the same time avoiding SOM depletion.

Since we found the highest leaching after termination of grass-clover, it appears critical to specifically control build-up of soil organic N stocks under grass-clover and take prolonged mineralization upon its termination into account. For the crop rotation grass-clover – maize – winter wheat, which is common under temperate climate not

only in Switzerland, the maize crop followed by winter cereal was not capable to take up the large amounts of mineralized soil N. Probably, fertilization of maize after grass-clover could be reduced which should be further tested and possibly included in fertilization recommendations. Furthermore, mineralization of soil N likely continues after harvest of maize. As winter wheat usually does not have a major capacity for N uptake before winter, in regions sensitive to nitrate leaching, undersown cover crops and/or shifting to barley or summer cereals could be further assessed as an improved management option.

Chapter 4

Increasing nitrogen use efficiency of cattle slurry: Potential of anaerobic digestion, biochar and a nitrification inhibitor

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Abstract

Animal manures are valuable multi-nutrient fertilizers, but their N use efficiency (NUE) by plants is low, bearing the potential of N losses such as nitrate (NO_3^-) leaching. For developing strategies to increase the NUE of cattle slurry, we need a refined understanding of slurry N fluxes in the soil-plant system for which ^{15}N labelling presents a suitable method. In a microcosm experiment in the greenhouse during 57 days, we assessed the effect of treating ^{15}N labelled cattle slurry by anaerobic digestion, biochar and/or the nitrification inhibitor DMPP (3,4-dimethyl-1H-pyrazole monophosphate) on slurry N turnover in soil and uptake by ryegrass (*Lolium multiflorum* var. Westerwoldicum). Thereby, we hypothesized that all tested treatments reduced residual N leaching compared to untreated slurry. ^{15}N recovery, hence NUE, in cumulated biomass was higher for digested (SLA) than for undigested cattle slurry (SLU), while recovery in soil at 55 days after set-up showed an opposite trend, with more than 45 % of N from SLU still recovered in soil. Despite this high ^{15}N recovery in soil, residual N leaching from SLU was low (< 1 % of added N). We could link this to the fact that very little N from slurry was recovered in mineral forms at this time point and most of it could be found in the non-microbial organic soil N pool. DMPP and biochar only had marginal effects on NUE, but their effects might have been impaired by hot temperatures in the greenhouse and the overall high NO_3^- levels in the soil. DMPP tended to increase biomass yield and total N uptake, but decreased the proportion of fertilizer N taken up by plants, which suggested that with DMPP plants took up more soil N than without DMPP. Between 17 and 22 % of added fertilizer N remained unaccounted for. This proportion was slightly higher for SLA than for the other treatments and presumably was lost via ammonia volatilization. Overall, in this experiment especially anaerobic digestion appeared suitable for increasing NUE of cattle slurry, but care must be taken to avoid increased N losses via ammonia volatilization. Furthermore, longer studies under field conditions would be necessary to confirm this finding and for including residual effects of the organic N.

Keywords: ^{15}N labelling, soil N dynamics, nitrate leaching, DMPP, digestate, NUE

4.1 Introduction

Animal manures are a valuable source of nutrients for crops, but their targeted use as nitrogen (N) fertilizer is challenging. The difficulty arises from the variable proportion of directly plant available mineral N and a considerable share of organic N (30 – 75 % of total N) in manure, which needs to be mineralized to become plant available (Webb et al., 2013). This makes it difficult to synchronize plant N demand with N supply from organic manures, resulting in low N use efficiencies (NUE) (Gutser et al., 2005, Webb et al., 2013). Mineral N not taken up by plants is prone to losses to the environment, such as nitrate (NO_3^-) leaching, deteriorating water quality (Grizzetti et al., 2011), ammonia (NH_3) emissions, leading to eutrophication of natural ecosystems (Guthrie et al., 2018), or nitrous oxide (N_2O) emissions, promoting global warming (IPCC, 2006). At the same time, using animal manures for fertilization is crucial for closing nutrient cycles and could replace considerable amounts of mineral fertilizer (Zavattaro et al., 2017), if application amount, type and timing are optimized and losses to the environment are minimized.

Anaerobic digestion of animal manures has been suggested as a means to facilitate synchronization between N supply and crop N demand (Möller et al., 2008). Compared to their feedstock, digestates have reduced dry matter and organic carbon (C) contents, but an increased ammonium (NH_4^+) to total N (Ntot) ratio and elevated pH (Möller and Müller, 2012). These changes potentially increase the short-term N availability to crops compared to undigested slurry, facilitating a more targeted application to the plants` needs both in amount and timing (Gutser et al., 2005, Möller et al., 2008), and, as a consequence, lower the potential for NO_3^- leaching. Nevertheless, estimates on the effect of anaerobic digestion on N transformation processes in soil, N availability to crops and leaching potential remain uncertain as contradictory results were reported (Nkoa, 2014). General conclusions are restrained as manures and digestates have quite variable properties, depending on factors such as animal feed (Sørensen et al., 2003) and conditions during the digestion process

(Möller and Müller, 2012). So far, most studies have focused on agro-industrial digestates, which are usually not produced from animal manure alone, but co-digested with other substrates such as corn silage, green waste or sewage sludge (e.g. Fouda et al., 2013, Nicholson et al., 2017, Svoboda et al., 2013) which is done in order to optimize biogas yield. For a better mechanistic understanding on the effect of digestion on fertilizer use efficiency of animal manure, digestion without co-substrate is necessary for which there are fewer studies (e.g. Cavalli et al., 2018, Huf and Olfs, 2020, Möller et al., 2008). Furthermore, we are unaware of any study using ^{15}N labelling in order to integrate both plant uptake, soil processes and potential losses, which is crucial to gain a realistic picture on interactions and to avoid pollution swapping.

Biochar, a solid by-product from pyrolysis of organic material, has gained considerable scientific and public interest for improving soil fertility and sequestering C in soil (Lehmann and Joseph, 2015). Adding biochar to digestates as a fertilizer additive might alleviate N losses by reversible adsorption of cations and anions to its highly porous structure (Sarkhot et al., 2013), and, with repeated applications, positively influence soil quality (Laird and Rogovska, 2015). In a recent meta-analysis, Borchard et al. (2019) found that biochar reduced NO_3^- leaching from soil by 13 %. According to them, the underlying mechanisms may involve, amongst others, sorption of NO_3^- either directly to the biochar (Yao et al., 2012), or to organic coatings of the biochar (Hagemann et al., 2017), and/or biochar-induced alterations in physical soil properties such as water retention (Clough et al., 2013). However, significant reductions were only observed at high biochar application rates and were dependent on soil type (reduction rather in coarse and sandy soils), pH (reduction rather at pH < 5.5) and on crop (reduction only in arable farming, but not in grassland). Furthermore, also fertilizer type affected the outcome, but organic fertilizers were clearly underrepresented in their meta-analysis. While there is broad evidence that biochar interacts with soil N transformations in several ways (Clough et al., 2013, Liu et al., 2018), the underlying drivers still remain largely unresolved (Fiorentino et al., 2019,

Bradley et al., 2015). ^{15}N labelling has been identified as a suitable method to disentangle several simultaneous and interconnected processes involving fertilizer and soil N cycling, directly and indirectly affected by biochar (Craswell et al., 2021, Schouten et al., 2012). However, to the best of our knowledge, the effect of biochar addition to ^{15}N labelled anaerobically digested cattle slurry has not yet been studied.

Nitrification inhibitors (NIs), which are synthetic or biological compounds reducing microbial nitrification in soil, have been proposed to reduce N losses from agriculture. Within this work, we will focus on a synthetic NI. Usually, NH_4^+ added to soil with fertilizers gets rapidly nitrified. Delaying this transformation and keeping the added N in the form of NH_4^+ for a prolonged time span, could reduce N leaching as NH_4^+ is less mobile in the soil profile than NO_3^-, while at the same time N_2O emissions both from nitrification and denitrification could be reduced. A recent meta-study showed the potential of NIs to reduce total N losses by on average 16.5 %, while NO_3^- leaching was found to be reduced by even 47 % (Qiao et al., 2015). The producers of these synthetic compounds not only claim lower N losses, they also promise higher yields due to an increased NUE (Sanz-Gomez, 2017). However, it appears that these effects depend, amongst others, on the form of NI (Qiao et al., 2015, Yang et al., 2016), the formulation and way of application (Ruser and Schulz, 2015), the type of fertilizer (Qiao et al., 2015), the amount of fertilizer (Rose et al., 2018, Rowlings et al., 2016), as well as abiotic soil conditions such as texture (Barth et al., 2019), temperature, or pH (Zerulla et al., 2001). While there has been a wide range of potential NIs identified (Ruser and Schulz, 2015), only few are currently commercially utilized of which 3,4-dimethyl-1H-pyrazole monophosphate (DMPP) appears superior as it is less phytotoxic, effective at lower applications rates and potentially over longer time spans than most other NIs (Yang et al., 2016, Zerulla et al., 2001). Most studies, however, have combined DMPP with mineral fertilizers. Its use with different organic fertilizers has been less widely studied.

The objective of this study was to assess the potential of anaerobic digestion, biochar, and DMPP as well as their interactions for increasing NUE, defined as ^{15}N fertilizer recovery in plant biomass, and for reducing N losses from cattle slurry. To this end, a microcosm experiment with the following ten treatments was established: 0N-control, ^{15}N ammonium sulphate, ^{15}N cattle slurry, ^{15}N anaerobically digested cattle slurry, and ^{15}N anaerobically digested cattle slurry plus biochar, each with/without DMPP. We measured N uptake from the fertilizers by annual ryegrass and traced N fluxes in soil. After 57 days of ryegrass growth, microcosms were oversaturated with water and flushed in order to assess the effect of the treatments on N leaching from the residual N. We hypothesized that i) N uptake by plants was greater from anaerobically digested slurry than from undigested slurry due to a higher NH_4^+-N share, ii) biochar addition to the digested slurry increased NUE by reversely binding NH_4^+, iii) DMPP addition to the fertilizers delayed their nitrification leading to higher NH_4^+ to NO_3^- ratios in soil solution, and prolonged N uptake, irrespective of fertilizer type, and iv) consequently all mentioned treatments (anaerobic digestion, biochar and DMPP) would reduce residual N leaching compared to untreated slurry.

4.2 Material and Methods

4.2.1 Experimental approach

A microcosm experiment was established with ten treatments: five fertilizer treatments were combined in a fully factorial design with and without the nitrification inhibitor DMPP: 0N-control (0N), ^{15}N ammonium sulphate (MIN), ^{15}N cattle slurry (SLU), ^{15}N anaerobically digested cattle slurry (SLA), and ^{15}N anaerobically digested cattle slurry plus biochar (SLA+), each with/without DMPP. The treatments were replicated four times.

In order to allow for repeated soil sampling during the experiment while preserving undisturbed microcosms for other measurements, the experiment was duplicated into a destructive set (D) for soil sampling and a non-destructive set (G) for other analyses

such as soil solution sampling and N_2O measurements (Efosa et al., in prep-a). This resulted in a total of 80 microcosms (5 fertilizer treatments x 2 nitrification inhibitor treatments x 4 replicates x 2 sets for destructive/non-destructive sampling). The microcosms were arranged in a complete randomized block design on movable tables in the greenhouse. Corresponding columns from the G- and D-set were placed next to each other. Tables within each block as well as the entire blocks were rotated weekly.

4.2.2 Characteristics of soil, fertilizers and additives

For the experiment, top soil from an organically managed field (47°35'50.5"N 8°11'57.7"E) was sampled. The soil was a silty loam with a pH of 6.4 (**Table 4.1**). Soil was sieved field moist to 5 mm, air-dried, and stored at room temperature. Nine days before set-up, approx. 400 kg of dry soil were re-wetted with demineralized water to ~40 % maximum water holding capacity (maxWHC) and pre-incubated under a plastic sheet in the greenhouse to allow the microbial community to revive and adjust to the conditions in the greenhouse.

^{15}N labelled cattle slurry was produced by feeding a young heifer with ^{15}N labelled ryegrass for eight days after an adaptation phase (Frick et al., in revision). Faeces and urine were sampled separately and frozen daily. Later, faeces and urine fractions with the highest ^{15}N label were recombined and diluted 1:1 with demineralized H_2O in order to achieve a representative slurry (**Table 4.2**).

Table 4.1*: Soil characteristics*

Clay	**Silt**	**Sand**	**Ntot**	**Corg**	**pH_{H2O}**	**maxWHC**
		g kg^{-1} dry soil			-	g H_2O g^{-1} dry soil
140	260	560	1.9	19.8	6.4	0.40

Table 4.2: *Characterization of ^{15}N slurry (^{15}N-SLU) and anaerobically digested ^{15}N slurry (^{15}N-SLA)*

	DM[3]	**Corg**[3]	**Ntot**[3]	**NH_4^+-N**[3]	**NDF**[1]	**NDF-N**[1]	**pH**[3]	**^{15}N-Ntot**[2]	**^{15}N-NDF**[1]
	%	g kg^{-1} dry matter					-	atom% excess	atom% excess
^{15}N-SLU	3.3	393	68.4	42.0	268	3.2	7.9	7.504	7.731
^{15}N-SLA	2.7	313[4]	94.6	62.0	214	4.5	8.0	7.019	6.365

[1] neutral detergent fibre (NDF), nitrogen in neutral detergent fibre fraction (NDF-N) and ^{15}N enrichment in NDF-N (^{15}N-NDF) were analysed in slurry dried at 60 °C
[2] parameters were determined on acidified and freeze-dried subsamples
[3] parameters were determined on subsamples of fresh slurry
[4] calculated based on loss on ignition

A subsample of the same slurry was anaerobically digested on an Automatic Methane Potential Test System (AMPTSII, Bioprocess Control), usually used for batch fermentation experiments. ^{15}N slurry was inoculated with 4 % (w/w) of an external digestate from an agricultural biogas plant and split up in 500 mL Schott bottles. Slurry was fermented under mesophilic conditions (40.5 °C) with regular stirring (45 sec stirring every 300 sec) over a period of 37 days. The process was stopped when daily methane yield over three consecutive days had dropped below 1 % of the total produced methane (Holliger et al., 2016). Batches were recombined and thoroughly mixed. Average cumulative methane yield was 369 ± 15 L kg^{-1} organic DM (Standard Temperature and Pressure), indicating that the digestion process was complete in all batches and comparable to fermentation of cattle slurry in an agricultural biogas plant (KTBL, 2013). Both, slurry and digested slurry were stored frozen at -20 °C until two days before set-up of the experiment, when they were slowly thawed and kept at 4 °C.

For the mineral fertilizer treatment, ^{15}N ammonium sulphate (Sigma-Aldrich, St. Louis, MO, USA, 10.7 atom% ^{15}N abundance) was diluted with non-enriched ammonium sulphate to a final enrichment of 7 atom% ^{15}N abundance.

Biochar was produced from tree and shrub cuttings at 500 – 600 °C in a PYREG reactor (PYREG GmbH, Dörth im Hunsrück, Germany). It contained 7.1 g N kg^{-1} dry

matter and 790 g Corg kg^{-1} dry matter and had a pH value in water of 8.7. It fulfilled the guidelines of the European Biochar Certificate. Milled biochar (< 1 – 2 mm) was used in order to facilitate mixing with the fertilizer and to get more homogeneous subsamples.

3,4-dimethyl-1H-pyrazole monophosphate (DMPP) (CAS: 202842-98-6) was used as nitrification inhibitor. The compound had been stored at -20 °C. A DMPP solution was prepared in the evening before set-up of the experiment and stored at 4 °C before. The solution contained 8.4 mg DMPP mL^{-1} and 1 mL of it was added to the fertilizers shortly before they were mixed into the soil in order to apply DMPP at a rate of 2 % of the total N added with the fertilizers.

4.2.3 Set-up and maintenance of the microcosms

Each microcosm consisted of a cylindrical PVC tube with 15 cm diameter and 25 cm length. The bottom was closed with a PVC plate with a drain tap in the middle to allow for leachate collection (Bender et al., 2015). In order to avoid water-saturated conditions in the soil, a drainage layer of 400 g moist sand (0.2 to 0.6 mm grain size) was added to the bottom of the columns and separated from the soil by a thin fleece layer (Sana milk filter disk).

All fertilizer treatments were supplied at a rate of 90 mg Ntot kg^{-1} dry soil; N addition with DMPP or biochar was negligible. Mixing of soil and fertilizers was done separately for each column. Immediately before mixing with the soil, fertilizers were mixed with DMPP solution, where applicable, and demineralized water to achieve the same amount of liquid as added with the SLU treatment. Biochar had been added to the SLA+ treatment at a rate of 2.2 % (w/w) of the fresh weight of SLA 13 hours before set-up to allow for appropriate contact between SLA and biochar. Upon set-up, all microcosms also received a basal micro- and macronutrient fertilization with a modified N-free Hoagland solution supplying the following amounts of nutrients (mg

kg^{-1} dry soil): K 250, P 50, Ca 102, Mg 48, Zn 1, Mo 0.1, Fe 1, B 1, Mn 2, Cu 2, and Co 0.1.

Soil was thoroughly mixed with fertilizer treatments and the Hoagland solution and packed into columns at a bulk density of 1.3 g cm^{-3} (split in four equal layers for more homogenous compaction). In order to reach 60 % maxWHC, additional demineralized water was added on top of each layer after compaction. 60 % maxWHC was chosen as this was shown to represent optimal conditions for mineralization and nitrification while denitrification was minimized (Drury et al., 2003, Linn and Doran, 1984). In the G-set, rhizon suction samplers (Rhizosphere Research Products, Wageningen, The Netherlands) were installed at 5 cm and 15 cm depth for non-destructive soil solution sampling in order to follow the development of NH_4^+ and NO_3^- concentrations in the soil solution at a high temporal resolution. In one column per treatment of the D-set, a tensiometer (MPS6, Meter Environment, Pullman, USA) was installed at 10 cm depth in order to monitor water potential and soil temperature during the experiment. Ryegrass (*Lolium multiflorum* var. Westerwoldicum, Pulse) was sown at a seed density of 30 g m^{-2} (i.e. 0.53 g $column^{-1}$) on the top of each column and covered with a small layer of vermiculite. The columns were additionally covered with plastic wrap during the first five days in order to facilitate germination.

The experiment was conducted between 5th of August and 1st of October 2020 in a greenhouse at the Research Institute of Organic Agriculture (FiBL), Frick, Switzerland (47°31'02.3"N 8°01'35.5"E). Average temperature was 21.5 °C (range: 10.2 – 39.2 °C, median: 20.5 °C) and average relative humidity was 61 % (range: 22 – 89 % rel. humidity, median: 63 % rel. humidity). No supplemental light was provided. Columns were watered daily with demineralized water back to the weight upon set-up in order to keep a constant moisture content. Due to increased water uptake and evapotranspiration of the grass, or due to additional water added before soil solution sampling (see below), daily water content fluctuated. It reached a

minimum of 40 % maxWHC shortly before harvest of the grass or a maximum of 65 % maxWHC before taking soil solution samples.

4.2.4 Soil solution sampling

Soil solution samples were taken 1, 2, 3, 6, 9, 14, 22, 28, 36, 43, 49, and 56 days after set-up (DAS) by attaching syringes to the plug of the rhizon suction samplers and installing a vacuum by inserting a wooden retainer (**Fig. 4.1**). In the beginning, syringes were attached in the late afternoon, one hour after watering back to 60 % maxWHC and left overnight. However, as it was difficult to extract enough soil solution for analysis with this procedure, from Day 9 onwards, sampling was performed during the day where vacuum could be applied repeatedly. In addition, 100 to 150 mL of water (depending on the growth stage of the grass) were added about two hours before start of sampling, in order to facilitate soil solution extraction. Soil solution samples from 5 cm and 15 cm depth of the same column were pooled and stored frozen. Analysis for mineral N (NH_4^+ and NO_3^-) was conducted as described below. Since it was not always possible to extract soil solution from both depth layers, in these cases, samples only consisted of pore water from one depth layer. However, concentrations tended to deviate from pooled samples, with lower values for the 5 cm rhizons and higher values for the 15 cm rhizon. For this reason, rhizon data only gives semi-quantitative information.

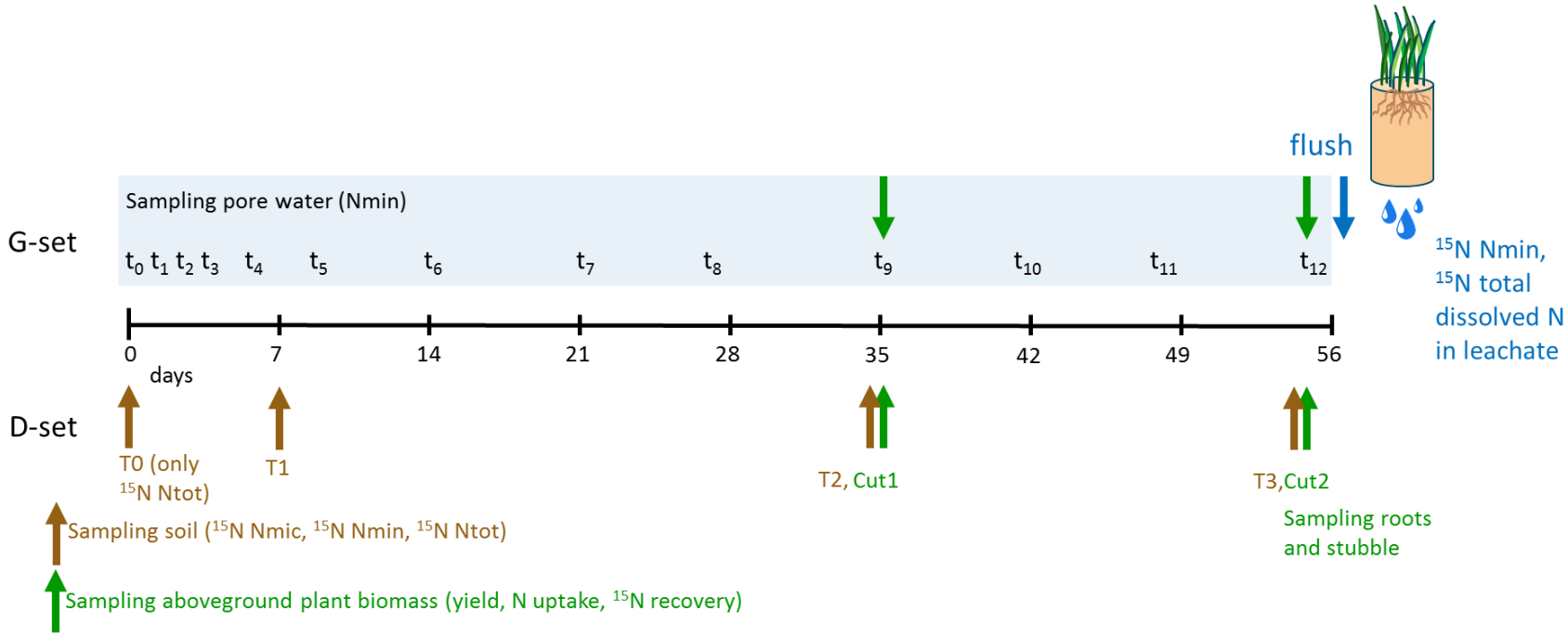

Fig. 4.1: *Sampling scheme of column study. Nmic = microbial N, Nmin = mineral N (NH_4^+-N + NO_3^--N), Ntot = total N. D-set was used for soil sampling and biomass sampling. G-set was used for non-destructive sampling of pore water with rhizons and leachate collection ("flush")*

4.2.5 Soil and biomass sampling

At 7, 35, and 55 DAS, soil samples were taken over the whole soil depth from the D-set for analysis of the ^{15}N label, both in Ntot as well as the microbial (Nmic) and mineral N pool (Nmin). At each sampling time, soil from three cores per microcosm (20 mm diameter) was pooled, thoroughly homogenized, and stored in a cooling box until extraction. Holes from soil coring were refilled with closed PVC-tubes.

On the sampling day, soil samples were extracted using the chloroform fumigation extraction method (CFE) in order to determine Nmic (Brookes et al., 1985, Vance et al., 1987). In short, from each sample, two subsamples of 20 g dry weight equivalent each, were weighed. One subsample was extracted immediately with 80 mL 0.5 M K_2SO_4, while the other subsample was fumigated with chloroform for 20 to 24 hours and extracted thereafter. Extracts were filtered through folded paper filters (Macherey Nagel Type 615, Ø 185 mm) and stored at -20 °C until analysis. Additionally, Nmin was measured on the non-fumigated extracts. The remaining soil was air dried, pulverized on a ball mill (MM200 Retsch, Haan, Germany) and analysed for ^{15}N-Ntot.

For analysis of ^{15}N enrichment in the soil Nmic and Nmin pools, extracts from the CFE extraction were prepared using a diffusion technique adapted from Goerges and Dittert (1998): For analysis of ^{15}N-Nmic, both fumigated and non-fumigated extracts were oxidized by autoclaving with $K_2S_2O_8$ (Cabrera and Beare, 1993) and afterwards diffused on acidified quartz filter traps (Whatman QM/A) by adding Devarda's alloy (0.4 g per sample), 4 mL 5M NaCl, and 0.75 mL 5M NaOH per 10 mL of extract (Goerges and Dittert, 1998, Mayer et al., 2003). NH_4^+ and NO_3^- were diffused together on the same filter from non-fumigated extracts to determine ^{15}N-Nmin following a similar procedure, but by adding 0.2 g MgO, instead of NaCl and NaOH (Douxchamps et al., 2011). After drying, filters were encapsulated in tin capsules and analysed for ^{15}N.

Aboveground biomass of all columns (D- and G-set) was harvested twice, at 35 and 55 DAS at a height of approx. 2.5 cm. At the final sampling (55 DAS), stubble biomass and root biomass of the D-set were sampled as well. Quantitative root washing was accomplished by washing the whole column content through a 1 mm sieve. The residue on top of the sieve, was separated from mineral debris and exogenous organic material by combined decantation and manual sorting with tweezers (Hirte et al., 2017). Shoot and stubble biomass samples were dried at 40 °C, while root biomass was dried at 60 °C due to high moisture content after root washing. Dried biomass was milled on a centrifugal mill (ZM200, Retsch, Haan, Germany), followed by pulverization on a ball mill and analysed for N and ^{15}N.

4.2.6 Flush

In order to assess how much of the residual N in soil at the end of the experiment could be leached, two days after the last biomass cut, the non-disturbed columns (G-set) were oversaturated with demineralized water and the leachate was collected. Since the drain tap had been filled with glass wool upon set-up, obtained leachate was already clear and not filtered afterwards (Bender et al., 2015). We aimed at slowly adding demineralized water to reach 105 % maxWHC. However, since infiltration varied between columns, we only could add water to reach 94 % maxWHC on average (range 82 to 103 % maxWHC) over a time course of 12 hours. After drainage of the first flush (approximately 12 hours later), a second flush was conducted by adding another 500 mL of demineralized water at once and immediately starting drainage. This second flush showed similar or lower concentrations in Nmin than the first flush and, thus, indicated that concentrations of the first flush represented equilibrium concentrations of the saturated soil extract. Water content in the columns after the first flush ranged between 76 and 95 % maxWHC, indicating that both infiltration and drainage did not work equally well in all columns. Therefore, we present the cumulated amount of residual N leached over both flushes.

Leachates collected from the first flush were diffused as described for the CFE samples to both determine ^{15}N recovery in Nmin as well as in dissolved organic N (DON; calculated as total dissolved N minus Nmin). We assumed that the ^{15}N enrichment did not change between the first and the second flush.

4.2.7 Chemical analyses

NH_4^+ and NO_3^- concentrations in soil solution, non-fumigated CFE-extracts, and leachate from the flush were analysed spectrophotometrically on an automated discrete analyser (Smartchem 450, AMS Alliance, Rome, Italy). NO_3^- concentrations of the extracts were determined according to Keeney and Nelson (1982). NH_4^+ was determined using the modified indophenol blue reaction (Krom, 1980). Total dissolved N in fumigated and non-fumigated soil extracts was measured with a TOC/TNb-analyser (multi N/C 2100S, Analytik Jena, Jena, Germany). Nmic was calculated as the difference between fumigated and non-fumigated extracts using a conversion factor of $k_{EN} = 0.54$ (Joergensen and Mueller, 1996). All ^{15}N analyses (soil samples, biomass samples, diffusion filters) were performed on an elemental analyser coupled with a continuous flow isotope ratio mass spectrometer (Pyro cube + isoprime100, Elementar, Germany). International standards (IAEA-N1, IAEA-N2) as well as internal reference material were included in each run. Characterization of ^{15}N labelled slurry and anaerobically digested slurry (dry matter, pH, N, NH_4^+, macro- and micronutrients, volatile fatty acids (VFA), heavy metals) was performed by bonalytic GmbH (Troisdorf, Germany).

4.2.8 Calculations

For all ^{15}N data, isotopic excess was calculated by subtracting the mean ^{15}N abundance (i.e. percentage of ^{15}N relative to total N) of non-labelled reference samples from the measured ^{15}N abundance. For MIN, the natural abundance of ^{15}N in air was subtracted as a reference (i.e. 0.3663 atom%), while for SLU and SLA the weighted mean ^{15}N abundance of non-labelled faeces and urine samples from the same heifer shortly before starting to feed with ^{15}N labelled feed was used as non-labelled

reference (0.386 atom%). For plant biomass, soil, soil extracts and leachate, the mean of the N0 treatment at the corresponding sampling time in the corresponding sample type (plant, soil, extracts, leachate) was used as a reference.

The ^{15}N excess was used to calculate the N fraction derived from fertilizer (Ndff) in the corresponding compartment (Hauck and Bremner, 1976):

$$Ndff_{rel}\,[\%] = \frac{atom\%\quad {}^{15}Nexcess\ sample}{atom\%\quad {}^{15}Nexcess\ fertilizer} \times 100 \qquad \textbf{Eq. 4.1}$$

where atom% ^{15}Nexcess sample is the ^{15}N enrichment of the considered compartment (i.e. plant (part), soil, extracts) and atom% ^{15}Nexcess fertilizer refers to N enrichment of either mineral fertilizer, slurry or digested slurry.

The amount of N derived from the fertilizer was calculated as:

$$Ndff\ [g\ kg^{-1}] = \frac{Ndff_{rel}[\%]}{100} \times TN_i \qquad \textbf{Eq. 4.2}$$

where TN_i is the total amount of N in the considered compartment expressed in g N kg^{-1} soil. For biomass samples taken from the D-set, TN_i was corrected for the amount of soil removed from the column by soil sampling.

The ^{15}N enrichment in the Nmic-pool was calculated according to Mayer et al. (2003):

$$^{15}N_{mic}\,[atom\%] = \frac{total\ N_{fum} \times atom\%\quad {}^{15}Nexcess_{fum} - total\ N_{non-fum} \times atom\%\quad {}^{15}Nexcess_{non-fum}}{total\ N_{fum} - total\ N_{non-fum}} \qquad \textbf{Eq. 4.3}$$

where “fum” indicates fumigated samples while “non-fum” indicates non-fumigated samples.

The recovery of the applied fertilizers in the different compartments was then calculated as:

$$recovery[\%] = \frac{Ndff}{N_{applied}} \times 100 \qquad \textbf{Eq. 4.4}$$

where $N_{applied}$ is the total amount of N applied with the labelled fertilizers. Fertilizer N taken out from the D-set by soil sampling was less than 1.5 mg kg^{-1} soil and was, thus, not considered in calculations.

4.2.9 Statistical analysis

Data preparation and statistical analysis were performed using R (Version 3.5.3) (R Core R Core Team, 2019). Throughout, a significance level of $p < 0.05$ was applied.

Statistical analysis were performed using mixed effect linear models (lmer within package *lme4*). Model validation was performed by qq-plotting and Shapiro Wilk Normality test. In case the assumptions of normal distribution or homoscedasticity of residuals were violated, analysis was performed on transformed data (log or square root). *emmeans*-package was used for pairwise comparisons. p-value adjustment for multiple comparisons was performed according to the Tukey-method.

For dry matter yield, TN uptake, Ndff and recovery of shoot biomass, the mixed effect linear model included the fertilizer *treatment*, *DMPP*, and *cut* as well as their twofold and threefold interactions as fixed effects and *block* as a random effect. Due to repeated measurements upon cuts, also *ID* was added as a random factor which specified the individual columns. The same approach was used for Ndff and ^{15}N recovery in Ntot, Nmic, organic N (Norg) and Nmin. Since root and stubble biomass were only sampled once at the end of the experiment, a simplified model with *treatment, DMPP*, and their interaction as fixed effect and *block* as a random effect was fitted. The simplified model was also applied on leached total dissolved N upon the flush. Since Nmin concentrations in the leachate were very low (for NO_3^-, more than half of the samples was below the limit of detection of 0.13 mg L^{-1}), no statistical analysis was performed. Also, due to difficulties with the extraction of pore water with rhizons (as described in 4.2.4), data was not analysed statistically and must be considered semi-quantitative.

Plant biomass and soil parameters are shown for the D-set, while analysis of the leachate and the rhizon extracts was only possible on the G-set. Only shoot biomass was sampled for both sets and dry matter yield did not differ between D and G set (data not shown). However, for N uptake and Ndff there was a significant interaction between *cut* and *set,* even though values for the D-set had been corrected for the amount of soil taken out upon soil sampling. Overall, treatment effects within each cut were similar between D- and G-set.

4.3 Results

4.3.1 Biomass production, N derived from fertilizer and fertilizer recovery in biomass

Biomass dry matter yield and N uptake was highest for MIN and lowest for N0, both for the first and second cut (**Table 4.3**). The organic fertilizer treatments performed intermediate and differences between them were marginal, with SLU tending to have slightly lower yield and N uptake than the digested slurry. Thereby, differences between the fertilizer treatments increased from the first to the second cut. Biochar did not have a significant effect on N uptake or yield from digested slurry. The interaction of DMPP and fertilizer treatment was significant ($p = 0.006$), with higher stubble biomass for both SLU and SLA when combined with DMPP, but significantly lower stubble biomass when DMPP was added to unfertilized soil. DMPP increased N-uptake upon the first cut, but the effect was not statistically significant. Furthermore, it decreased $Ndff_{rel}$ for shoots and for stubble ($p = 0.01$), with significant differences for SLA in shoot biomass of the first cut and for SLU in stubbles (**Table 4.3**).

Plants took up almost half of their N demand from the ^{15}N labelled mineral fertilizer, while $Ndff_{rel}$ for both the digested and undigested slurry treatments was about 40 % (**Table 4.3**). Thereby, $Ndff_{rel}$ in shoot biomass was significantly affected by the interaction of treatment and cut ($p = 0.003$). However, differences in $Ndff_{rel}$ between the two cuts were small, while the decline in absolute N uptake upon the second cut

was much more pronounced for all treatments. Cumulated over both cuts and all plant parts, almost 70 % of MIN were recovered in plant biomass (**Fig. 4.2**). As for biomass yield, recovery of the organic fertilizers was significantly lower. For SLU, cumulated recovery of ^{15}N in biomass was 36 %. It was significantly increased by anaerobic digestion to reach about 42 to 44 % of applied N for SLA and SLA+. Neither DMPP nor biochar significantly affected recovery of fertilizer N in plant biomass.

Table 4.3: *Biomass yield, total nitrogen (TN) uptake and N derived from fertilizer ($Ndff_{rel}$) over the two consecutive cuts. Stubble and roots were only sampled upon the second cut. Data represents mean ± standard deviation, n = 4; N0 = no N fertilizer, MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry.*
*Letters indicate significant differences between fertilizer treatments ($p < 0.05$), separately for different biomass samples. Pairwise comparisons were averaged over the levels of DMPP whenever DMPP did not have a significant effect; * indicates significant differences induced by DMPP. When DMPP had a significant effect or when there was a significant interaction between fertilizer treatment and DMPP, statistical analysis over the treatments was performed separately for each level of DMPP.*

	Treatment	Dry matter yield			TN uptake			$Ndff_{rel}$		
		DMPP	no		DMPP	no		DMPP	no	
		[g kg^{-1} soil]			[mg kg^{-1} soil]			[%]		
Shoot (Cut 1)	N0	1.06 ± 0.27	1.15 ± 0.14	*a*	38.5 ± 7.4	40.7 ± 9.1	*a*	[-]	[-]	
	MIN	1.50 ± 0.21	1.23 ± 0.25	*b*	79.4 ± 6.5	68.6 ± 12.9	*c*	48.0 ± 2.8^{b}	48.2 ± 3.1^{b}	
	SLA	1.20 ± 0.18	1.05 ± 0.35	*ab*	62.1 ± 6.5	54.5 ± 13.7	*b*	37.4 ± 4.0^{a}	42.6 ± 1.5^{a}	*
	SLA+	1.34 ± 0.30	1.24 ± 0.16	*ab*	68.8 ± 12.3	63.3 ± 4.6	*bc*	41.1 ± 1.6^{a}	42.9 ± 1.5^{a}	
	SLU	1.21 ± 0.31	1.13 ± 0.29	*ab*	57.0 ± 6.4	56.5 ± 11.4	*b*	38.0 ± 2.6^{a}	40.5 ± 5.1^{a}	
Shoot (Cut 2)	N0	0.42 ± 0.07	0.38 ± 0.01	*a*	9.3 ± 1.3	8.1 ± 0.8	*a*	[-]	[-]	
	MIN	0.93 ± 0.11	1.11 ± 0.13	*c*	34.8 ± 8.6	43.9 ± 8.7	*c*	45.4 ± 2.1	48.1 ± 2.0	*c*
	SLA	0.82 ± 0.11	0.73 ± 0.10	*bc*	26.3 ± 10.9	26.0 ± 11.8	*b*	36.3 ± 2.2	38.0 ± 1.3	*b*
	SLA+	0.75 ± 0.11	0.76 ± 0.07	*bc*	20.9 ± 5.9	20.7 ± 4.9	*ab*	37.0 ± 1.7	38.6 ± 1.6	*b*
	SLU	0.70 ± 0.14	0.63 ± 0.16	*b*	20.9 ± 11.0	20.0 ± 8.6	*ab*	32.4 ± 0.9	33.9 ± 2.9	*a*
Stubble	N0	0.30 ± 0.02^{a}	0.36 ± 0.03^{ab}	*	3.0 ± 0.6	3.4 ± 0.6	*a*	[-]	[-]	
	MIN	0.46 ± 0.04^{b}	0.47 ± 0.04^{c}		10.0 ± 2.1	10.1 ± 0.6	*c*	45.0 ± 2.1^{c}	46.8 ± 2.1^{b}	
	SLA	0.45 ± 0.06^{b}	0.36 ± 0.04^{ab}	**	8.0 ± 2.9	7.5 ± 3.0	*bc*	35.8 ± 1.4^{ab}	37.8 ± 0.6^{a}	
	SLA+	0.39 ± 0.04^{b}	0.42 ± 0.02^{bc}		5.9 ± 0.9	6.2 ± 1.2	*b*	39.0 ± 2.2^{b}	39.0 ± 1.4^{a}	
	SLU	0.40 ± 0.05^{b}	0.34 ± 0.02^{a}	*	6.0 ± 1.7	5.6 ± 1.2	*b*	32.8 ± 1.5^{a}	36.4 ± 2.7^{a}	*
Roots	N0	0.50 ± 0.07	0.52 ± 0.11		5.0 ± 0.5	4.9 ± 0.8		[-]	[-]	
	MIN	0.40 ± 0.18	0.37 ± 0.07		6.0 ± 1.3	6.1 ± 0.8		41.7 ± 1.6	43.1 ± 3.2	*c*
	SLA	0.41 ± 0.13	0.36 ± 0.18	*ns*	5.4 ± 1.1	4.9 ± 1.1	*ns*	31.7 ± 1.9	33.9 ± 1.7	*ab*
	SLA+	0.41 ± 0.14	0.50 ± 0.11		4.9 ± 1.3	6.0 ± 0.9		33.1 ± 2.4	33.6 ± 2.5	*b*
	SLU	0.41 ± 0.20	0.35 ± 0.07		5.7 ± 1.5	4.8 ± 0.4		27.9 ± 3.6	30.6 ± 3.3	*a*
Total biomass	N0	2.29 ± 0.31	2.41 ± 0.14	*a*	55.7 ± 6.9	57.1 ± 9.5	*a*	[-]	[-]	
	MIN	3.29 ± 0.46	3.18 ± 0.35	*c*	130.3 ± 6.6	128.8 ± 13.3	*c*	46.8 ± 2.5^{b}	47.9 ± 2.5^{b}	
	SLA	2.88 ± 0.29	2.50 ± 0.50	*ab*	102.0 ± 8.2	92.9 ± 3.4	*b*	36.8 ± 1.6^{a}	40.5 ± 1.6^{a}	*
	SLA+	2.89 ± 0.47	2.92 ± 0.16	*bc*	100.4 ± 9.8	96.3 ± 3.4	*b*	39.8 ± 1.2^{a}	41.3 ± 1.2^{a}	
	SLU	2.77 ± 0.43	2.46 ± 0.32	*ab*	89.6 ± 7.9	86.8 ± 7.8	*b*	35.9 ± 4.5^{a}	38.3 ± 4.5^{a}	

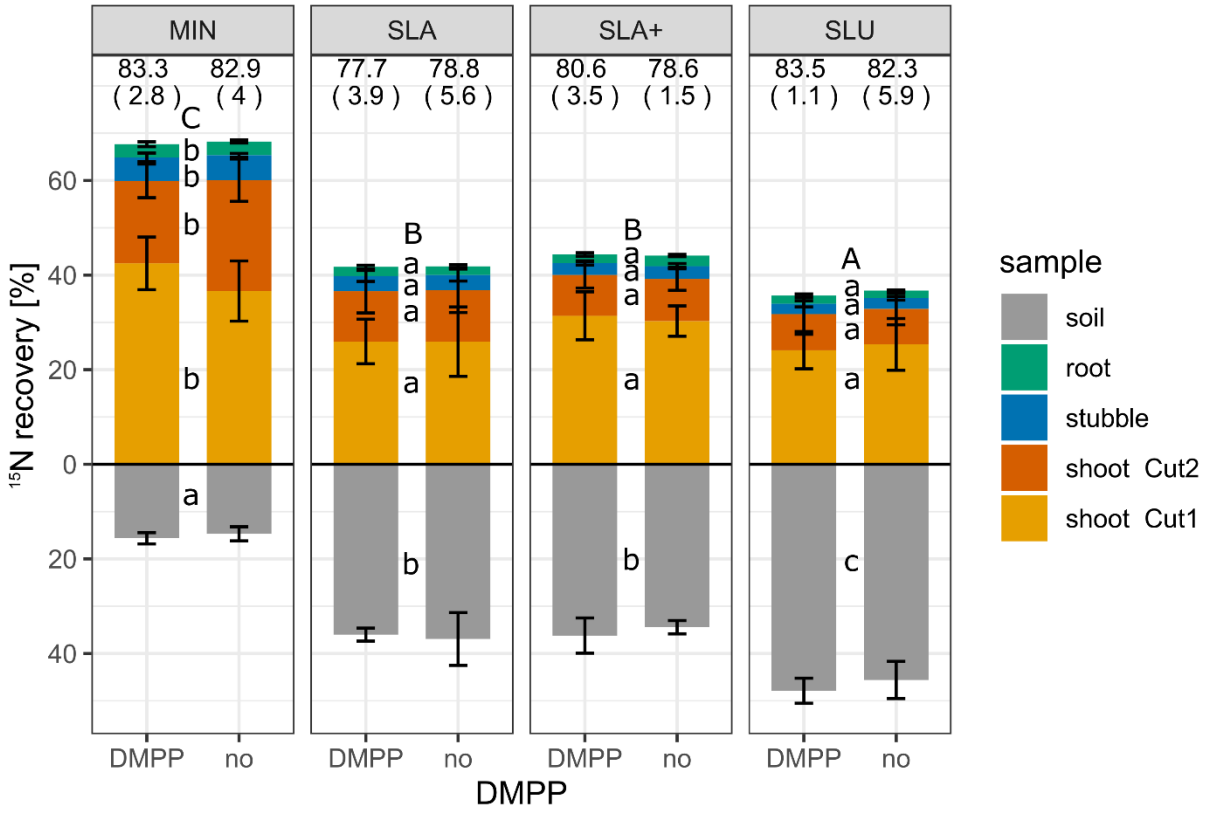

Fig. 4.2: *^{15}N balance after 55 days. Soil data refers to the final sampling at 55 days after set-up (same days as Cut 2 biomass sampling including stubbles and roots). Numbers above bars indicate cumulated recovery (mean (standard deviation)). MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry*
Letters indicate significant differences between fertilizer treatments ($p < 0.05$). Capital letters refer to the cumulated plant biomass. Pairwise comparisons were averaged over the levels of DMPP, because DMPP did not have a significant effect.

4.3.2 ^{15}N fertilizer recovery and distribution in soil N pools

^{15}N recovery in soil showed a decreasing trend over time (**Fig. 4.3**). The decrease was strongest between 7 DAS and 35 DAS (Cut 1) and less pronounced thereafter until 55 DAS (Cut 2). There was a significant interaction between treatment and sampling time ($p < 0.001$), with increasing differences between treatments over time. Upon 7 DAS, recovery of all fertilizers in soil was almost the same and equal to 80 to 88 % of total N added. Upon the last sampling, recovery of MIN had declined to less than 16 %, while for SLA and SLA+ 36 % of ^{15}N were still found in soil (**Fig. 4.3** and **Fig. 4.2**). For SLU, ^{15}N recovery in soil remained even higher (approx. 47 % of added N)

and was significantly different from that of the digested slurries (SLU vs SLA p = 0.04, SLU vs SLA+ p = 0.02).

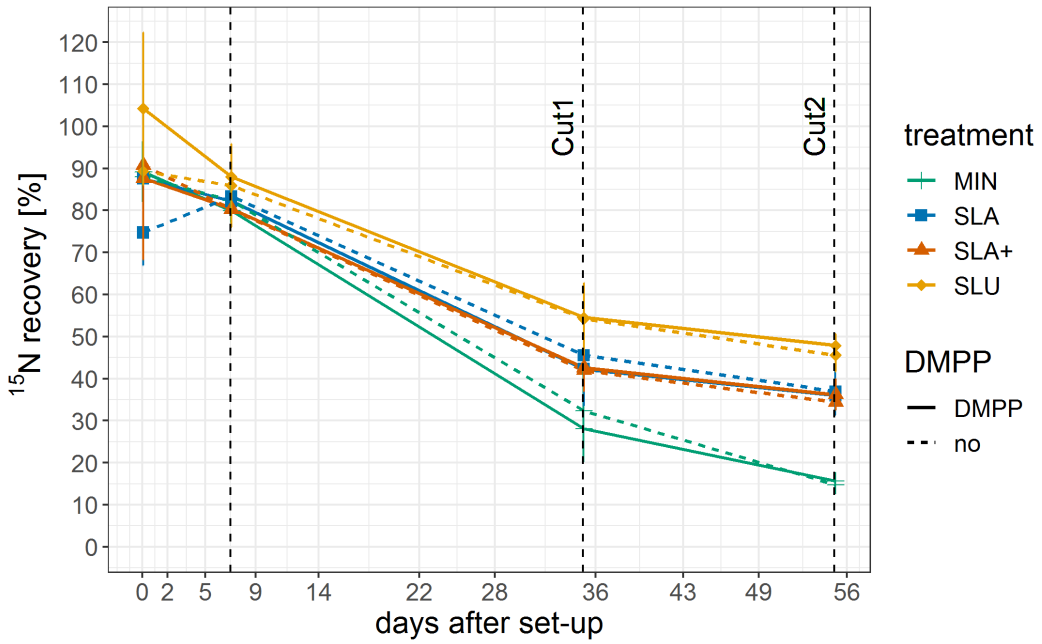

Fig. 4.3: *^{15}N recovery in total soil N; data represents mean ± standard deviation, n = 4 MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry*

DMPP did not affect ^{15}N recovery in Ntot nor the distribution in different soil N pools. Upon the first sampling at 7 DAS, most of the ^{15}N labelled mineral fertilizer could be found in the Nmin pool (**Fig. 4.4**). For the organic fertilizers, already about a third of the ^{15}N recovered in soil was part of the non-microbial organic N pool (**Fig. 4.4** and **SI 4 Fig. 4**). Except for SLU and partly MIN, no ^{15}N could be detected in the Nmic pool at 7 DAS. With time, recovery in Nmin decreased while it increased in Nmic and Norg. At 35 DAS, recovery in Nmic was significantly lower for SLA than for SLU (p = 0.002) and decreased even further with biochar (significant difference between SLA and SLA+, p = 0.01). Recovery in Nmin was highest for MIN and similar among the other treatments. Upon the last sampling, there was less than 0.5 % of added ^{15}N left in the Nmin pool, irrespective of fertilizer type. SLU had the highest recovery in all N pools.

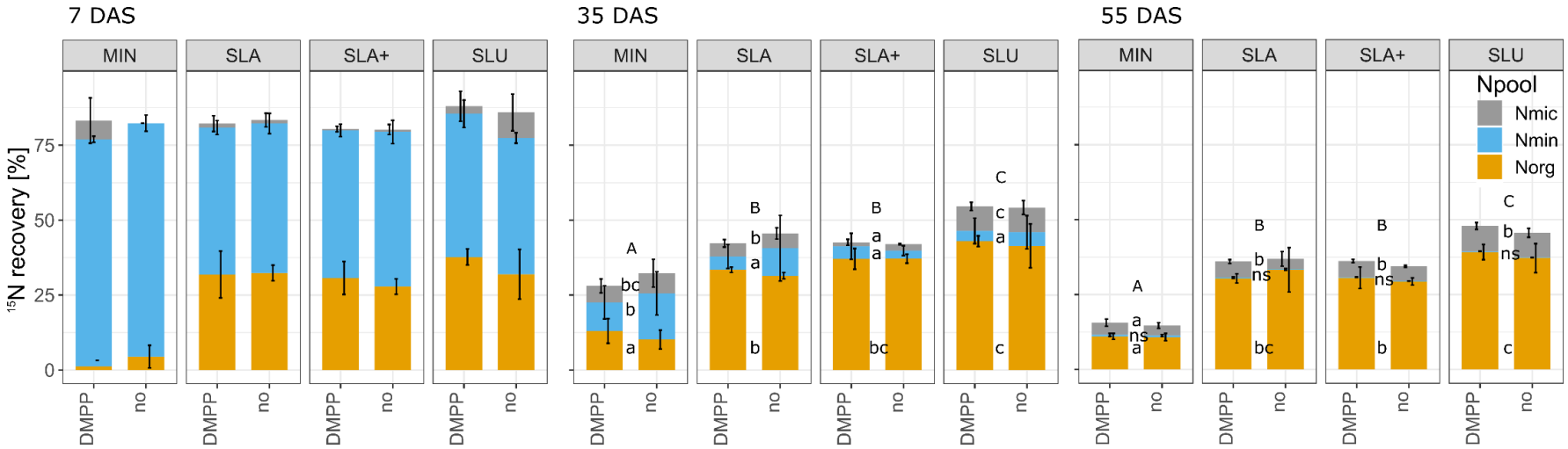

Fig. 4.4: *^{15}N recovery in different soil N pools at 7, 35 and 55 days after set-up (DAS); data represents mean ± standard deviation, n = 4. As DMPP did not have a significant effect, different letters indicate significant differences between fertilizer treatments within each soil N pool, averaged over the levels of DMPP ($p < 0.05$). Capital letters refer to ^{15}N recovery in Ntot; ns = not significant*
Nmic = microbial N, Nmin = mineral N (NH_4^+-N + NO_3^--N), Norg = non-microbial organic N
MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry
Note: Upon 7 DAS, (almost) no ^{15}N could be detected in Nmic, causing negative values for ^{15}N recovery in Nmic. For graphical illustration and for calculation of ^{15}N recovery in Norg, negative values were replaced by 0. For this reason, statistical analysis was only performed for the later time points (35 DAS and 55 DAS).

4.3.3 Mineral and microbial N dynamics in soil and soil solution

At 7 DAS, NH_4^+ content in soil clearly reflected the amounts of NH_4^+ that had been added with the fertilizers, with the highest values for MIN, intermediate for SLU, SLA and SLA+ and very low for N0 (**SI 4 Fig. 1**). For NO_3^-, there were no significant treatment differences, with even N0 reaching the same level as the other treatments (**SI 4 Fig. 2**). Columns treated with DMPP tended to have higher NH_4^+ contents, but lower NO_3^- contents than columns without DMPP at 7 DAS. However, these differences were not statistically significant. NH_4^+ and NO_3^- contents in soil decreased drastically over time. The decrease was even more pronounced for NH_4^+ than for NO_3^-. Contrary, Nmic significantly increased over time ($p < 0.001$), but did not show significant differences between fertilizer or DMPP treatments (**SI 4 Fig. 3**).

In addition to the three time points of soil sampling, we sampled pore water at a high temporal resolution using rhizon suction samplers. Although these results are potentially influenced by the difficulty to extract enough pore water at all time points (see 4.2.4), they confirm the observed decline in both NH_4^+ and NO_3^- in soil extracts over time as well as differences between treatments (**Fig. 4.5**). They also showed that NO_3^- concentrations were very high, even for N0. Unlike the soil extracts, rhizon extracts revealed a sharp decline in NO_3^- for SLU during the first two weeks of the experiment, both with and without DMPP. Furthermore, all other treatments peaked in both NO_3^- and NH_4^+ at 5 DAS. Thus, soil sampling and extraction upon 7 DAS almost represent peak levels. DMPP did not have a clear effect on neither of the fertilizers.

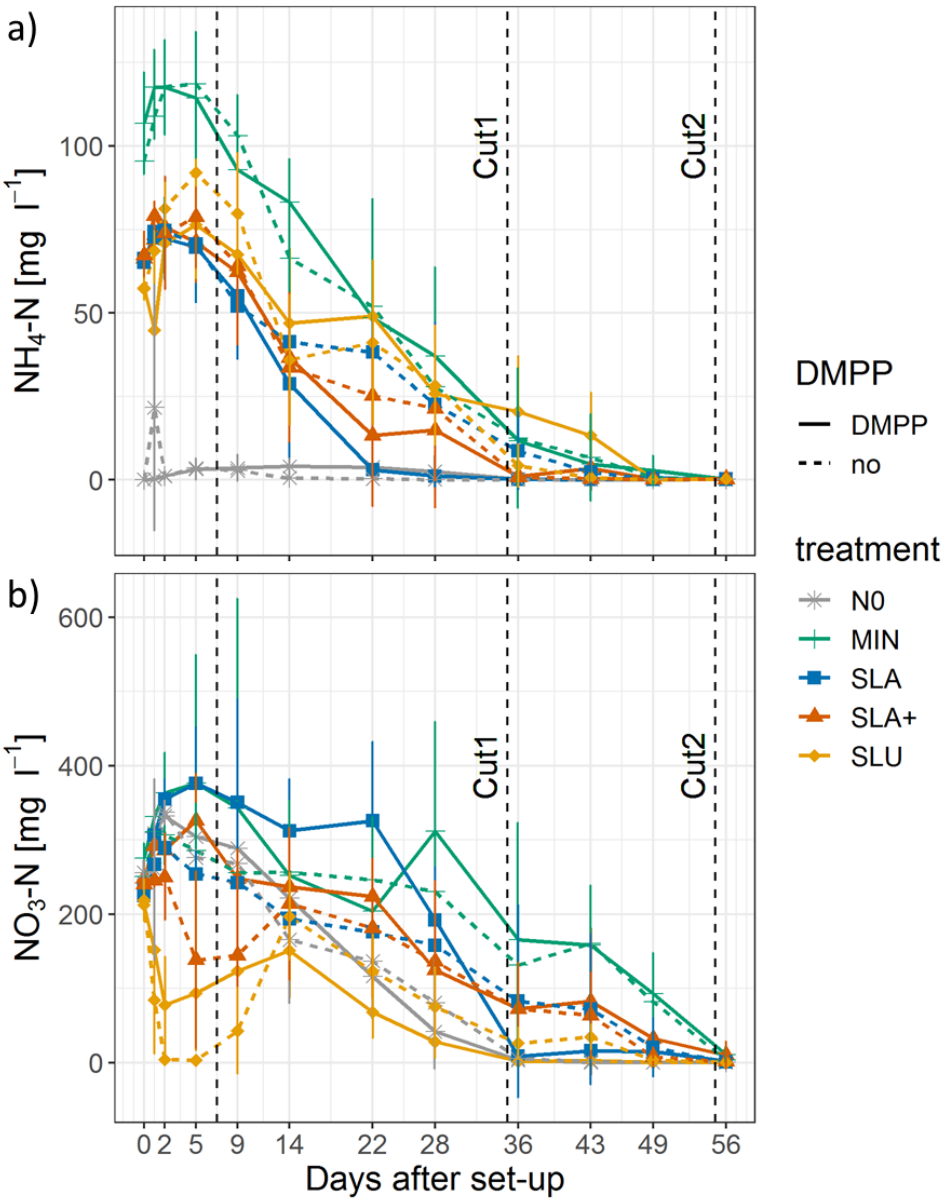

Fig. 4.5: *Development of ammonium (NH_4^+) (a) and nitrate (NO_3^-) (b) concentrations in pore water sampled with rhizon suction samplers (G-set); mean ± standard deviation, n = 4). Vertical dashed lines indicate time points for soil sampling (D-set). Cut1 and Cut2 refer to the two biomass cuts. N0 = no N fertilizer, MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry*

4.3.4 Cumulative ^{15}N recovery

Cumulative recovery of ^{15}N in all biomass samples and in soil at the last sampling ranged between 78 and 84 % and was similar for all treatments (**Fig. 4.2**). However, treatments differed in the distribution between recovery in biomass and in soil.

Overall, these numbers indicate that up to 22 % of added N remained unaccounted for. Likely, this amount was lost via NH_3 volatilization. It can be assumed that NH_3 emissions usually occur during the first days after fertilizer application. Estimated NH_3 emissions based on the difference between ^{15}N recovered in soil at 7 DAS and the amount we originally applied ranged between 12 and 20 % of applied N and were lower for SLU than for the other treatments (**SI 4 Fig. 5**). This approach is corroborated by the fact that cumulative recovery of biomass and soil at the end of the experiment reached 95 to 103 % when expressed relative to ^{15}N remaining in soil at 7 DAS (data not shown).

4.3.5 Leaching of residual N

Total dissolved N leaching was highest for MIN, with significant differences to N0 ($p = 0.002$) and SLU ($p = 0.03$), while leaching of residual N for SLA and SLA+ was intermediate (**Fig. 4.6**). NO_3^- leaching followed the same trend, however, no statistical analysis was performed since concentration of NO_3^- in leachate for several microcosms (21 out of 40) was below the limit of detection. NH_4^+ leaching was negligible. Surprisingly, also DON leaching was highest in MIN, but similar in all other treatments. Thereby, DON was in the same range than NO_3^- for MIN and tended to be the greatest share of residual N leached for the other treatments. Overall, data showed a high variability. DMPP did not significantly affect Ntot leaching, however, NO_3^- leaching in MIN, SLA and SLA+ tended to be higher with than without DMPP.

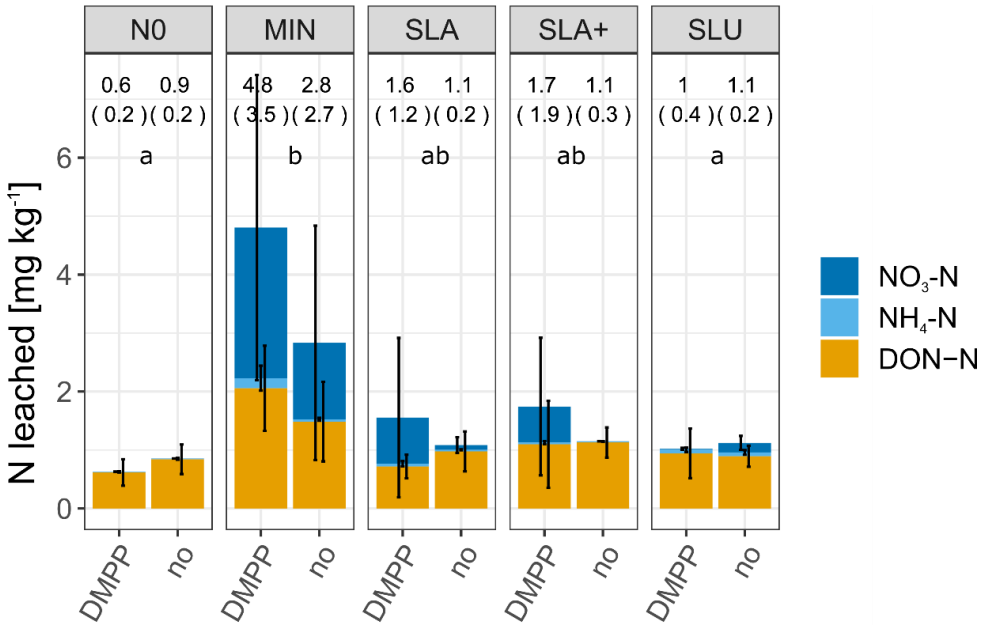

Fig. 4.6: *Leaching of nitrate (NO_3^-), ammonium (NH_4^+) and dissolved organic N (DON) at 57 days after set-up of the experiment. Cumulated values over both consecutive flushes are shown. Mean ± standard deviation; n = 4 (except MIN_no, SLA_no, SLA+_no: n = 3); MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry; Numbers on top indicate total N leached (mean (standard deviation)). Different letters indicate statistically significant differences between fertilizer treatments in total N leached. Since DMPP did not have a significant effect, pairwise comparisons were averaged over the levels of DMPP.*

In MIN, up to 50 % of leached residual N was derived from the fertilizer, while $Ndff_{rel}$ in leachate ranged between 8 and 40 % for the other treatments (data not shown). Cumulated over both consecutive flushes, about 2 % of mineral fertilizer N was recovered in total dissolved N, while it was less than 1 % for the other treatments, but the differences were not statistically significant (p = 0.07) (**Fig. 4.7**). When expressing leaching relative to residual ^{15}N in soil, differences between treatments declined, but the recovery in leached N was still highest for MIN (data not shown).

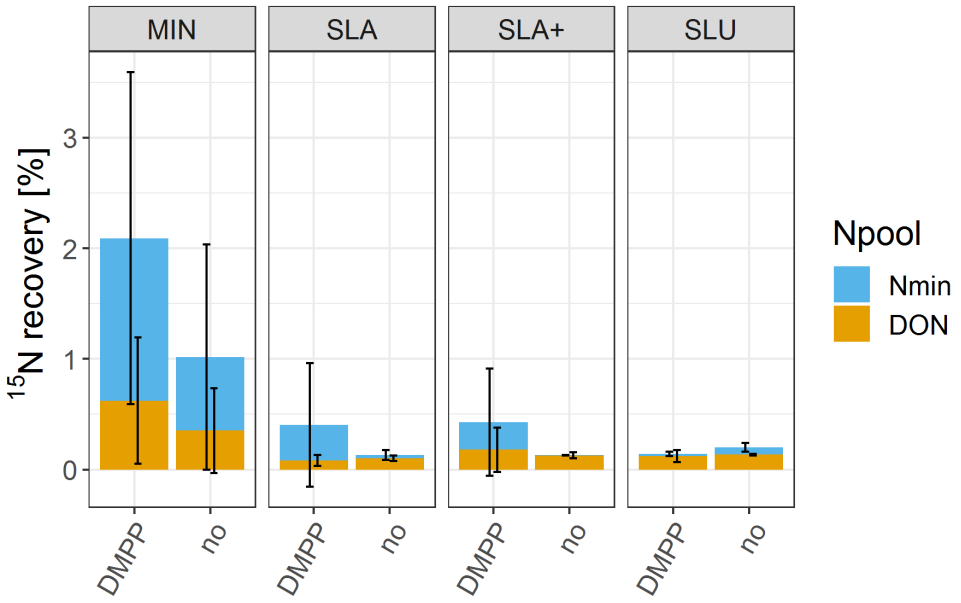

Fig. 4.7: *^{15}N fertilizer recovery in leachates collected at 57 days after set-up. Cumulated values over both consecutive flushes are shown. Mean ± standard deviation; n = 4 (except MIN_no, SLA_no, SLA+_no; n = 3); MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry*
For recovery in total leached N, neither differences between fertilizers nor DMPP levels were statistically significant.

4.4 Discussion

4.4.1 Anaerobic digestion increased fertilizer recovery in biomass and reduced recovery in soil

We expected anaerobic digestion to increase NUE of cattle slurry due to a greater NH_4^+-N content than in undigested slurry (**Table 4.2**). Indeed, cumulated ^{15}N recovery in all plant biomass was about 15 % higher with digested than with undigested slurry (**Fig. 4.2**). However, there was no significant difference between SLA and SLU in dry matter yield or total N uptake (**Table 4.3**). Overall, differences in NUE were less pronounced than reported by others (e.g. Walsh et al., 2012, Nkoa, 2014, Messner and Amberger, 1988). In contrast to most of the previous studies, our digestates were produced from the same feedstock as the undigested cattle slurry and were not co-digested with other organic waste material, allowing to link any observed difference directly to the digestion. SLA and SLU were quite similar in their NH_4^+:Ntot mass ratio (0.62 for SLU compared to 0.65 for SLA), which was reported to

be a good predictor for N availability (Svoboda et al., 2013). Higher NH_3 emissions from SLA and SLA+ than from SLU – estimated as the deviation of the ^{15}N recovery in soil from 100 % at 7 DAS (see **SI 4 Fig. 5**) – may have contributed to reduced differences in fertilizer recovery in biomass. Möller and Stinner (2009) also reported higher NH_3 emissions from digested slurries which could be linked to a higher NH_4^+ content and an increased pH. Overall, ^{15}N recovery in biomass was comparable to a pot study with ^{15}N labelled cattle slurry and mineral fertilizer (Langmeier et al., 2002).

^{15}N recovery in soil was significantly lower for digested slurry than for undigested slurry, at least from 35 DAS onwards (**Fig. 4.3**). Higher soil N recoveries could indicate a higher residual fertilizer value of undigested slurry, but also might bear the potential of increased long-term nitrate leaching (Messner and Amberger, 1988, Sørensen and Jensen, 2013). Within our study we only could assess the leaching of residual N after 57 days of ryegrass growth, but for evaluating differences in the residual fertilizer effects linked to the organic N in the fertilizers another one or two growth cycles would have been necessary. After the first cut, there was still fertilizer N in the Nmin pool left and overall NO_3^- levels in soil remained at a level of about half to two thirds of the N uptake in plant shoot biomass during the second growth phase (**Table 4.3**, **SI 4 Fig. 2**). Presumably, heat in the greenhouse had accelerated soil N mineralization and nitrification, leading to high amounts of available N from soil, which explains that ^{15}N recovery in biomass upon the second cut was still similar for SLU, SLA and SLA+, albeit lower than for MIN (**Fig. 4.2**).

The residual effect of fertilizers is determined not only by the amount, but also by the distribution of the residual fertilizer N in different soil N pools with different mineralization rate. While at 7 DAS the distribution of recovered ^{15}N in different soil N pools reflected the original composition of the fertilizers, already at 35 DAS significantly more ^{15}N was recovered in Nmic for SLU than for the other treatments (**Fig. 4.4**). An immobilization of N from SLU during the first two weeks of the

experiment was also indicated by reduced NO_3^- concentrations in pore water (**Fig. 4.5**). Also others found increased immobilization with undigested compared to digested slurry (Hossain et al., 2021). It can be explained by reduced contents of available C in digested slurry, reducing immobilization (Alburquerque et al., 2012). Furthermore, lower pore water NO_3^- concentrations in SLU compared to SLA are also in accordance with Risberg et al. (2017) who found a negative correlation between VFA content and nitrification due to an inhibitory effect on ammonia oxidation, which is the initial step of nitrification. Thereby, SLA has lower VFA contents than SLU (**SI 4 Table 1**).

Overall, the temporal development and fate of the residual labelled fertilizer N in soil is comparable to Frick et al. (in revision), finding under field conditions a rapid decline in recovery in Nmin and the major fraction of residual fertilizer N in Norg, irrespective whether it originated from mineral fertilizer or cattle slurry. Thereby, the remaining fertilizer N in soil showed a similar mineralization rate over two residual years (Frick et al., in prep). However, this might be different for digested slurry as the remaining organic N might be more recalcitrant than from undigested slurry (Wentzel et al., 2015, Möller, 2015) and further research would be needed on the mineralization rate of organic N from digested and undigested slurry. In our study, ^{15}N recovery in Norg did not change significantly over time (**Fig. 4.4**), but this does not necessarily allow for the conclusion that organic N did not start to mineralize. Likely, rather immobilization of NH_4^+-N was compensated by mineralization of organic N from the slurries (Sørensen, 2001). For clear conclusion on this, assessment of gross N transformation rates would have been necessary, which were, however, not targeted within our study. Overall, anaerobic digestion increased ^{15}N recovery in plant biomass, hence NUE, even though NH_3 losses tended to be greater than for undigested slurry (**SI 4 Fig. 5**). The resulting decrease in ^{15}N recovery in soil for SLA and SLA+ compared to SLU indicated a potential for reducing both NO_3^- leaching and the residual fertilizer effect.

4.4.2 Minor effect of DMPP on N transformation processes and losses

We investigated the effect of DMPP on N fluxes and N forms as well as on NUE in order to evaluate its potential for reducing NO_3^- leaching through inhibiting nitrification when added to different fertilizer types. Contrary to our expectations, we did not see clear effects of DMPP on NH_4^+ or NO_3^- concentrations in pore water (**Fig. 4.5**). Also the effect of DMPP on NH_4^+ and NO_3^- contents in soil extracts was marginal (**SI 4 Fig. 1**, **SI 4 Fig. 2**). Upon 7 DAS, NH_4^+ content in soil was slightly higher and NO_3^- content slightly lower in all fertilized treatments with DMPP. However, the effects were not statistically significant. This is in contrast to others, finding significantly more NH_4^+ and less NO_3^- in soil when treated with DMPP (e.g. Merino et al., 2005, Guo et al., 2021, Huf and Olfs, 2020). In the past, DMPP was reported to be effective up to six weeks, depending on soil type and environmental conditions (e.g. Peschke et al., 2004, Barth et al., 2001). In our setting, DMPP appeared to be effective only to a limited extent and for a short time span. This might be linked to several factors. First, a heat wave struck shortly after the start of the experiment, and temperatures in the greenhouse were quite high, with soil temperatures of up to 39 °C during the first days (mean soil temperature throughout the whole experiment was 21.5 °C). It was reported that DMPP was less effective and its degradation accelerated under warm conditions (Lan et al., 2018, Zerulla et al., 2001). Second, the way of application of NIs plays a crucial role, ensuring NH_4^+ from fertilizers and DMPP to remain closely associated. All our fertilizers came in a liquid form. Thus, we had to mixed DMPP solution with liquid ^{15}N labelled fertilizers, which was reported to be less effective than when formulated as granules (Ruser and Schulz, 2015). Third, NO_3^- levels in our soil were quite high already from the beginning and even in the non-fertilized control (**Fig. 4.5**). Likely, heat in the greenhouse had enhanced soil N mineralization and nitrification even before application of fertilizer treatments, overlaying the effect of DMPP. Overall, we did not see a clear interaction between fertilizer type and DMPP. However, from this we cannot unequivocally conclude that DMPP is equally effective with all tested fertilizers due to the outlined obstacles.

Dry matter production and N uptake tended to be slightly higher with than without DMPP. This was unexpected as increased yields or NUEs were usually only reported at sites with high leaching potential where DMPP could reduce losses and, thus, increase mineral N remaining in soil (Abalos et al., 2014, Tauchnitz et al., 2018). In our experimental set-up, leaching losses were avoided during the phase of plant growth (until 57 DAS). Nevertheless, plants fertilized with MIN, SLU or SLA produced more biomass and took up more N when DMPP was added, although the effects were not statistically significant in most cases. For SLA+, the effect of DMPP on dry matter yield and N uptake appeared to be weakened, as also described by Fuertes-Mendizábal et al. (2019). At the same time, we found $Ndff_{rel}$ in biomass to be slightly reduced upon addition of DMPP. Similar effects were also observed by others, reporting Ndff to be lower with DMPP for cereals in a pot study and for pasture in a field study (Peschke et al., 2004, Peschke et al., 2001, Rowlings et al., 2016). Lower $Ndff_{rel}$ under DMPP treatment combined with higher biomass yield and N uptake indicates that plants grown with DMPP must have taken up more N from soil. In short-term soil incubation studies using ^{15}N labelling, increased gross mineralization rates were observed under the addition of NIs (Shi et al., 2016, Ernfors et al., 2014). Increased mineralization represents a non-target effect of NIs, but could explain the lower Ndff, combined with overall higher yield and N uptake with DMPP. This should be further investigated.

Contrary to minor effects on soil N dynamics and plant N uptake, DMPP reduced N_2O emissions (Efosa et al., in prep-a). This is in accordance with other studies finding less pronounced and less clear effects of DMPP on soil N dynamics than on N_2O emissions (Guardia et al., 2018, Nair et al., 2020, Misselbrook et al., 2014). Likely, N_2O emissions are a more sensitive measure. It was shown that DMPP affects N_2O emissions originating from autotrophic nitrification, but in soil, clear effects might have been prevented as NO_3^- leaching was kept minimal in this study.

4.4.3 Biochar did not increase NUE of anaerobically digested slurry

There is some evidence that biochar can reduce N losses from liquid fertilizers by providing sorption sites for cations such as NH_4^+ (see also SI 4.5). Sorbed N is protected from being lost through NH_3 emissions and from nitrification and, thus, also protected from leaching while still being available to plants (Bradley et al., 2015, Craswell et al., 2021). We tested only the combination of anaerobically digested cattle slurry and biochar as digestates usually have higher NH_4^+ to Ntot ratios than undigested slurries and N in digested slurry is therefore more prone to getting lost, if not taken up by plants.

Biomass yield, N uptake and recovery were not different between SLA and SLA+, although cumulative recovery in all biomass parts tended to be slightly higher for SLA+ (~44 %) than for SLA (~42 %) (**Fig. 4.2**). Similarly, Foereid et al. (2021) found only insignificant differences in N uptake when digestates were amended with biochar. However, their digestate had a high dry matter content and thus they suspected that NH_4^+ was already sorbed to digestate particles so that biochar addition made no difference. Overall, any described effects of biochar are usually highly dependent on feedstock and pyrolysis conditions and therefore hard to predict (Craswell et al., 2021). Furthermore, the application rate was reported to be crucial and major effects on NO_3^- leaching were only observed at applications rates of > 10 t ha^{-1} (Borchard et al., 2019). We applied biochar at a much lower rate (~1.8 t ha^{-1}, if the amount applied in our columns would be equally mixed into the top 20 cm of a soil). Our application rate was intended to reflect realistic application amounts used as a fertilizer amendment.

Unlike negligible effects on biomass and NUE, biochar reduced the recovery of ^{15}N from SLA in the soil Nmic pool at 35 DAS (**Fig. 4.4**). However, this effect was transient and vanished until 55 DAS. Likely, reduced recovery in Nmic was caused by sorption of NH_4^+ to the biochar surface, limiting access by microbes. This was also suggested by others (e.g. Knowles et al., 2011). Indeed, in a batch sorption experiment we could

confirm the capacity of the biochar used in our study to effectively sorb up to 20 to 40 % of NH_4^+ added with an ammonium sulphate solution (**SI 4 Fig. 6**). As we pre-mixed SLA with biochar about 13 hours before set-up of the experiment, biochar likely also effectively could be loaded with NH_4^+ from SLA. Nevertheless, biochar did not affect absolute Nmic contents in soil (**SI 4 Fig. 3**). Since fertilizer recovery in Nmic was reduced, more soil N must have been immobilized and incorporated into Nmic. Thus, biochar might have induced a transient immobilization of soil N, which could be explained by increased short term gross transformation rates following biochar addition as also found by Nelissen et al. (2015).

However, apparently neither sorption of NH_4^+ to biochar nor enhanced mineralization-immobilization turnover evoked any significant effect on yield or N losses, or the effect was masked by other processes, such as an overall high fertilizer efficiency and low losses. Long-term effects of repeated biochar applications or applications of higher amounts of biochar might differ and should be further investigated.

4.4.4 Only small amounts of residual ^{15}N leached, independent of fertilizer type or treatment

After the second biomass cut, columns were oversaturated with demineralized water in order to assess the proportion of residual fertilizer N that could be leached. Overall, only small amounts of residual N were leached after growing ryegrass for 57 days, and recovery of fertilizer N in leachate was less than 2 % of the applied amount in all treatments (**Fig. 4.6**, **Fig. 4.7**).

Surprisingly, the amount of Ntot leached (MIN > SLA = SLA+ > SLU) followed an opposite trend to total ^{15}N recovery in soil at 55 DAS (SLU > SLA = SLA+ > MIN) (**Fig. 4.2**, **Fig. 4.6**). This emphasizes the importance of the N pool in which the ^{15}N prevails. Indeed, ^{15}N recovery in the Nmin pool at 55 DAS was still highest for MIN and lowest for SLU, though overall small (**Fig. 4.4**). Although total N recovery in soil was highest for SLU, residual N leaching was lowest for it, indicating that the residual

slurry N was stabilized in soil. This might be due to the observed higher microbial immobilization (**Fig. 4.4**), as also suggested by Sørensen (2004), but also due to the higher amount of both organic N and organic C applied with the slurry compared to the other treatments (**Table 4.2**). Our results further indicate that in the timeframe considered in this study, leaching of residual N was still mostly controlled by differing amounts of mineral N applied with the fertilizers.

Our study was too short to evaluate the mineralization rate of the residual fertilizer N in soil. However, this is an important factor to consider especially under field conditions where fertilizers are usually applied repeatedly over the years and residual fertilizer N accumulates in soil. Model predictions, calibrated on data from a four-year field study, showed that undigested cattle slurry has a lower short-term, but higher residual fertilizer effect than digested slurry (Schröder et al., 2007). However, whether this translates into an increased NO_3^- leaching potential depends also on the mineralization rate, which in turn is a function of the composition of the originally applied slurry, as well as of soil, climate and crop (Berntsen et al., 2007). Cumulating the residual effect of a single application over 50 years, Jørgensen and Petersen (2006) simulated a decrease in soil C upon application of digested pig slurry compared to undigested slurry likely due to a lower C:N ratio in digested slurry inducing soil organic matter mineralization. In their simulation, NO_3^- leaching could only be reduced when the increased mineral N content of digestate was taken into account and the application amount reduced accordingly. Especially when applied repeatedly, the residual effect should be considered for future fertilization in order to minimize leaching potential (Jarosch et al., 2018).

We found equal or even higher amounts of DON than Nmin leached and even recovered considerable amounts of ^{15}N from mineral fertilizer in leached DON (**Fig. 4.6**, **Fig. 4.7**). DON leaching was previously suggested to be an important, though often neglected N loss pathway (Van Kessel et al., 2009). Our results highlight the importance to also consider DON leaching as potential loss pathway. However, the

higher amounts of DON and ^{15}N-DON leached under MIN than under the organic fertilizers were unexpected and are difficult to explain.

In our experimental setting, all tested treatments (anaerobic digestion, DMPP and biochar) only had minor effects on residual N leaching. Under field conditions, NO_3^- leaching was found to be driven mainly by amount and timing of inputs, whereas previous digestion of slurry had little effect (Möller, 2015, Svoboda et al., 2013). Anaerobic digestion could offer assets in this respect as it allows for more flexible timing of application and usually the N contents of the digestate are known to the farmer. Overall, reduced inputs might be necessary for avoiding losses. Anaerobic digestion and DMPP both could allow for reduced Ntot input rates with cattle slurry while alleviating potential negative effects on yield (Rose et al., 2018, Rowlings et al., 2016).

It must be kept in mind that 17 to 22 % of ^{15}N remained unaccounted for (**Fig. 4.2**). Most of this share might be ascribed to NH_3 losses (**SI 4 Fig. 5**), indicating that under the conditions of our study, NH_3 volatilization was a much more important N loss pathway than leaching. As these values tended to be higher for SLA than for SLU, special care must be taken to reduce NH_3 volatilization from digested slurry in order to avoid pollution swapping.

4.5 Conclusion

In this study we assessed the potential of anaerobic digestion, biochar and DMPP to increase NUE and reduce residual N leaching from cattle slurry. We found anaerobic digestion to increase plant N recovery while reducing recovery in soil. This might suggest a lower residual fertilizer value of anaerobically digested slurry. It also indicates that anaerobic digestion might be a feasible way to reduce soil N accumulation and the associated potential for NO_3^- leaching, if the higher NH_4^+ content is considered and input amounts are reduced accordingly. However, estimated NH_3 emissions were higher for SLA than for SLU, indicating that care must be taken

to reduce NH_3 emissions and to avoid pollution swapping. Although more than 45 % of N from SLU were still recovered in soil at 55 DAS, this did not translate into increased N leaching. It highlights the importance of whether N is present in organic or mineral form. Further research is needed to evaluate the long-term mineralization rate of the residual N from both digested and undigested cattle slurry in soil. Biochar tended to augment the observed effect of anaerobic digestion with regard to increased N uptake from digested slurry, but the effects were not statistically significant. There was some evidence that biochar reduced the ^{15}N recovery in Nmic likely by adsorption of the NH_4^+ applied with the fertilizer, but the effect was transient. Also DMPP only induced small changes, but especially the reduced relative proportion of N derived from the fertilizers in plant biomass combined with higher absolute N uptake and dry matter yield warrants further research. Overall the effects of all investigated treatments (anaerobic digestion, biochar and DMPP) were rather small, likely due to high temperatures in the greenhouse during the first days of the experiment leading to high soil N mineralization and the rather short experimental duration of the experiment.

Chapter 5 General Discussion and Conclusion

This thesis aimed at investigating the N fluxes, nitrate leaching potential, and the residual fertilizer effect of cattle slurry in comparison to mineral fertilizer under on-farm conditions. In addition, the effects of anaerobic digestion, biochar and the nitrification inhibitor DMPP on the NUE of cattle slurry and its leaching potential were tested. To this end, ^{15}N labelled mineral fertilizer and ^{15}N labelled cattle slurry were used and traced through the soil-plant-system, both in a field experiment and in a column trial in the greenhouse. I hypothesized that under field conditions and when applied according to current fertilizer recommendations, i.e. at the same rate of mineral N, cattle slurry would cause higher nitrate leaching than mineral fertilizer, but also would have a higher residual fertilizer effect (**Chapter 3**). Tracing fertilizer N into plant uptake as well as its fluxes and forms in the soil system in the year of application, I presumed to gain a better understanding of the short-term fate of the two fertilizers, which would also facilitate predicting their long-term fate (**Chapter 2**). Furthermore, I expected that anaerobic digestion, biochar and DMPP would enhance plant N uptake from cattle slurry and consequently reduce its leaching potential (**Chapter 4**).

In this chapter, the results of the experiments described in **Chapter 2** to **Chapter 4** will be jointly discussed in order to identify main drivers of nitrate leaching from animal manure at field level as well as to identify measures to reduce nitrate leaching losses and improve the efficiency of using cattle slurry as a fertilizer.

5.1 Does animal manure cause higher nitrate leaching than mineral fertilizer?

To answer this questions it is important to acknowledge that nitrate leaching from fertilizers (irrespective whether applied as animal manure or mineral fertilizer) occurs at different time scales and has different drivers. Direct leaching of fertilizer N results from a mismatch between plant N demand and easily available N input with the fertilizer – either in amount, timing, or both – and usually happens shortly after

application. In the long-term, nitrate leaching is mostly a function of the residual fertilizer N amount in soil, which in turn is influenced by crop N uptake.

Animal manure is often suspected to be a major source of nitrate leaching as the applied amounts of total N are usually higher with animal manure than with mineral fertilizer (e.g. Sørensen and Jensen, 2013, Bergström and Kirchmann, 2006). Indeed, in the field experiment, absolute amounts of leached nitrate deriving from the ^{15}N labelled fertilizers over a sequence of three crops were larger for cattle slurry than for mineral fertilizer (**Chapter 3**). However, the ^{15}N recovery in leachate was not different between the fertilizers. Overall, only little N in leachate (< 5 %) derived from the labelled fertilizers while the rest derived from soil N mineralization, both under field conditions (**Chapter 3**) and in the greenhouse experiment (**Chapter 4**), which is consistent with findings by others (e.g. Macdonald et al., 1989).

Increased nitrate leaching from animal manure could be related to the fact that ^{15}N recovery in plant biomass was smaller for cattle slurry than for mineral fertilizer in the year of application and consequently more ^{15}N from cattle slurry remained in soil (**Fig. 3.3**). With continued (re-)mineralization of (immobilized) organic N in the following seasons, a higher amount of slurry N than mineral fertilizer N got released, resulting both in a higher residual fertilizer effect and higher nitrate leaching (**Fig. 3.5**). Although being temperature dependent, both mineralization and nitrification continue at temperatures down to 0 °C and, thus, also happen during autumn and winter when plants do not take up the released N (Sørensen et al., 2019).

Anaerobic digestion was tested as an approach to increase the short-term N availability of slurry N to crops and thereby reduce the residual N in soil and with it the potential for leaching losses (**Chapter 4**). Indeed, anaerobic digestion enhanced plant N uptake from slurry and reduced recovery in soil (**Fig. 4.2**). Nevertheless, nitrate leaching from the residual fertilizer N in soil after 57 days of ryegrass growth tended to be higher for digested than for undigested slurry (**Fig. 4.6**). As in the field experiment, most slurry N in soil, regardless of digestion, was found in the non-

microbial organic N pool, and, thus, did not end up in nitrate leaching (**Fig. 4.4**). This observation emphasizes that the higher observed leaching in the field experiment can be explained only by continued mineralization of organic N over several months/years and that direct leaching of cattle slurry was low (**Fig. 3.5**) (Sørensen and Jensen, 2013). Considering repeated applications, though, the lower ^{15}N recovery in soil from anaerobically digested slurry compared to undigested slurry could hint to less N accumulation in soil and thereby reduced long-term leaching losses.

Nevertheless, the results presented here must be seen in relation to the specific experimental conditions. Under different agro-pedoclimatic conditions or manure applications schemes, there is also a risk of direct nitrate leaching from animal manure. As outlined above, direct leaching of manure N might happen when plant N demand and the input of easily available N are not well synchronized. In this respect, the following aspects should be considered:

i) Application amounts of mineral N exceeding the crop demand were found to lead to an exponential increase in nitrate leaching, irrespective whether applied as mineral or organic fertilizer (e.g. Wang et al., 2019, Goulding et al., 2000). Although legally restricted, excessive N application amounts with animal manure might happen in areas with a large structural N surplus resulting from local separation of crop and livestock production (Garnier et al., 2016, Oenema and Tamminga, 2005) (see also **1. 2**). It might also happen when animal manure is applied at times when plants are not readily taking up the nutrients, e.g. in autumn or early in spring.
ii) Animal manures having a high ammonium-N to total N ratio are specifically prone to direct leaching, if applied at amount or time not matching plant N demand (Sørensen and Jensen, 2013).
iii) Direct leaching of fertilizer N plays a bigger role on sandy soils (e.g. Wachendorf et al., 2005, Jayasundara et al., 2010, Thomsen et al., 1997) than on soils with a finer texture such as prevailing in the Gäu region.

iv) If plant growth is limited by other factors, for example by drought or a deficiency in another nutrient, fertilizer N accumulation in soil can be substantial and might be at risk of being leached under subsequent precipitation or irrigation (Feigenbaum et al., 1984, Smith and Chalk, 2018). Despite strongly reduced precipitation in summer 2018 (between April and October, precipitation was about 30 % lower than the long-term average) (**Fig. 3.2**), this did not translate into massive losses of fertilizer N in the following winter, though (**Fig. 3.5**). Likely, the clay-rich soil (**Table 3.1**) prevented major losses as described by Jayasundara et al. (2010). They found that suboptimal timing of manure application only translated into higher leaching on sandy soil, but not on a loamy soil where overall direct leaching of fertilizer N was much lower.

Otherwise, there is evidence that when applied according to recommendations, usually the major part of leached nitrate under arable fields does not originate directly from the fertilizer – irrespective whether it is animal manure or mineral fertilizer – but from mineralization of soil organic N (Macdonald et al., 1989, Christensen, 2004, Jayasundara et al., 2010, Cookson et al., 2000). By using the natural abundance of ^{15}N and ^{18}O in nitrate from leachate samples collected in the Gäu region, also Gilbert (2021) identified soil N mineralization as the major source of nitrate leaching and found only low contributions of direct leaching of fertilizer N. This highlights the importance of better understanding and predicting soil N mineralization and of taking it into account for locally adapted fertilization guidelines (see **5.4**).

Still, repeated slurry applications bear the potential of N accumulation in soil and with it increased long-term leaching losses. Thus, an improved understanding of both the restitution of soil N with fertilizer N not taken up by the crop in the year of application as well as of the turnover dynamics and the long-term N fertilization effect of cattle slurry is required. These aspects should be taken into account in fertilization recommendations. Within the following section, the prerequisites as well as

knowledge gaps that have to be addressed for efficient use of animal manure as a fertilizer will be discussed.

5.2 Understanding animal manure as an N fertilizer

Understanding animal manure as a nutrient source to crops requires considering both its short-term effect and its residual effect. Often the ammonium-N to total N ratio in manure is considered decisive for its plant availability in the year of application (Sørensen et al., 2003). In my experiments, the fertilizer effect in the first year was lower for Slu than for Min. Despite both fertilizers had been applied at the same rate of mineral N, both ^{15}N recovery and total Ndff in shoot biomass were lower for Slu than for Min (**Table 3.3**, **Table 3.4**). It can be explained by higher NH_3 losses and increased immobilization of cattle slurry N due to simultaneous addition of organic C, which resulted in higher Ndff in microbial biomass N in soil (**Chapter 2**). This finding is consistent between the field study (**Chapter 2**) and the column experiment (**Chapter 4**) as well as observations by others (Sørensen, 2004, Cavalli et al., 2014, Cavalli et al., 2016a, Schröder et al., 2005).

In contrast to Min, Slu also contains organic N which gets mineralized simultaneously. The difference between gross immobilization of mineral N and gross mineralization of organic N is termed net mineralization. Net mineralization was observed to turn negative within the first one to four weeks after application, especially for animal manures with a C to N ratio above 15 (Sørensen, 2004). In the greenhouse study, net mineralization of undigested slurry was also found to turn negative during the first two weeks, but it was not the case for the digested slurry which had a slightly lower C to N ratio (**Fig. 4.5**). In the mid- to long-term, net mineralization turns positive, both from mineralization of organic N but also re-mineralization of previously immobilized N. However, in the field study mineralization obviously could not compensate for increased immobilization after slurry application, or plant uptake from slurry was reduced by other factors. These could be: 1) higher NH_3 losses for Slu than for Min (**Chapter 2**, **SI 2.2**), and 2) clay

fixation of ammonium. NH_3 losses ranged between 2 and 19 % of the applied N and were, thus, rather low compared to other studies (**SI 2 Fig. 4**) (Sommer and Hutchings, 2001). Ammonium sorption seems likely in the field experiment due to the high clay content in the soils (**Table 3.1**). Cavalli et al. (2016b) observed that after repeated application of cattle slurry around 20 % of ammonium-N remained adsorbed to clay. The mineral fertilizer in the field experiment was applied as ammonium nitrate, thus, part of the applied amount of N with Min was in the form of nitrate and therefore not directly affected by clay sorption.

As a considerable part of N from both Slu and Min was recovered in the soil after harvest of the first crop (**Fig. 3.4**), their residual effect on the succeeding crops has to be taken into account. Less than 5 % of the originally applied fertilizers were recovered in the second and less than 3 % in the third crop which is consistent with the literature (**Table 3.4**) (Cusick et al., 2006, Smith and Chalk, 2018). However, in order to better predict and consider the rate of mineral N release from the fertilizers, not only plant uptake in the succeeding crop, but fertilizer N losses via leaching should be considered.

Net mineral N release from Min vs Slu can be estimated for each of the three cropping seasons by summing up Ndff in the harvested plant biomass and Ndff in collected nitrate leaching (**Fig. 5.1**, **Fig. 5.2**). The net mineralization rates were obtained by dividing this sum (Ndff in plant + Ndff in leachate) by the residual fertilizer N in the soil (**Eq. 5.1**).

$$net\ mineralization\ rate = \frac{Ndff_{crop} + Ndff_{leachate}}{residual\ fertilizer\ N} = \frac{Ndff_{crop} + Ndff_{leachate}}{N_{applied} - NH_3 - \left(Ndff_{crop} + Ndff_{leachate}\right)_{precrop}} \quad \textbf{Eq. 5.1}$$

where residual fertilizer N is the amount of residual fertilizer N in soil, calculated as difference between the applied amount of fertilizer N ($N_{applied}$), the NH_3 emissions upon fertilizer application (NH_3) (compare **SI 2.2**) and the amount of fertilizer N

already taken up by crop(s) or leached during the preceding crop(s). Since the field experiment was conducted as an on-farm study, the experimental conditions reflect realistic agricultural conditions including further supply of unlabelled fertilizer and manure according to the Swiss fertilization guidelines (Richner and Sinaj, 2017) (**Table 3.2**). The calculated residual fertilizer N amount in soil was higher than the measured values for the residual fertilizer N in soil (in italics and parentheses) (**Fig. 5.1**, **Fig. 5.2**). This can be explained because soil sampling in 2018 and 2019 took place in October and not at the same time as biomass sampling and exchange of the SIA devices (**Fig. 3.1**).

From the first to the second crop, 7 to 14 % of the residual N were released (**Fig. 5.1**, **Fig. 5.2**). At Field A, these values decreased to less than 6 % in the succeeding crop, while they remained stable at 8 to 9 % at Field B. Net mineralization relative to the originally applied amount of fertilizer N followed a similar pattern, but was obviously lower than when related to the remaining fertilizer N in soil. Overall, the net mineralization rates are in accordance with others (Gutser and Dosch, 1996) and were similar between Min and Slu. This indicates that residual N from Min and Slu has a similar degradability. This is also emphasized by the fact that already in the next spring after application, the residual fertilizer N from both Min and Slu was found in the non-microbial organic N pool (**Fig. 2.6**). Slightly higher release rates of residual N for Min than for Slu might hint to the fact that residual organic N from slurry such as undigested dietary N could be still more recalcitrant than immobilized N from mineral N in slurry or mineral fertilizer (see **Chapter 2**) (Bosshard et al., 2011). Overall, mineral N release, was likely more controlled by crop rotation and soil tillage (higher values found after termination of grass-clover) than by fertilizer type. Absolute amounts of N turnover, however, were higher for Slu than for Min due to higher amounts of slurry N remaining in soil. These must be considered for further fertilization in order to reduce leaching losses.

Knowledge of these release rates can help to gain an improved understanding not only on the effect of a single fertilizer application, but also of the repeated application of both mineral fertilizer and animal manure over several years and decades. The release rates are also an important prerequisite to predict the long-term fertilizer effect using modelling approaches (e.g. Berntsen et al., 2007, Sørensen et al., 2017). Over a time range of several decades, an increase both in soil N stocks and in the release of mineral N were found (e.g. Jensen, 2013). In theory, a steady-state could be reached when cumulated mineral N release equals annual organic N input. This might take up to 100 years, though (Sebilo et al., 2013, Schröder et al., 2013, Jarosch et al., 2018).

Considering the long-term manuring history of most agricultural fields, these aspects are also important to take into account within fertilization guidelines. The Swiss fertilizer guidelines assume 50 to 70 % of total N in bovine slurry to be available, taking repeated manure applications already into account (Richner and Sinaj, 2017). The assumptions in the Swiss fertilizer guidelines can be challenged by the fact that they presume rather high NH_3 losses as "unavoidable". Nowadays, with improved manure storage conditions as well as low-emission application techniques becoming the standard, N amounts entering the soil system might be higher and with it the N availability to plants. Schröder et al. (2007) suggested to consider 70 to 80 % of manure N to be available on fields with a long-term manuring history. However, a reduction in inputs should not happen at the cost of a long-term depletion of soil fertility (see also **5.4**).

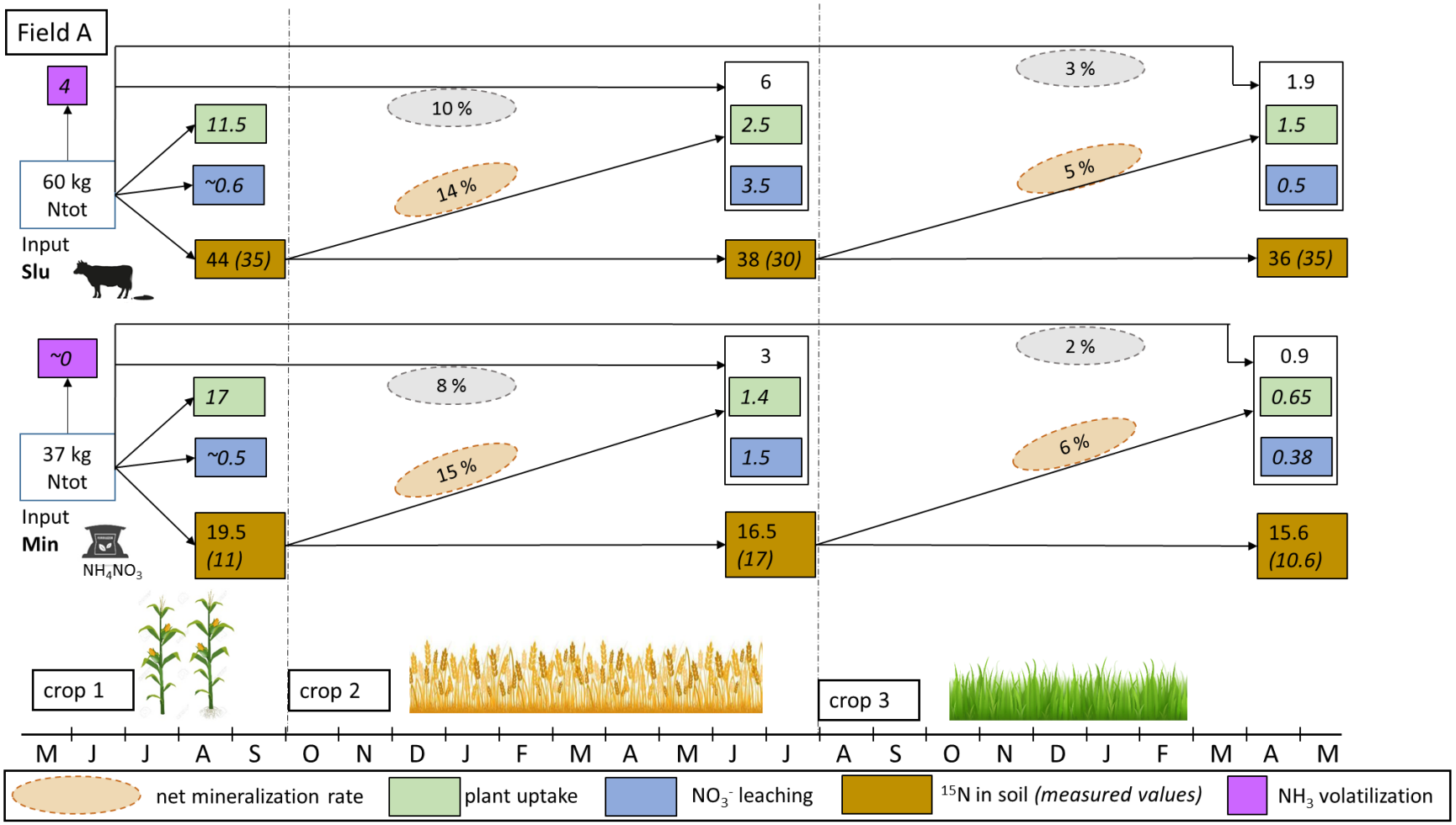

Fig. 5.1: *Net mineralization rate of ^{15}N labelled mineral fertilizer (Min) or ^{15}N labelled cattle slurry (Slu) over the crop sequence silage maize – winter wheat – grass-clover at Field A between May 2018 and April 2020 as proportion of residual fertilizer N in soil (orange circles) or as proportion of originally applied fertilizer N (grey circles). Measured values within the field experiment are shown in italics. Net mineralization rate was estimated as the sum of fertilizer N in plant shoot uptake (green) and fertilizer N in leachate collected in SIAs (blue) relative to the residual amount of fertilizer N in soil (brown) or relative to the originally applied amount of fertilizer N. Numbers are given in kg N ha^{-1}.*

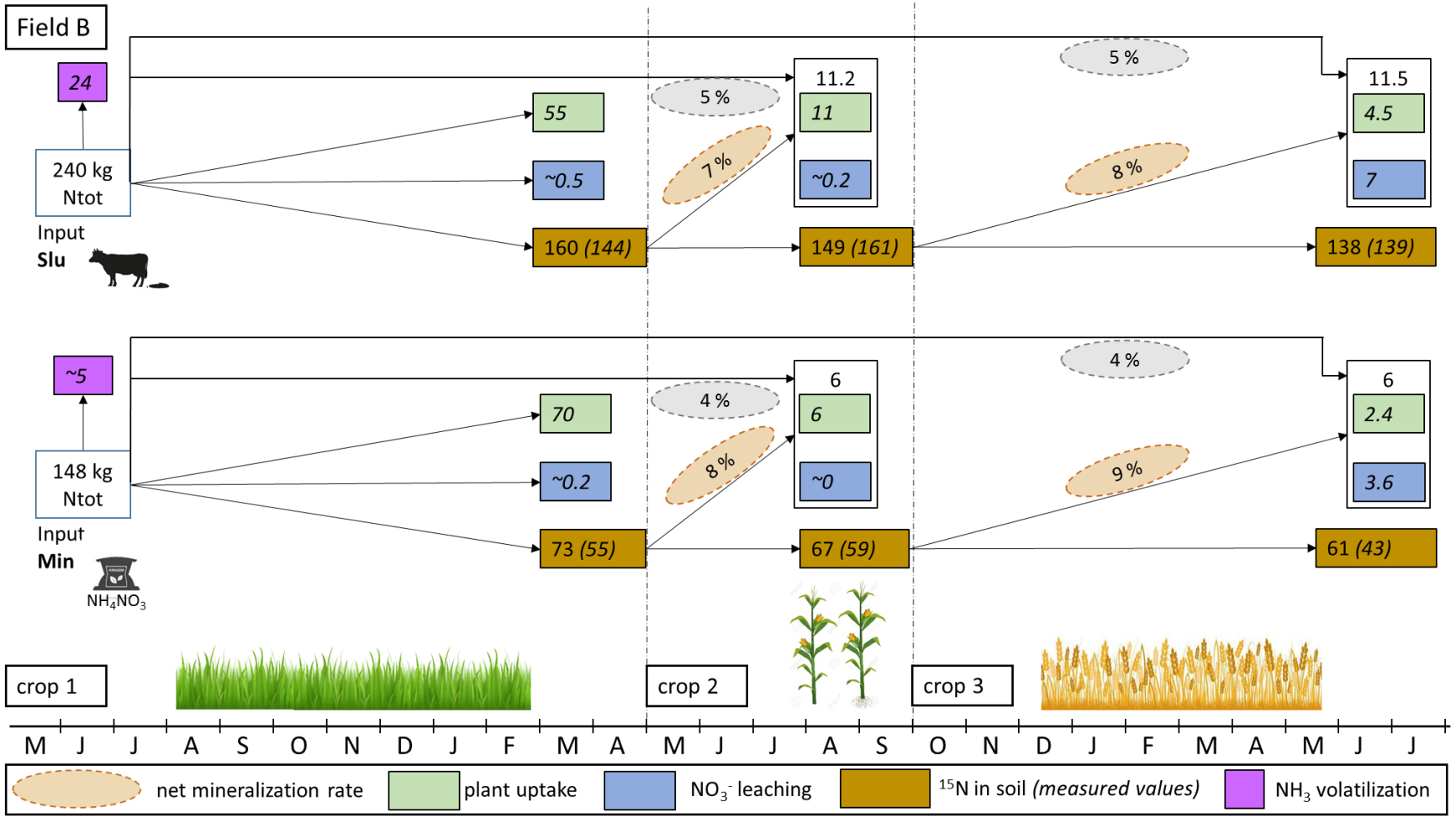

Fig. 5.2: *Net mineralization rate of ^{15}N labelled mineral fertilizer (Min) or ^{15}N labelled cattle slurry (Slu) over the crop sequence grass-clover – silage maize – winter wheat at Field B between May 2018 and July 2020 as proportion of residual fertilizer N in soil (orange circles) or as proportion of originally applied fertilizer N (grey circles). Measured values within the field experiment are shown in italics. Net mineralization rate was estimated as the sum of fertilizer N in plant shoot uptake (green) and fertilizer N in leachate collected in SIAs (blue) relative to the residual amount of fertilizer N in soil (brown) or relative to the originally applied amount of fertilizer N. Numbers are given in kg N ha^{-1}.*

5.3 Reducing nitrate leaching requires a multifaceted approach

At appropriate application rates and timing, reducing nitrate leaching from animal manure is mostly a function of managing its residual effect by i) predicting the residual N release and take it into account for upcoming fertilizer applications and, ii) ensuring sinks for released mineral N during times when the main crops do not take up N, especially during winter (Sørensen et al., 2019). However, despite considerable efforts in better predicting both immediate and long-term fertilizer effect of organic manures, anticipating mineral N release dynamics and even more so synchronizing mineral N release with crop N demand remain challenging. In the following, both technical approaches as well as agronomic measures for better matching N release and uptake are discussed.

5.3.1 Improved manure management by technical approaches

Nowadays, several technical possibilities for manure management are at hand, usually aiming at increasing the short-term N availability of manure. Increasing short-term manure N availability will both enhance predictability of its fertilizer effect and reduce residual effects that are hard to synchronize with crop N demand and therefore at risk of being lost to the environment. Stevens (2020) gives a detailed overview on current approaches to recover mineral nutrients from organic material. Discussing all of them goes beyond the scope of this thesis, but I will highlight a selection that I consider relevant to this thesis and the situation in the Gäu.

Anaerobic digestion

Anaerobic digestion makes use of C in the animal manure as energy source and leaves a digestate with lower C, but an increased ammonium-N to total N ratio. As a consequence and in accordance with others (Foereid et al., 2021), I found cumulated N recovery in plant biomass higher for digested than for undigested slurry (**Fig. 4.2**). However, this did not translate into a reduction in nitrate leaching (**Fig. 4.6**, **Fig. 4.7**). It can only be speculated that in the long term, lower accumulation of organic N in soil after repeated application of digested slurry as compared to undigested slurry

might reduce leaching risk. A 3-year field study in Switzerland comparing undigested cattle slurry, anaerobically digested slurry from an on-farm biogas plant, and a liquid digestate from a commercial biogas plant likewise found slightly higher crop NUE for digestates than for undigested slurry (Bünemann and Mayer, unpublished). Differences were small, though. However, in the same experiment, Efosa et al. (in prep-b) found elevated NH_3 emissions from digestate, highlighting the importance of suitable application technique (injection, placement or incorporation) in order to avoid pollution swapping.

Besides effects on N-cycling, replacing animal manure with digestates will also affect C return to soil, which might affect long-term fertility of soil. Digestates have a lower C content, but the remaining C after digestion usually is much more recalcitrant (Möller, 2015). Thomsen et al. (2013) found similar retention of C in soil when plant material was applied directly to soil compared to either cow manure or biogas digestate produced from the same amount of plant material.

Biochar addition

Along with anaerobic digestion, the potential of biochar to reduce losses and further enhance NUE of digested cattle slurry was tested (**Chapter 4**). Beneficial effects of biochar on N uptake or a reduction in nitrate leaching as suggested elsewhere (Borchard et al., 2019) could not be confirmed within my thesis. It was reported that biochar usually is most effective in soils with a low cation exchange capacity, i.e. in sandy soils or soils with a low content of organic matter. Both is not the case on most of the fields in the Gäu region (Wey, 2021). Thus, large scale application of biochar appears a debateable measure for reducing nitrate leaching losses in the Gäu region.

Inhibiting nitrification

Inhibiting nitrification of ammonium-rich fertilizers has the potential to reduce nitrate leaching by keeping the added fertilizer longer in the form of ammonium (Qiao et al., 2015). In my experiment, I could not prove the effectiveness of the nitrification inhibitor DMPP when combined with either cattle slurry, anaerobically digested

cattle slurry or mineral fertilizer in reducing leaching or increasing yields (**Chapter 4**). Partly, the high soil temperatures caused by the heat wave at the beginning of the greenhouse experiment might explain the low effectiveness of DMPP (Zerulla et al., 2001). Furthermore, increased yields or N uptake upon addition of DMPP was mostly associated with a reduction in leaching losses and the resulting increase in available N (Abalos et al., 2014). In the greenhouse study, only the leaching of the residual fertilizer N at the end of the experiment was assessed. Leaching losses during the experiment did not occur and, therefore, available N was likely the same whether DMPP was added or not.

Ideally, the application of nitrification inhibitors should go along with a reduction in N inputs. Only then N losses could be drastically reduced, while yield levels would stay unaffected (Rose et al., 2018, Rowlings et al., 2016). In case nitrification inhibition will be further pursued as a nitrate leaching mitigation strategy, this aspect should be further investigated and tested within field settings in the Gäu region. It must be noted, though, that nitrification inhibitors were reported to be less effective in clay-rich soils than on lighter soils (Barth et al., 2019). Furthermore, it was indicated that the formulation and way of application of DMPP could have an important effect (Ruser and Schulz, 2015). Currently, in Switzerland DMPP is mostly used within the commercial fertilizer Entec, which is a mineral fertilizer formulation. For animal slurries or digestates, the liquid formulation Vizura® is available (Sanz-Gomez, 2017), but currently much less used than Entec. Therefore, the effectiveness of the combination of DMPP with liquid organic fertilizers should be further assessed.

Although nitrification inhibitors must undergo excessive risk assessment before being legally approved, they might still have unwanted side effects. As other agrochemicals, nitrification inhibitors could leach to the groundwater and become water pollutants themselves (Marsden et al., 2016). Furthermore, they might have unwanted side-effects on the soil microbial community (Florio et al., 2016). Biological nitrification inhibitors, produced by plants and introduced in the soil environment via plant

exudates or through incorporation of their biomass might be an interesting alternative (Coskun et al., 2017). However, the effectiveness and the conditions of biological nitrification inhibitor production under N-rich environments are a field of active research.

Solid-liquid separation and further processing

Separating the solid and liquid phase of cattle slurry might be another option for a more targeted application of the nutrients within animal manure as a fertilizer. As shown by Pedersen et al. (2021), the direct N fertilizer value of slurry within the year of application decreases with increasing contents of dry matter due to a higher N immobilization. Separation of a liquid fraction with strongly reduced dry matter content, containing most of the N and potassium (K), and of a solid fraction, containing most of the phosphorus (P), could allow for a more targeted application of the individual nutrients (Fangueiro et al., 2012). Compared to the unseparated slurry, the liquid phase infiltrates easier into soil and has an increased availability of N. Furthermore, by additional processing steps it is possible to manufacture fertilizer products similar to mineral fertilizer with known nutrient contents (Sigurnjak et al., 2020). These techniques involve ammonia stripping from the liquid phase, or the application of reverse osmosis (Bosshard et al., 2010). All these approaches appear viable in reducing N losses, facilitating transport and easing targeted application, but they also use quite sophisticated technology, are costly and have a high energy demand, which must be considered. Furthermore it must be kept in mind that also the solid fraction, containing most of the C should be returned to the soil in order to preserve soil fertility.

Slurry acidification, either of full slurry or the liquid phase from slurry separation, can prevent NH_3 volatilization (Fangueiro et al., 2015). However, acidification also influences microbial processes, enzyme activities as well physical characteristics of the slurry. It might reduce mineralization of organic matter during slurry storage

(Sørensen and Eriksen, 2009) and could limit nitrification (Fangueiro et al., 2016), but clear conclusions on the effect acidification on crop NUE as well as on nitrate leaching from slurry warrant further research (Fangueiro et al., 2015). Furthermore, handling of concentrated acids such as sulfuric acid is also a health risk for the farmer, thus, alternative acidification strategies should be investigated (Regueiro et al., 2016).

5.3.2 Agronomic improvements for less nitrate leaching

The above-described technical improvements can facilitate manure management and offer possibilities for more targeted application of animal manure. However, as found within **Chapter 3**, most nitrate leaching does not originate directly from the fertilizers, but from mineralization of soil N. This indicates a legacy effect of long-term inputs of organic fertilizers which requires further management adaptations.

Reduce fertilizer N input

Lowering N inputs from surplus to about optimum levels is effective in reducing direct nitrate leaching losses (e.g. Goulding et al., 2000), but further lowering inputs is ineffective with this regard (Heumann et al., 2013). Suboptimal N inputs can impair yields quite immediately, although yield reductions vary between crops, with much stronger effects on cereals (Thomsen et al., 2003) than on silage maize (Kayser et al., 2011). This was also observed in the Gäu region within the work of Wey (2021). Heumann et al. (2013) explained this by variable soil N mineralization rates, being higher during summer and lower during winter (see also **5.4**). While maize has its major N demand during summer when soil N mineralization rate is high, this is not the case for winter cereals. For the Gäu region this suggests that fertilization to silage maize could be strongly reduced or even abandoned without any impairment on maize yield. This is even more true as silage maize often follows the termination of grass-clover ley (Hoffmann et al., 2018).

Adapt crop rotations

Termination of grass-clover is the hot moment within an arable crop rotation both in terms of gaseous N losses and nitrate leaching (Wagner-Riddle et al., 2020). In my work, this could be seen by elevated nitrate leaching losses under winter wheat which followed in the winter after grass-clover termination (**Fig. 3.5**) and is consistent with Wey (2021). Grass-clover is usually fertilized with high amounts of animal manure containing up to 315 kg N ha^{-1} (Richner and Sinaj, 2017), leading to accumulation of soil organic matter over the years. Kayser et al. (2008) highlighted that not the inputs and management during grass-clover cultivation, but rather management after ley-termination is the decisive factor for nitrate leaching. Thereby, nitrate leaching and/or soil Nmin levels haven been reported to remain elevated after ley-termination for at least two years (Eriksen et al., 2004, Helfrich et al., 2020).

Besides a reduction of N inputs, crop rotation is a crucial factor in managing N release after ley termination. Especially catch crops – either sown after harvest of row crops such as maize or cereals or even undersown to them – have been reported to effectively reduce the risk of nitrate leaching (De Notaris et al., 2018, Thorup-Kristensen et al., 2003). For one of the typical crop rotations in the Gäu region (grass-clover – silage maize – winter cereals) this could mean using undersown catch crops to maize and shifting to spring sown cereals. It is important to consider that catch crops must release N at times when the main crop can readily take it up and fertilizer N inputs can thus be substituted (Sørensen and Jensen, 2013). This requires a well thought choice of locally adapted catch crop species, incorporation time and crop rotation. Using a catch crop species mixture instead of a single species can improve nutrient capture, mineralization and transfer to the following crop (Gentsch et al., 2021).

The way of grass-clover termination seems to be less important for nitrate leaching losses. Compared to ploughing, rotary band seeding of maize after broadband

herbicide treatment has been promoted in Switzerland as a way to reduced nitrate leaching losses and to prevent soil erosion (Anken, 2004). Wey (2021) investigated leaching losses on neighbouring fields with the SIA method, but with ley-termination by ploughing rather than rotary band seeding as in the microplot study (**Chapter 3**). Leaching losses under winter wheat were similar between my study (**Fig. 3.5**) and the work of Wey (2021), questioning the effectiveness of rotary band seeding for reducing nitrate leaching. Also Helfrich et al. (2020) found elevated soil Nmin levels after grass-clover termination irrespective whether it was terminated mechanically or purely chemically.

The role of mineral fertilizer – to supplement or to replace?

Animal manures are multi-nutrient fertilizers, containing besides N also P, K, and other (micro-)nutrients. The ratio of the nutrients contained in the manure usually does not match the nutrient requirements of the crops, resulting in overfertilization with some nutrients while others might be still limiting. For example, applying cattle slurry targeting to match the N demand results in an overfertilization with P for most crops.

An alternative could be to target the P-demand of the crop with manure application and supplement with mineral N fertilizer in order to avoid N-limitation (Jensen, 2013). While this approach reduces negative environmental impacts caused by excessive P loads, it is also a way to increase the NUE of animal manure and to get benefits from both mineral fertilizer and animal manure (Schröder and Sørensen, 2011). It is also emphasized by the finding of Kirkby et al. (2014) that nutrient stoichiometry determines the maintenance and build-up of soil organic matter which in turn is responsible for soil fertility. However, contrasting results on the interactive effect of the application of animal manure and mineral fertilizer have been reported and warrant further research (Chalk et al., 2020). With overall better management of the nutrients in animal manure, ultimately the need for mineral fertilizer could be reduced, leading to a lower N surplus and consequently lower losses (Spiess, 2011).

Support farmers in their fertilization decisions

Applying the right type and amount of fertilizer at the right moment is not an easy task and heavily influenced by local soil and farming conditions as well as the weather. General guidelines cannot fully cope with these variabilities and uncertainties. Several tools and approaches are available that could assist informed and locally adequate fertilization.

Targeted application of manure is often restrained by the fact that farmers have difficulties in determining both quantity and nutrient content of manure on the farm (Spiess, 2011). As the C to N ratio often explains a large part of the short-term N availability (Delin et al., 2012), near infrared spectroscopy sensors, which are available to directly mount to the slurry tank, might be a cheap and easy technology (Stenberg and Gustafsson, 2013).

Decision support tools, taking both local weather and soil conditions into account could be a viable option as well. MANNER-NPK is an example of such a software-tool, developed for estimating the nutrient availability from animal manure under UK farming conditions (Nicholson et al., 2013). Adapting such a tool to local conditions in the Gäu region appears promising. However, maintaining and optimizing these tools requires modelling of local soil mineralization (also see **5.4**).

Ultimately, local advisory service and educational support could help to achieve a better adaptation of current best practice management options. Encouraging models can be found for example within the Swedish Advisory Service programme "Focus on nutrients" which offers farm-specific advice (Greppa Näringen, 2011).

Grassland-based ruminant feeding

Adaptation of the feeding strategy for ruminants, can have impacts both on the manure composition (Sørensen et al., 2003), but also N fluxes in general as well as on

a broad range of socio-economic factors. Stolze et al. (2019) suggested that in Switzerland, with its large share of grassland, complete abandonment of concentrates and maize silage in ruminant feeding has the potential to reduce the national N surplus by 24 % and GHG emissions by 10 %. Nevertheless, it is important to also consider that methane emissions originating from enteric fermentation within the digestive system of the ruminants are usually higher when animals are fed solely on roughages compared to diets with a higher digestibility (Hristov et al., 2013). Therefore, in order to avoid pollution swapping, this strategy would also require to reduce animal numbers. While calorie production for human consumption would remain unaffected, this would result in lower protein production (Schader et al., 2015, Stolze et al., 2019). The potential of these strategies (grassland-based ruminant feeding, lower animal numbers) for alleviating the nitrate leaching problem in the Gäu region warrant further investigations.

5.3.3 Revisit our view on the soil-fertilizer-plant system

As scientists we are also challenged by a need to rethink our current view on the soil-fertilizer-plant system. Focusing on supplying plants with soluble inorganic nutrients decreased rather than increased NUE and has uncoupled natural biogeochemical cycles of C, N and P (Drinkwater and Snapp, 2007, Daly et al., 2021). My work emphasized that plants only take up a minor part of their N demand from current fertilizer addition and that most of N added with fertilizers is incorporated into soil organic matter (**Table 3.4**, **Fig. 3.4**). In turn, plants take up more than half of their N demand from sources other than fertilizers, likely from soil N mineralization (Yan et al., 2020). This finding highly questions the current assumption that with fertilization we would nourish our crops. Rather, we must see our inputs as a refilling of soil resources which will then provide nutrients to our crops. The ^{15}N recovery in soil was higher for cattle slurry than for mineral fertilizer in both field (**Fig. 3.4**) and greenhouse experiment (**Fig. 4.3**), indicating that cattle slurry application might be better capable in maintaining soil organic N due to the simultaneous addition of C

(Gardner and Drinkwater, 2009). This was also suggested by Daly et al. (2021) proposing a revised framework on the drivers of "bioavailable N". According to them, bioavailable N is not the release of mineral N but controlled by "interactions between organic N depolymerisation, mineral sorption-desorption dynamics, and the actions of plants and microbes" (Daly et al., 2021). Thus, minimizing N leaching losses would require an improved management of soil organic matter, fulfilling the challenging task of enhancing soil C storage while allowing crop nutrition from organic N turnover in soil (Cotrufo et al., 2021).

With this respect, also a better understanding of the incorporation mechanisms of both mineral fertilizer and animal manure into physical soil organic matter fractions might help. In continuation of the work done within this thesis, Fuchs et al. (in prep) showed that most of the residual N two years after field application of either ^{15}N labelled mineral fertilizer or ^{15}N labelled cattle slurry could be found in the mineral associated organic matter fraction (MAOM) (**Fig. 5.3**), confirming results by Bosshard et al. (2008). In a pot study using the soil from the field experiment, plants took up more residual fertilizer N from MAOM, than from the particulate organic matter fraction (POM) (Fuchs et al., in prep) (**Fig. 5.4**). While this might be related to the higher recovery of residual fertilizer N in MAOM than in POM, this finding is also in line with the framework proposed by Daly et al. (2021) and contrasts previous assumptions that MAOM was a recalcitrant organic N fraction. Rather, this finding highlights that MAOM-N dynamics together with POM-N turnover should be considered for future fertilization schemes.

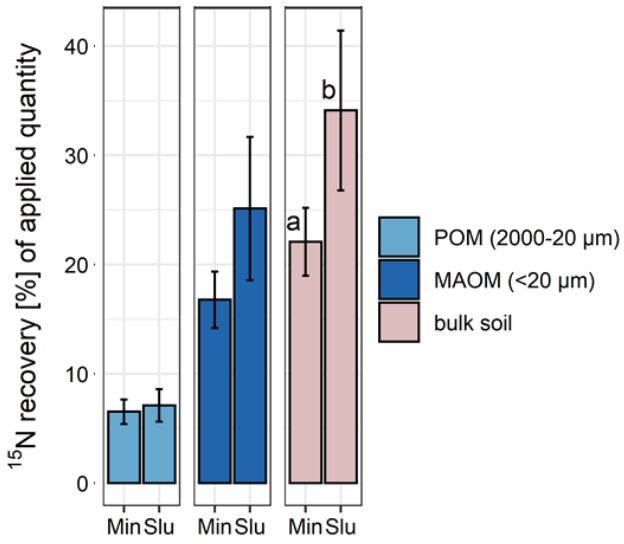

Fig. 5.3: *^{15}N recovery in physical soil organic matter fractions (particulate organic matter (POM), mineral associated organic matter (MAOM)) two years after field application of ^{15}N labelled mineral fertilizer (Min) or ^{15}N labelled cattle slurry (Slu). Topsoil (0 to 30 cm) from the final sampling at Field B of the field study (see **Chapter 3**) was fractionated according to the scheme of Cotrufo et al. (2019). Error bars represent standard deviation (n = 4) and different letters indicate significant differences between fertilizer treatments (p < 0.05). (adapted from Fuchs et al., in prep)*

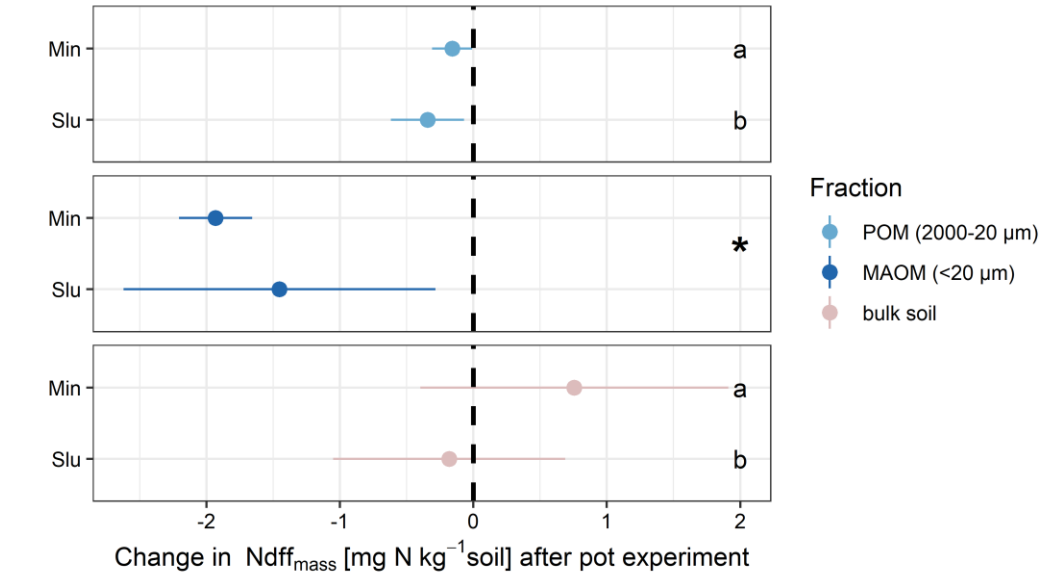

Fig. 5.4: *Change in N derived from the labelled fertilizers (Ndff) in physical soil organic matter fractions (particulate organic matter (POM), mineral associated organic matter (MAOM)) soil before and after a pot study cultivating ryegrass (*Lolium multiflorum*). The pot experiment was conducted on topsoil (0 to 30 cm), collected upon the final sampling at Field B from the field study (see **Chapter 3**). Error bars represent standard deviation (n = 16), different letters indicate significant differences between fertilizer treatments (p < 0.05) and asterisk indicates significant difference of the intercept from 0 (Fuchs et al., in prep).*

5.4 Soil N mineralization: a key factor in understanding nitrate leaching and its mitigation measures

To understand the drivers of nitrate leaching in the Gäu region and elsewhere requires not only to consider current fertilization, but also soil N mineralization under local climate, soil, and farming conditions. The data obtained in **Chapter 3** allows to estimate local net soil mineralization by two different approaches.

A simple approximation on the soil N net mineralization rate can be achieved by calculating a soil surface balance (**Eq. 5.2**), both individually for the duration of the single crops and cumulated over the crop rotation of the three crops.

$$net\ mineralization_{balance} = TNuptake_{shoot} + N\ leaching\ - fertilizerN\ - BNF \qquad \textbf{Eq. 5.2}$$

with $TNuptake_{shoot}$ being total N uptake in harvested shoot biomass (for grass-clover cumulated over all cuts within the respective time period), *N leaching* being nitrate-N leaching measured with SIAs, *fertilizerN* being total N input with both organic and inorganic fertilizers, including ^{15}N labelled and unlabelled inputs, and BNF being N from biological N fixation (all given in kg N ha^{-1}). Thereby, BNF was estimated as the sum of fixed N in clover shoots ($Ndfaclover_{shoots}$) (**SI 2.3**) and the transfer of fixed N from clover to non-leguminous species, especially grasses. A transfer factor of 1.3 x $Ndfaclover_{shoots}$ was used, estimated based on the work by Nyfeler et al. (2011).

Gaseous losses via NH_3 volatilization, N_2O and N_2 were not considered as I assumed that N deposition would compensate for them (Heumann et al., 2013). Furthermore, this balance neglects stubble and roots. However, as roots and stubble are usually kept in the system, they will enter the soil N pool through re-mineralization and averaged over the years, their role should be negligible. In fact, these simplified balance approaches can barely give exact estimates when assessed on a yearly base or based on a single cropping season, but might yield representative insights when cumulated and averaged over several years (Buczko et al., 2010).

Alternatively, soil N net mineralization can be estimated considering total N uptake and N leaching and subtracting from it N uptake and leached nitrate directly derived from fertilizer inputs (**Eq. 5.3**). Thereby, I used Ndff values based on ^{15}N labelled fertilizer in the year of application (**Table 2.4** and **Table 2.5**) and assumed for the unlabelled fertilizers that 45 % of mineral fertilizer N and 20 % of slurry N would be recovered in shoot biomass, thus, contribute to Ndff. Furthermore, I presumed that N not taken up in the year of application gets incorporated into the soil organic matter pool (compare **Chapter 2**). Hence, re-mineralization of fertilizer N was not distinguished from soil N mineralization and residual fertilizer effects beyond the year of application were not considered. Again, root and stubble N turnover as well as gaseous losses and deposition were neglected.

$$net\ mineralization_{15N} = (TNuptake_{shoot} - BNF) + N\ leaching\ - Ndff_{shoot} - Ndff_{leaching} \quad \textbf{Eq. 5.3}$$

Average annual net mineralization rates based on the balance approach (**Eq. 5.2**) were 70 kg N ha^{-1} year^{-1} for Field A and 138 kg N ha^{-1} year^{-1} for Field B (**Table 5.1**). These values are slightly higher than the average annual net mineralization rate reported in the work by Wey (2021), but overall fall in the wide range of values reported. Wey (2021) determined soil N net mineralization by a similar soil-surface balance approach for 11 fields in the Gäu region and found an average net mineralization rate of 59 kg N ha-^{1} year^{-1} (range: -5 to 278 kg N ha^{-1} year^{-1}). Overall, these values are also supported by estimates from a continuous wheat rotation in France on a loamy soil where soil N net mineralization was estimated to equal 120 kg N ha^{-1} year^{-1} (Mary and Recous, 1994).

The balance approach revealed that in most cases (except for under grass-clover where negative net mineralization rates indicate an N surplus with fertilization), N uptake by the crops exceeded N inputs, even more when N leaching losses were also considered. Thus, in the long term the current farming approach will deplete soil organic N stocks. This trend is also confirmed by long-term trials in Switzerland

finding declining soil N stocks under arable cultivation especially when fertilized with mineral fertilizer (Charles et al., 2018). Only under grass-clover, which is usually repeatedly fertilized with animal manure, some soil organic N could accumulate. This was, however, lower than the soil N output during the other crops so that the balance over the whole crop rotation indicates a tendency for soil organic N mining and likely a depletion of soil fertility. Furthermore, as shown in my work, but also elsewhere (Davies et al., 2001), mineralization is enhanced after grass-clover termination. With the current crop rotation and fertilization strategy, a big share of the accumulated soil N during grass-clover ley cultivation is not recycled but lost.

The net soil N mineralization rates based on the ^{15}N approach (**Eq. 5.3**) give a more realistic indication for "real" soil N mineralization as the ^{15}N approach accounts for the fact that not all fertilizer N is taken up by the crop (see **Chapter 3**). These rates are therefore higher, ranging on average between 200 and 300 kg N ha^{-1} $year^{-1}$ (**Table 5.2**). Lower monthly mineralization rates under grass-clover compared to the other crops indicate efficient re-cycling of N, including low leaching losses and additional N inputs from biological N fixation. Overall, these mineralization rates could be useful to inform and refine fertilization guidelines. Currently, the Swiss fertilization guidelines offer several options to correct the standardized N inputs and to account for pre-crops as well as local soil conditions. The guidelines intend that N inputs could be reduced (or increased) depending on the mineralization potential of the soil, which in turn is estimated from the clay content and the organic matter content of the soil. These estimates are rather rough, though, and for the conditions on the two fields investigated within this thesis (about 20 % clay and 3 % organic matter) they would not suggest any adaptation in the standard N fertilization recommendations. After grass-clover termination in spring, N inputs are recommended to be reduced by 10 to 40 kg N ha^{-1}. Likely, with modelling, such as suggested by Heumann et al. (2013), local soil N mineralization dynamics could be better taken into account.

***Table 5.1**: Net N mineralization rate based on total N balance (**Eq. 5.2**) (all numbers are given in kg N ha^{-1})*

Field	Treatment	FertilizerN	BNF	TN uptake shoot	N-Leaching	Net_mineralization	mineralization rate per month	FertilizerN	BNF	TN uptake shoot	N-Leaching	Net_mineralization	mineralization rate per month	FertilizerN	BNF	TN uptake shoot	N-Leaching	Net_mineralization	mineralization rate per month	Cumulated mineralization	average mineralization rate per month
		In		Out		Out - In		In		Out		Out - In		In		Out		Out - In			
		Silage maize 5 months						Winter wheat 10 months						Grass-clover 9 months						Cumulated 24 months	
A	Con	69	-	137	2	70	14	152	-	192	73	113	11	190	36	130	43	-53	-6	130	5
A	Min	106	-	140	5	39	8	152	-	183	91	122	12	190	19	118	60	-31	-3	130	5
A	Slu	129	-	150	6	27	5	152	-	193	106	147	15	190	22	144	57	-12	-1	162	7
		Grass-clover 12 months						Silage maize 5 months						Winter wheat 10 months						Cumulated 27 months	
B	Con	95	56	254	16	119	10	198	-	195	2	-1	~0	109	-	220	128	239	24	357	13
B	Min	243	25	297	8	37	3	198	-	193	4	-2	~0	109	-	223	194	308	31	343	13
B	Slu	335	18	287	9	-57	-5	198	-	211	3	16	3	109	-	221	161	273	27	232	9

BNF = biological N fixation; TN = total N, Ndff = N derived from fertilizer

***Table 5.2**: Net N mineralization rate based on ^{15}N approach* (**Eq. 5.3**) *(all numbers are given in kg N ha^{-1})*

Field	Treatment	TN uptake shoot	BNF	N-Leaching	Ndff shoot	Ndff leaching	Net_mineralization	mineralization rate per month	TN uptake shoot	BNF	N-leaching	Ndff shoot	Ndff leaching	Net_mineralization	mineralization rate per month	TN uptake shoot	BNF	N-leaching	Ndff shoot	Ndff leaching	Net_mineralization	mineralization rate per month	Cumulated mineralization	average mineralization rate per month
		Silage maize 5 months							Winter wheat 10 months							Grass-clover 9 months							Cumulated 24 months	
A	Con	137	-	2	31	1	108	22	192	-	73	68	2	200	20	130	36	43	38	2	97	11	400	17
A	Min	140	-	5	47	1	96	19	183	-	91	68	2	204	20	118	19	60	38	2	112	13	419	17
A	Slu	150	-	6	43	1	112	22	193	-	106	68	2	229	23	144	22	57	38	2	138	15	480	20
		Grass-clover 12 months							Silage maize 5 months							Winter wheat 10 months							Cumulated 27 months	
B	Con	254	56	16	19	1	194	16	195	-	2	70	2	125	25	220	-	128	49	1	298	30	617	23
B	Min	297	25	8	88	1	191	16	193	-	4	70	2	123	25	223	-	194	49	1	367	37	682	25
B	Slu	287	18	9	74	1	202	17	211	-	3	70	2	141	28	221	-	161	49	1	332	33	676	25

BNF = biological N fixation; TN = total N, Ndff = N derived from fertilizer

5.5 Conclusions and Outlook

This thesis emphasized that under current best practice management, only minor shares of nitrate leaching derived directly from recent N fertilizer addition – irrespective whether it was applied as animal manure or mineral fertilizer. However, managing the residual N effect of animal manure and adequately taking soil N turnover into account in fertilization remain crucial and understudied issues. Preserving groundwater quality might not be possible without accepting trade-offs. In regions with a high inherent soil fertility and/or long-term manuring history, such as the Gäu region, pronounced nitrate leaching reductions are likely only feasible under strongly reduced N inputs. This would probably both impair crop yields and lead to a depletion of soil organic matter in the long-term. Finding a balance between nitrate leaching reduction, crop production and soil fertility will require further steps which might entail:

- **Improve our understanding and management of soil organic N turnover.** As discussed above, soil organic N turnover is still poorly integrated into fertilization, although its central role in plant nutrition is corroborated. Further research should address the driving factors of SOM turnover (both with respect to built-up and depletion) as well as the release dynamics of plant available N from SOM. These factors might include nutrient stoichiometry of inputs (Kirkby et al., 2014), the role of microbial necromass (Coonan et al., 2020), different turnover dynamics of physical soil organic matter fractions (Daly et al., 2021), coupling of N and C dynamics (Cotrufo et al., 2021), climatic factors as well as agronomic practices. The gained knowledge could be used for site specific fertilization recommendations (e.g. through modelling).
- **Consider gross turnover rates of N.** Uncertainties in predicting and using the soil organic N turnover for plant nutrition might be reduced by taking gross turnover rates into account. These rates are more difficult to determine, but likely are decisive for the amount of N that is available to plants (Luxhøi et al., 2007). As suspected by Luxhøi et al. (2007) "mineral N in the transition between gross N mineralization and gross N immobilization is available for

assimilation by plants". Ideally, N turnover should be managed in a way that gross turnover rates are large enough to nourish plants, but net N mineralization is close to zero in order to reduce the potential for leaching losses.

- **Optimize N application rates with animal manure.** Currently, about half of the N excreted by animals does not appear in the Swiss fertilization balance and is considered to not be available to crops. With advances in reducing NH_3 emissions upon storage and application, less "unavoidable" losses occur and more N might enter the soil system. Therefore, a revision of the fertilization guidelines is discussed for which an N utilization rate of 70 to 80 % of total N in manure is proposed. However, the results obtained within my thesis do not allow for this conclusion and rather suggest that a reduction in N inputs with manure under current farming practices (crop rotations, soil tillage) would lead to a long-term N depletion of the soil.
- **Adapt crop rotations and find innovative systems capable in "catching" N lost from mineralization during winter.** Mineralization of both soil organic N and residual fertilizer N during winter are challenging to manage, especially after grass-clover termination. Likely, leaching losses can only effectively be prevented by integrating winter catch crops and/or adapting an agroforestry approach with perennial, deep rooting plants. An effective design for the Gäu region should be further elaborated.
- **Include social science.** The above described trade-offs between groundwater protection and agricultural production do not only affect natural ecosystem processes, but also have extensive socio-economic impacts. Therefore, an integrated approach also involving social sciences, is needed. Furthermore, finding and successfully implementing effective measures for the reduction of nitrate leaching require all concerned stakeholders to be actively involved (Kanter et al., 2020b). First and foremost, local farmers should be supported

and involved both with advanced training, farm specific advisory service, planning, and choice of methods.

- **Better use of existing knowledge.** Within this thesis, I investigated processes and problems that had been addressed at other places and in other circumstances before, but still there were no direct solutions to the nitrate leaching problem in the Gäu region at hand. The science community should strive for both, better making use of the huge existing and ever increasing body of knowledge and work on better strategies to break down general patterns to locally adapted solutions.

Cotrufo et al. (2021) nicely summarized what also could be the end of my thesis: "The world is looking to ecosystem ecologists to advise large-scale efforts to co-manage soil C and N stocks. Are we ready for the challenge? Globally, plant C inputs to soils need to increase and soil C outputs via microbial C mineralization need to decrease, while N mineralization and internal N recycling need to be maintained to support plant productivity and avoid detrimental environmental impacts. Do we know where and how to achieve these outcomes?"

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Supplementary Information – General Part

SI 1.1 Detailed documentation on the production of ^{15}N labelled cattle slurry

^{15}N labelled cattle slurry was produced by feeding a heifer with ^{15}N labelled ryegrass hay. Therefore, in a first step ^{15}N labelled ryegrass hay had to be produced. This was done by ryegrass cultivation and fertilization with ^{15}N labelled ammonium nitrate (NH_4NO_3), both in sand-filled container boxes in the greenhouse and on a ryegrass pure stand in the field, following approaches by Bosshard et al. (2011) and Langmeier et al. (2002).

^{15}N labelled ryegrass production in the greenhouse

80 container boxes with an area of 1 m^2 each were filled with a sand perlite mixture (80:20 w/w). The mixture with perlite was chosen in order to improve the water holding capacity of the substrate. The container boxes had a height of 0.8 m, but in order to save substrate, they were only filled with substrate up to 0.2 m. To prevent waterlogging at the bottom of the boxes, a drainage matt (Tricodrain® 823, Tegum, CH-Frauenfeld) was used. For irrigation, an automatic drip hose system was installed (**SI 1 Fig. 1**). The boxes had a hole at the bottom, from which – if needed – excess water could be released. This water then was collected and reapplied to the box.

Ryegrass (*Lolium multiflorum* var. Westerwoldicum) was sown at a seed density of 30 g m^{-2}. During the first days, boxes were covered with plastic film in order to keep moisture and to facilitate germination of the seeds. 55 boxes were used for the cultivation of ^{15}N labelled hay, while 25 boxes were kept under similar conditions, but fertilized with unlabelled ammonium nitrate in order to cultivate unlabelled hay with otherwise similar properties. This unlabelled hay was fed to the animal in the seven days before and in the three days after feeding with the ^{15}N labelled hay (see below).

The following approach was targeted:

- ^{15}N fertilization: N was applied as NH_4NO_3 with a ^{15}N enrichment of 19.4 atom% abundance. A total N application rate of 11 g N m^{-2} for the first cut and

7.6 g N m^{-2} cut^{-1} for the following cuts was targeted, split into weekly doses. Higher targeted N amount for Cut 1 was chosen in order to also allow for the establishment of the root system. The total dose was based on an expected dry matter yield of 300 g m^{-2} cut^{-1} with an N concentration of 2 mg g^{-1}.

- Micro- and macronutrients: Additionally, micro- and marconutrients were ought to be applied biweekly with an N-free Hoagland nutrient solution. Target [mg m^{-2} cut^{-1}]: 85 K, 14 Mg, 18 Ca, 17 P, 0.5 Mn, 0.3 Fe, 0.3 Cu, 0.005 Co.

- Correct for NO_3-N in irrigation water: Irrigation water in the greenhouse contained 7 mg NO_3-N L^{-1}. In order to keep the ammonium-N to nitrate-N ratio constant and to avoid dilution of the ^{15}N enrichment of the applied N, an equal amount of unlabelled NH_4-N L^{-1} was applied based on the amount of irrigation water used. This amount was factored in when preparing the fertilizer solution from ^{15}N labelled ammonium nitrate so that the enrichment of all entering N remained at 19.4 atom%.

- Harvest: I aimed at 4 cuts; the first one after 4.5 weeks, afterwards every 3 weeks.

Since the produced dry matter remained below the expected yield, the amount of N inputs (but also for all other nutrients) had to be reduced drastically in order to avoid overfertilization (**SI 1 Table 1**). The low productivity might relate to a lower light intensity than under field conditions, likely fostered due to the shading within the boxes.

SI 1 Table 1*: N fertilization, dry matter yield and ^{15}N abundance of ryegrass hay produced in the greenhouse.*

Cut	Growing duration weeks	N° of split doses for N application	Total N input g N m^{-2}	Dry matter yield g m^{-2}	^{15}N abundance atom%
Cut1	4.5	3	6.8	35.9	15.5 ± 1.1
Cut2	3.0	1	2.3	67.8	15.1 ± 1.8
Cut3	3.0	3	1.4	40.3	16.1 ± 3.8
Cut4	3.5	3	1.4	79.7	14.9 ± 6.6

^{15}N analysis of ryegrass hay was challenging as hay had a high N concentration (up to 6 mg g^{-1} for the first cut, around 4 mg g^{-1} for the other cuts) combined with a high ^{15}N enrichment. In order to facilitate isotope ratio mass spectrometer analysis, samples were diluted with unlabelled rice flour. However, this introduced an additional source of error and data must be seen semi-quantitative.

SI 1 Fig. 1: *Cultivation of ^{15}N labelled ryegrass (*Lolium multiflorum*) in container boxes in the greenhouse. Drainage layer (a), sand-perlite substrate mixture (b), ryegrass seedlings with automatic drip hose irrigation (c), fertilization with micro- and macronutrient solutions (d), growing ryegrass (e), and manual ryegrass harvest with scissors (f)*

^{15}N labelled ryegrass production in the field

Since the amount of ryegrass biomass produced in the greenhouse was not enough for feeding a heifer over several days, additionally a ryegrass pure stand, used for seed production was fertilized with ^{15}N ammonium nitrate. On an area of 144 m^2, 40 kg N ha^{-1} ammonium nitrate (35 atom% abundance) was applied. The application was performed using watering cans and split into two equal doses applied at the 4th and the 19th of September 2017 (**SI 1 Fig. 2**). On an area of 72 m^2, unlabelled ammonium nitrate was applied on the same dates, the same rates and in the same way in order to produce unlabelled hay of the same quality.

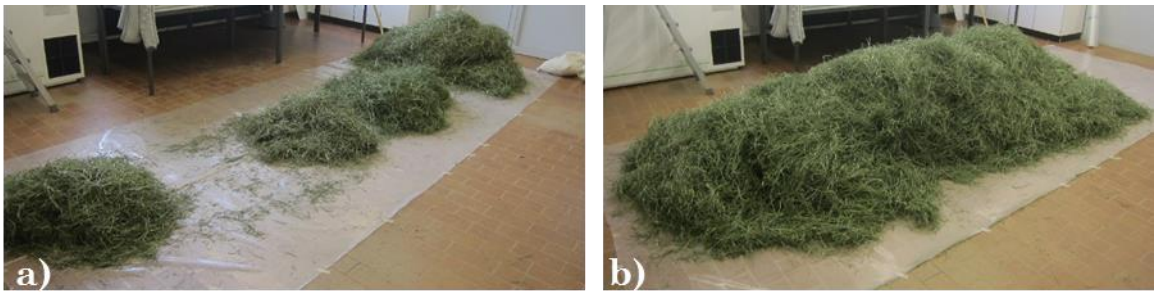
The grass was harvested on the 20th of October 2017 and dried in a grain drying facility at Agroscope Reckenholz (CH-Zürich). In total, 32 kg dry matter with a ^{15}N abundance of 9.1 atom% and a raw protein content of 146 g kg^{-1} were produced.

SI 1 Fig. 2*: ^{15}N labelled ryegrass production near Wünnewil, Fribourg. Application of ^{15}N labelled ammonium nitrate with watering cans (a) and harvest of the ^{15}N labelled grass (b)*

Mixing of animal feed

In order to obtain daily feed ratios of homogenous quality and average ^{15}N labelling, hay from the field and from the greenhouse were mixed (**SI 1 Fig. 3**). Mixing of the hay was also necessary in order to compensate for excessive raw protein contents in the hay from the greenhouse. The final feed was a mixture containing 26 % of hay

from the greenhouse and 74 % of hay from the field. It had a raw protein content of 167 g kg^{-1} and a ^{15}N enrichment of approximately 12.6 atom%.



SI 1 Fig. 3*: Mixing of ^{15}N labelled hay from greenhouse and field for achieving homogenous feed ratios with an adequate feed quality for the heifer*

Feeding of the heifer

In February 2018, a young heifer (~6 months old) was fed with the produced hay. The animal was kept at the Metabolic Centre of AgroVet Strickhof (CH-Lindau/Eschikon). No bedding material was used in order to avoid dilution of the excreta with it. During the first 7 days, the animal received unlabelled ryegrass hay. Initially, daily ratio of 5.5 kg hay day^{-1} was offered to the animal. Due to extremely cold temperatures during the feeding period, the daily ratio was increased to 6 kg hay day^{-1}. At Day 6, an urinal was attached to the hindquarters of the animal to start separate and quantitative sampling of urine and faeces (**SI 1 Fig. 4**). From Day 8 until Day 15, the animal received ^{15}N labelled hay, followed by another three days of unlabelled hay (**Fig. 2.3**). The heifer excreted 7 to 13 L urine day^{-1} and 8 to 12 kg faeces day^{-1}. The final slurry was mixed from 56 kg urine and 48 kg faeces and had an enrichment of 7.89 atom%.

SI 1 Fig. 4*: Feeding of ^{15}N labelled hay to a heifer (a) with separate collection of urine with a urinal (b) and faeces (c)*

SI 1.2 Incubation of ^{15}N labelled cattle slurry to study the temporal development of the ^{15}N label in mineralized N

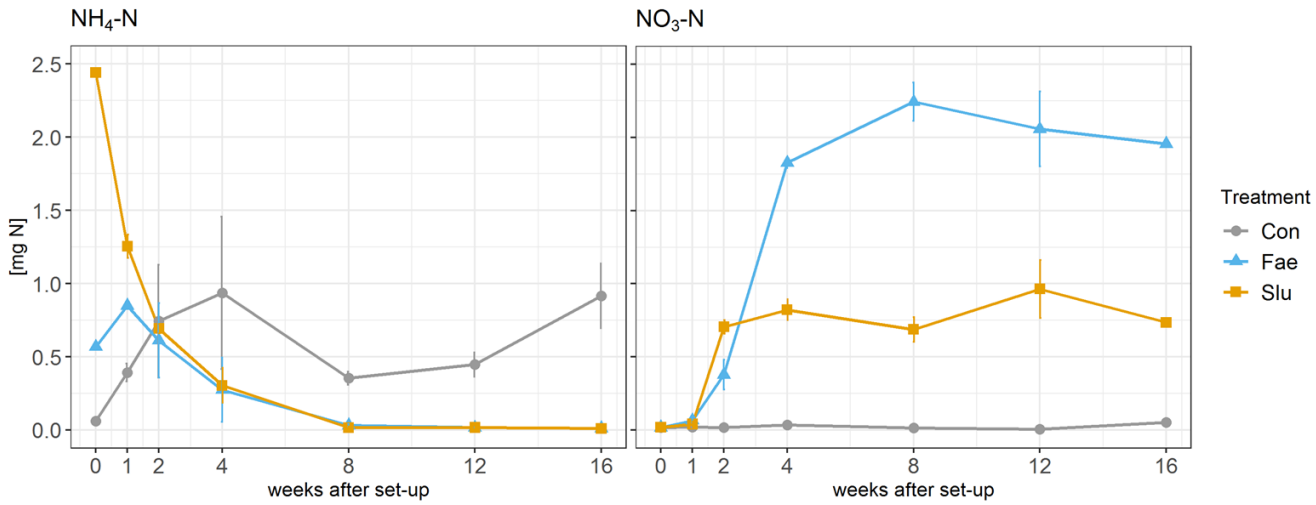
The ^{15}N label in faeces and urine is not the same and also labelling within faeces is inhomogeneous as expected from previous studies (e.g. Bosshard et al., 2011, Langmeier et al., 2002) (compare **Table 2.2**). Since the different faeces fractions have different N mineralization behaviour, an incubation study was conducted. This study aimed at investigating the temporal development of the ^{15}N label within the mineralized N from either faeces alone or from the slurry (faeces mixed with urine).

Similar as for Bosshard et al. (2011), the following procedure was applied: 25 g of quartz sand (pH in water 4.2) were inoculated with a small amount of soil (1.25 g dry weight equivalent) to ensure microbial activity. To this, 5 mg N, either with slurry or with faeces (faeces-mix, consisting of mixed fraction from Day 11 to Day 16 according

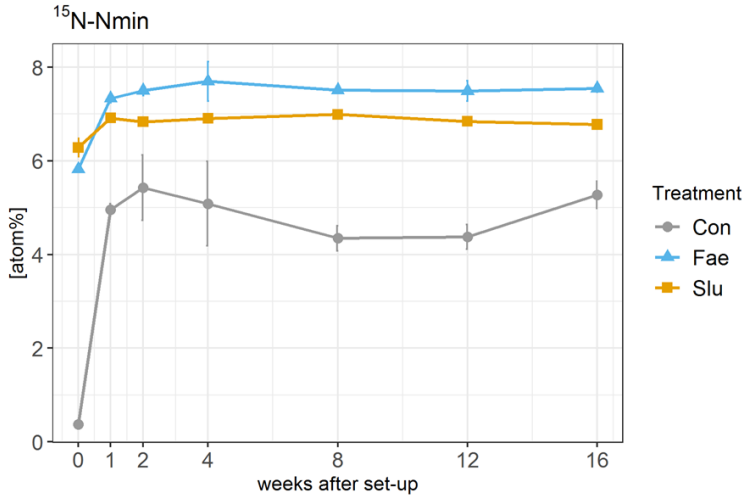
to relative amounts in the slurry) were added and incubated at 55 % WHC and 20 °C. Water losses were compensated weekly by adding demineralized water up to the initial weight.

Three treatments were tested: Con (sand + soil); Fae (sand + soil + ^{15}N faeces-mix); Slu (sand + soil + ^{15}N slurry). Of each treatment, 21 microcosms were prepared for 7 extraction time points in three replicates. Microcosms were extracted with 100 mL 0.5 M K_2SO_4 at 0, 1, 2, 4, 8, 12, and 16 weeks after set-up. Extracts were filtered and stored frozen until analysis of ammonium and nitrate (Keeney and Nelson, 1982, Krom, 1980). The ^{15}N enrichment in the mineralized N was determined using the microdiffusion method (Goerges and Dittert, 1998). Ammonium and nitrate were diffused together on the same filter.

As expected, the nitrate content in the samples increased while the ammonium content decreased over time (**SI 1 Fig. 5**). However, unfortunately, the ammonium content in the Con treatment strongly increased within the first week of incubation. Likely, ammonia was volatilized from the Slu treatment leading to a cross-contamination of not only the Con but likely also of the Fae treatment. This is emphasized by the high ^{15}N label of the mineral N also in the Con treatment (**SI 1 Fig. 6**). In previous studies, only the faeces fractions had been incubated and no such problem was reported (Sørensen et al., 1994, Bosshard et al., 2011). It indicates that either the approach is not suitable for slurry containing volatile N from urine or the incubation procedure has to include prevention for possible cross-contamination.



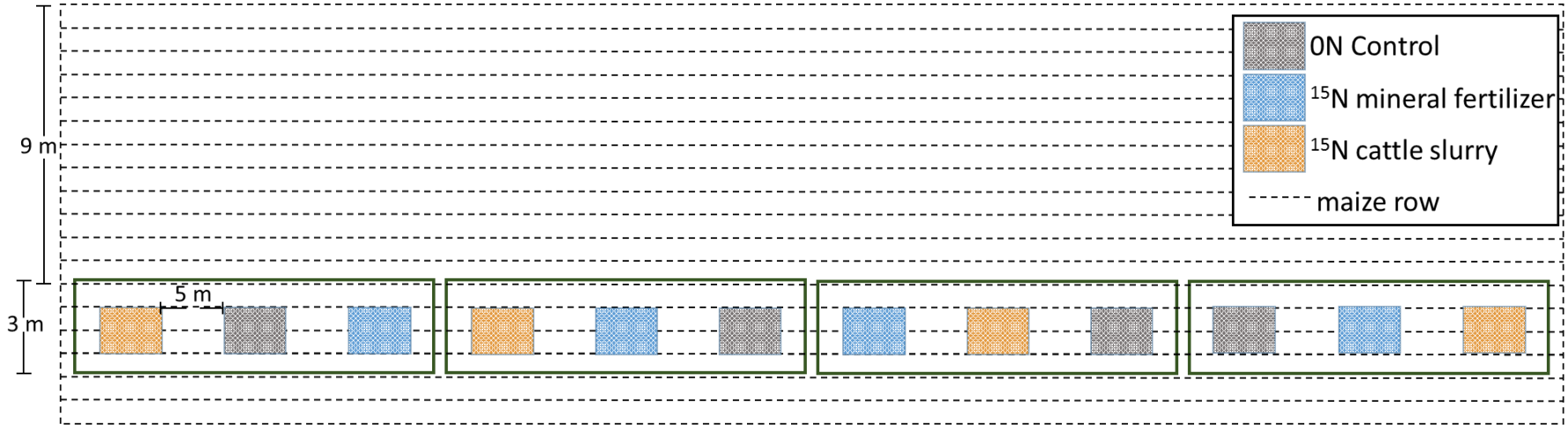
SI 1 Fig. 5*: Temporal development of ammonium-N (NH₄-N) and nitrate-N (NO₃-N) in microcosms in the incubation experiment*



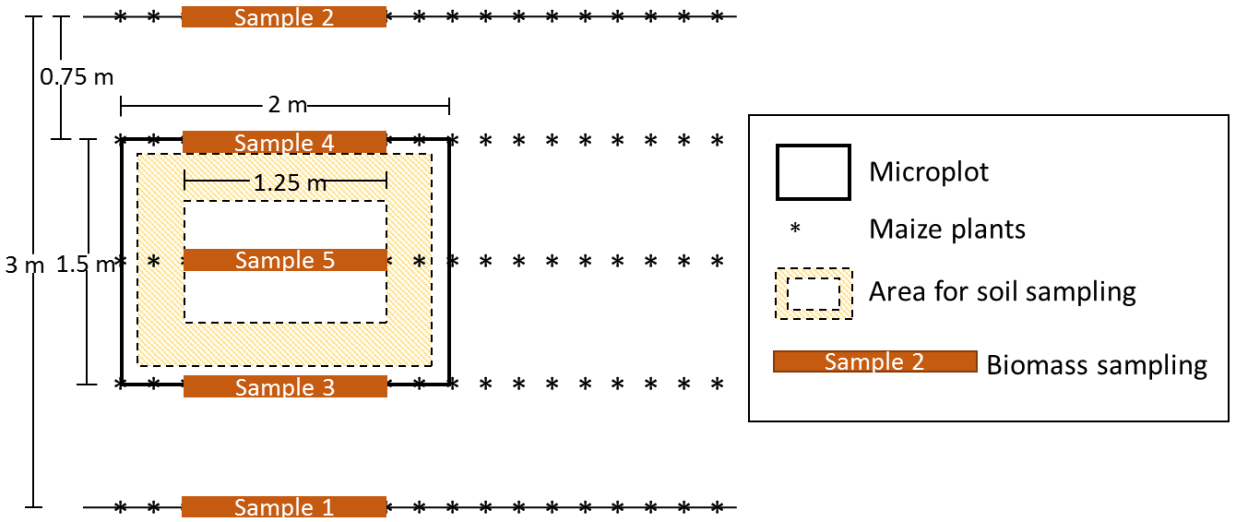
SI 1 Fig. 6: *Development of the ¹⁵N enrichment in mineral N for the incubation experiment*

Supplementary Information Chapter 2

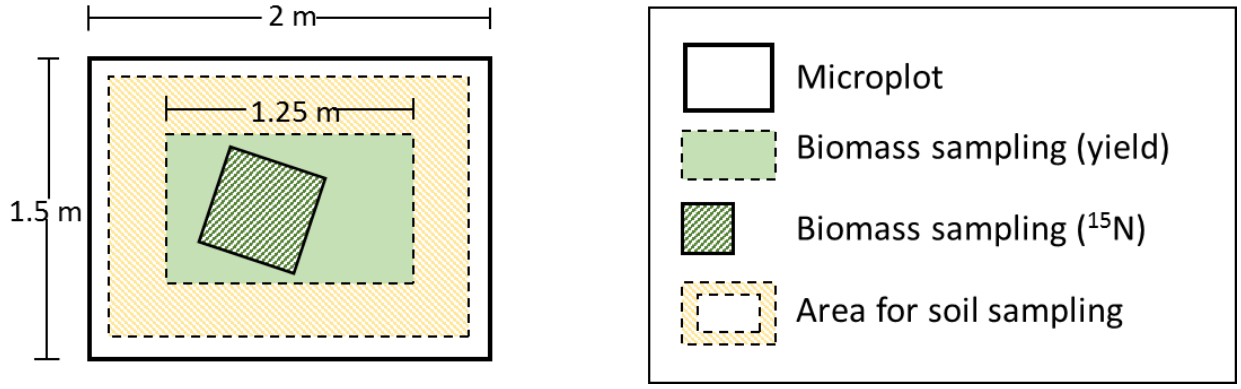
SI 2.1 Arrangement of microplots and sampling scheme



SI 2 Fig. 1: *Arrangement of microplots in randomized block design*



SI 2 Fig. 2: *Layout of microplots Field A (maize) including area for soil sampling and locations for biomass samples*



SI 2 Fig. 3: *Layout of microplots Field B (grass-clover) including area for soil sampling and area for biomass sampling*

SI 2.2 Methodological approach and data evaluation of ammonia volatilization from microplots

Choice of methodology

The volatilization of NH_3 from organic and mineral fertilizers is highly dependent on fertilizer properties, environmental conditions, application technique and timing (Sommer and Hutchings, 2001, Asman et al., 1998, Meisinger and Jokela, 2000). After volatilization, NH_3 is moved away from the source area by turbulent atmospheric transport (Sommer and Hutchings, 2001, Meisinger and Jokela, 2000). For measuring the volatilization of NH_3 from the microplots of the field experiment, the Standard Comparison Method (SCM) (Vandré and Kaupenjohann, 1998) was chosen. The SCM is designed to be applied on small plots and follows a micrometeorological approach – unlike enclosure approaches widely used to investigate trace gas fluxes from microplots. This ensures the NH_3 air-surface equilibrium, which is determined by meteorological parameters, to remain undisturbed N (Loubet et al., 2018, Sommer and Hutchings, 2001, Sommer and Olesen, 1991). NH_3 is sampled passively by exposing sulphuric acid to the ambient air close to the plot surface. Thereby, NH_3 is transported into the passive samplers by turbulent transport and dissolves in the solution according to Henry's law, where it is stabilized as NH_4^+ due to the low pH of the solution (acid trap). The conversion of sample NH_3 concentrations into NH_3 fluxes is based on the assumption of transport conditions being similar between microplots and adjacent reference flux plots. In principle, the method therefore does not require complex sensor equipment and in-depth micrometeorological knowledge.

Nevertheless, wind speed, temperature and relative humidity were logged in order to check spatial inhomogeneities in conditions of turbulent transport along the experimental field. Additionally, the option of predicting and temporarily replacing reference flux plot data by standard meteorological measurements and a sufficiently calibrated model was assessed.

Field and lab work

Strips of microplots both on Field A and Field B were oriented from west-southwest to east-northeast, which corresponds to the most prevailing wind directions.

Two replicate reference plots were installed in parallel alignment to the strip of microplots at a distance of 10 m. Reference plots consisted of a perforated tube system releasing NH_3 gas from gas cylinders in similar quantities as expected to volatilize from applied fertilizer.

Directly (only a few seconds) after fertilizer application, passive samplers filled with 20 mL of 0.05 M sulphuric acid were installed above microplots (Slu, Min, Con) as well as above reference plots and in the four edges of the measurement area (for tracking background NH_3 concentrations) in a height of 0.1 m above the soil surface (Field A) or the grass-clover canopy (Field B) under 0.2 x 0.2 m rain and sun protection roofs. Evaporation or dilution were tracked by adding a 10 ppm PO_4^{3-} spike to the acid. In total, NH_3 emissions were monitored 60 hours after fertilizer application by a sequence of six to seven sampling intervals of increasing duration (3 – 24 hours). At the end of each measuring interval, acid solution was extracted from the passive samplers and a clean passive sampler was installed, starting a new sampling interval. Samples were stored cool in the field and frozen once in the lab until colorimetric analysis for NH_4^+ and PO_4^{3-} concentrations on a Skalar Aqua Pro Segmented Flow Analyser (Skalar Analytical, 2005).

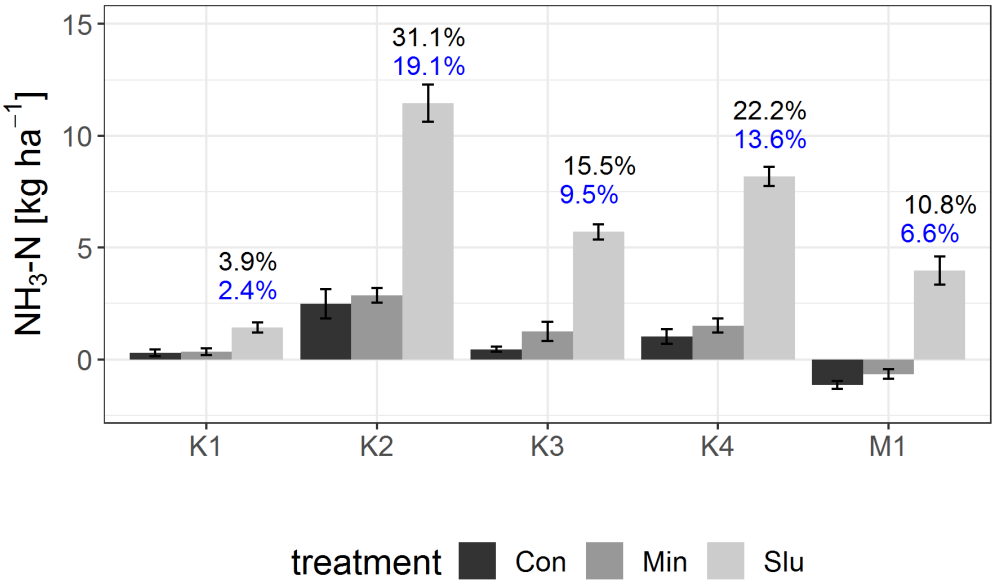
Data analysis and calculations

Measured concentrations of NH_4^+ (mg L^{-1}) were corrected for dilution or evaporation during the time of exposure based on the observed deviation in PO_4^{3-} concentrations. Further, NH_4^+ concentrations were normalized by the time of exposure and corrected by the mean of the observed background concentrations in order to only contain plot effects.

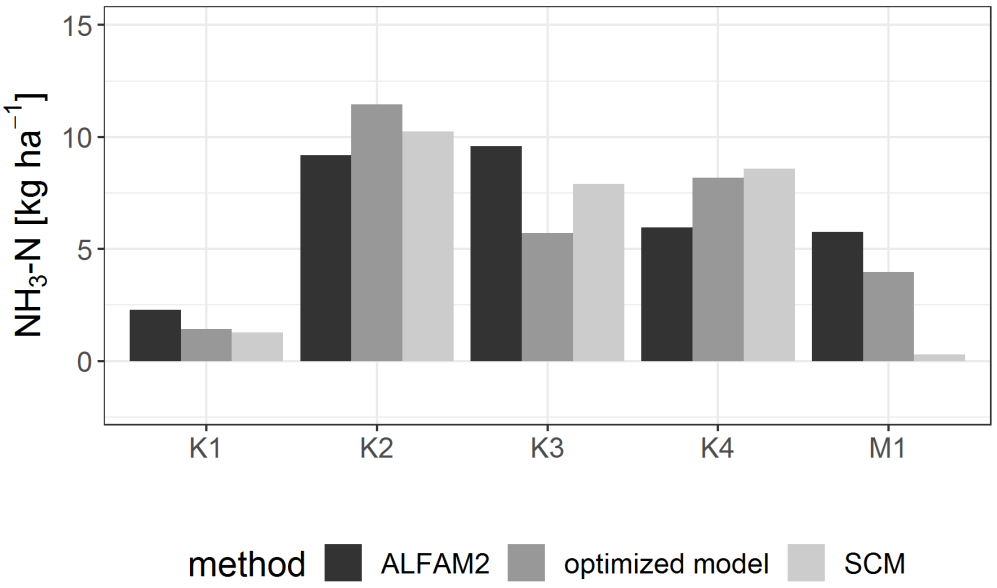
According to the SCM method, transfer factors (mg L^{-2}) were calculated for each sampling interval by dividing normalized NH_4^+ concentrations (mg L^{-1} min^{-1}) on

reference plots by the known reference NH_3 gas flux (L min^{-1}). Normalized NH_4^+ concentrations (mg L^{-1} min^{-1}) observed on experimental plots were then converted into fluxes of volatilized NH_3 (L min^{-1}) by dividing them by the transfer factor of the respective sampling interval (mg L^{-2}).

For both the first fertilizer application at Field A and at Field B, data from reference plots was only partially available due to failures of the NH_3 release system. In these cases, a model could be calibrated and optimized based on meteorological data (wind speed, temperature, relative humidity) and using the available reference plot data of each fertilizer application. This allowed for reconstructing probable transfer factors and the complete calculation of NH_3 volatilization from applied fertilizers.



SI 2 Fig. 4*: Total amounts of NH_3 volatilized from Slu, Min and Con plots, according to the optimized meteorological model. Additionally, the share of total slurry N (blue) or NH_4-N in slurry (black) lost via NH_3-N emissions is displayed for the Slu plots.*
M = sampling in silage maize (Field A), K = sampling in grass-clover (Field B)



SI 2 Fig. 5: *Total amounts of NH_3 volatilized from Slu plots according to the standard comparison method (SCM) as well as the optimized meteorological model, compared with predictions by the ALFAM2 model (Hafner et al., 2019)*

M = sampling in silage maize (Field A), K = sampling in grass-clover (Field B)

Results and interpretation

NH_3-emissions showed a large variability over the different fertilizer applications, ranging between 2.4 to 19.1 % of total slurry N (**SI 2 Fig. 4**). The high data variability can partly be explained by weather conditions during or shortly after fertilizer application. For instance, after the first slurry application at Field B, there was considerable rainfall, possibly explaining low emission values (main text **Fig. 2.2**). However, also some methodological issues cannot be excluded due to the failure of the reference system failed during several periods during the first fertilizer application both at Field A and Field B. Thus, for validation of the measured and modelled data, NH_3-emissions were also estimated using the ALFAM2 model (Hafner et al., 2019). Values obtained from this model confirmed the pattern of measured NH_3-emissions, overall yielding slightly higher emissions (**SI 2 Fig. 5**). Overall, the NH_3 emissions were rather low compared to other studies (Hoekstra et al., 2011, Sommer and Hutchings, 2001, Misselbrook et al., 2005). On the one hand, this might be surprising as slurry had a rather high share of NH_4-N (main text **Table 2.3**). On the other hand, the application with watering cans and subsequent rinsing of cans with water reduced

NH_3-losses and prevented fertilizers from remaining on plant and soil surfaces. Also, our experimental slurry did not contain any bedding material such as straw which would have impaired infiltration.

SI 2.3 Biological nitrogen fixation

Since Field B was cropped with a grass-clover mixture, also biological nitrogen fixation (BNF) added to the N inputs. Thereby, BNF can be calculated by different means (Unkovich and Pate, 2000): The simplest approach is to assume BNF to be the difference between N uptake of leguminous plants and non-leguminous reference plants. However, due to the low yield of the legumes in this experiment, this approach resulted in negative values. Alternatively, BNF can be calculated by isotopic approaches, which are independent from the yield.

For the fertilized treatments (Min and Slu), N derived from atmosphere (Ndfa) was calculated according to McAuliffe et al. (1958) using the enriched dilution (ED) method:

$$Ndfa_{ED}\ [\%] = (1 - \frac{atom\%\ 15Nexcess_{legume}}{atom\%\ 15Nexcess_{grass}}) \times 100 \qquad \textbf{SI 2 Eq. 1}$$

As the control treatment did not receive any ^{15}N labelled fertilizers, BNF was calculated using the natural abundance (NA) method (Shearer and Kohl, 1986).

$$Ndfa_{NA}\ [\%] = \frac{\delta^{15}N_{grass} - \delta^{15}N_{legume}}{\delta^{15}N_{grass} - B} \times 100 \qquad \textbf{SI 2 Eq. 2}$$

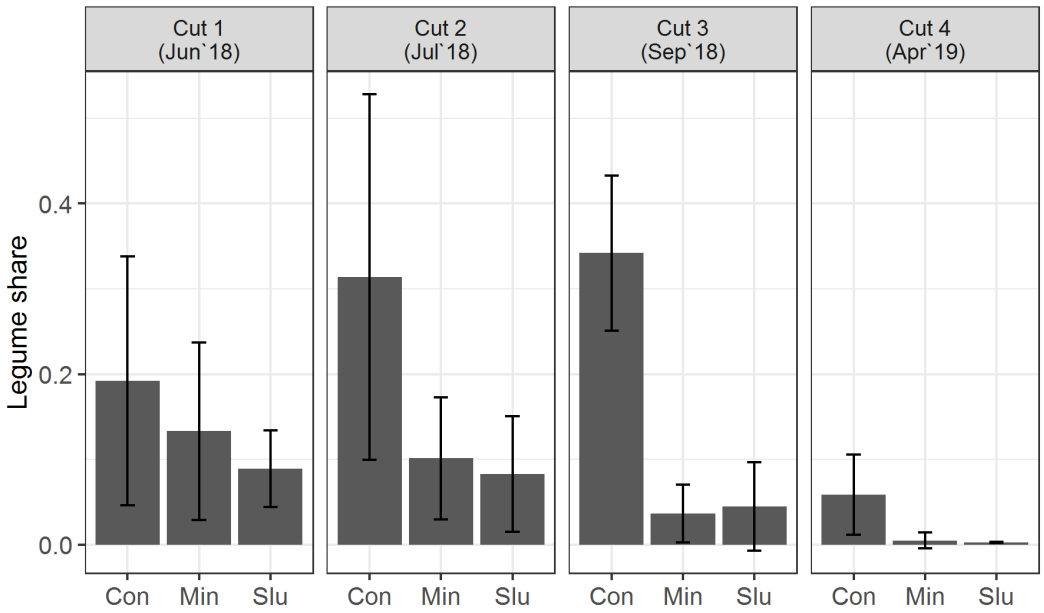
where $\delta^{15}N$ [‰] is the deviation (excess or depletion) over the ^{15}N abundance of N in air (0.36637 atom% ^{15}N).

According to Carlsson et al. (2009) the lowest measured $\delta^{15}N$ value for legumes in the field was used as B value, for this experiment B = -1.0. This value is also well in accordance with values considered by others under similar field conditions (Oberson et al., 2013).

For both, the ED and the NA method, grass grown in the same microplot as the legume was used as the corresponding non-N fixing reference plant. We acknowledge that this approach comprises methodological constrains by differing root morphology and probably temporal and spatial distinct N uptake patterns for legumes and grasses. Furthermore, N transfer between legumes and grasses must be expected, especially since Field B had been cropped with grass-clover already for four years when starting the experiment, leading to an underestimation in Nfix. Still, if N transfer is suspected, more reliable results can be expected with reference plants grown in mixture with legumes and not in adjacent pure stands (Carlsson and Huss-Danell, 2014).

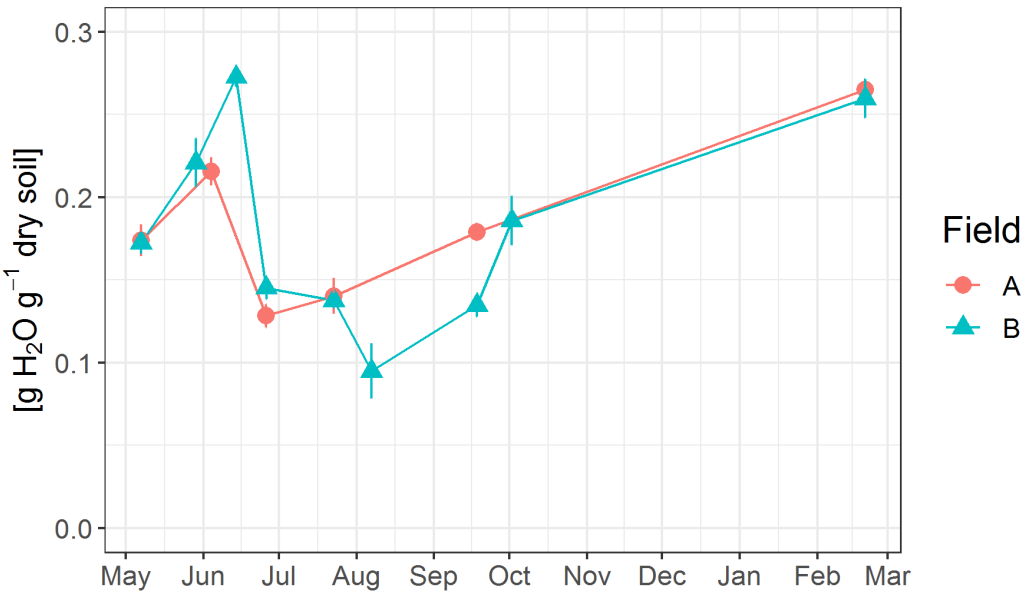
Our results, ranging between 14 to 40 kg Nfix ha^{-1} during one year, are lower than results reported elsewhere, where usually Nfix reached values between 60 and 160 kg N ha^{-1} at a legume share between 29 to 53 % of total biomass (Oberson et al., 2013). Besides underestimation due to N transfer between legumes and grasses, also the overall very low legume share, even further decreasing over the duration of our experiment, might explain the low values (**SI 2 Fig. 6**). Again, the low legume share might result from the rather old grass-clover stand, receiving substantial amounts of animal manure for several years.

Overall, we expected that the non-fertilized control would have higher Nfix values, than the fertilized treatments, as biological nitrogen fixation usually is hindered by large amounts of available N (McAuliffe et al., 1958). However, we could not statistically prove this, but identified one replicate within the control treatment as an outlier in which no legumes were growing at the specific location of this microplot. Exclusion of this value would have made the Nfix of the control treatment significantly higher than for the fertilized treatments. We did not exclude it from the analysis presented within this study, though, as it also was part of the heterogeneity of the field. Nfix did not differ between Min and Slu and overall strongly decreased over the time course of the experiment, possibly due to increased amounts of available N with repeated fertilization.



SI 2 Fig. 6: *Relative share of legumes in total biomass at Field B during the 4 cuts between June 2018 and April 2019 (mean ± standard deviation, n = 4)*

SI 2.4 Gravimetric soil moisture and correlation with Nmic and Nmin



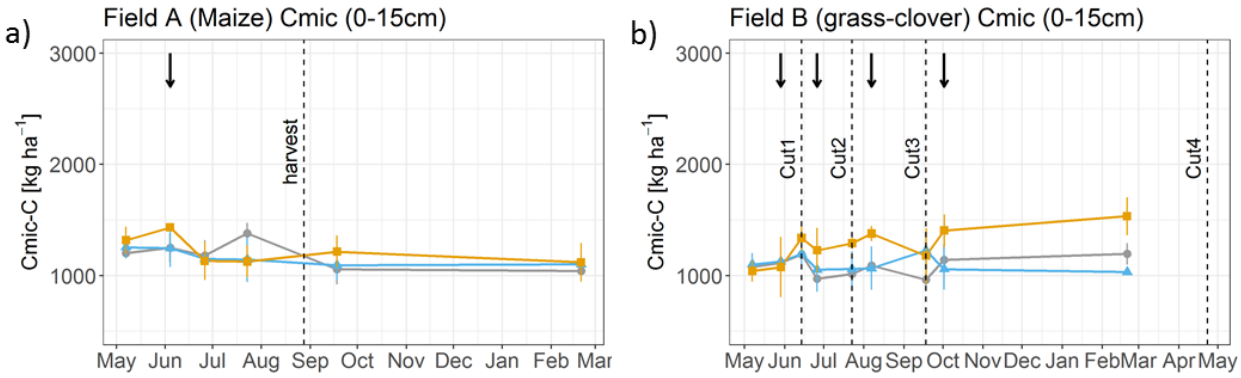
SI 2 Fig. 7: *Gravimetric soil moisture content (0 – 0.15 m) between May 2018 and February 2019 at the two field sites*

SI 2 Table 1: *Spearman`s rank correlation coefficient over both fields and all sampling points*

	Gravimetric moisture	Nmic [kg ha^{-1}]	Cmic [kg ha^{-1}]	NO_3 [kg ha^{-1}]	NH_4 [kg ha^{-1}]	NH_4:NO_3 ratio [-]
Gravimetric moisture		0.24***	0.13	-0.37***	-0.40***	0.11
Nmic [kg ha^{-1}]			0.74***	0.00	-0.03	0.03
Cmic [kg ha^{-1}]				0.17*	0.05	-0.13
NO_3 [kg ha^{-1}]					0.32***	-0.78***
NH_4 [kg ha^{-1}]						0.29***
NH_4:NO_3 ratio						

*, **, *** significant at $p < 0.05$, 0.01 and 0.001 probability level

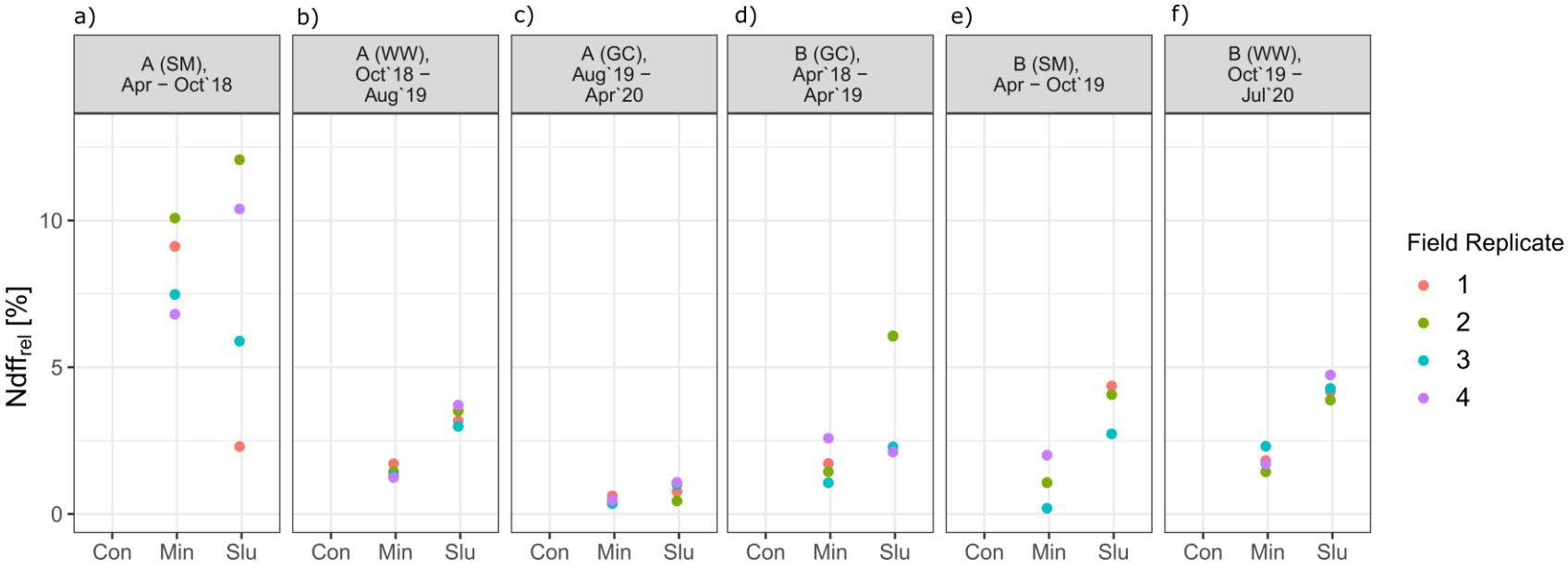
SI 2.5 Microbial Carbon



SI 2 Fig. 8: *Temporal development Cmic-C (mean ± standard deviation, n = 4). Black arrows indicate sampling(s) at one week after fertilizer application, dashed lines indicate aboveground biomass harvest.*

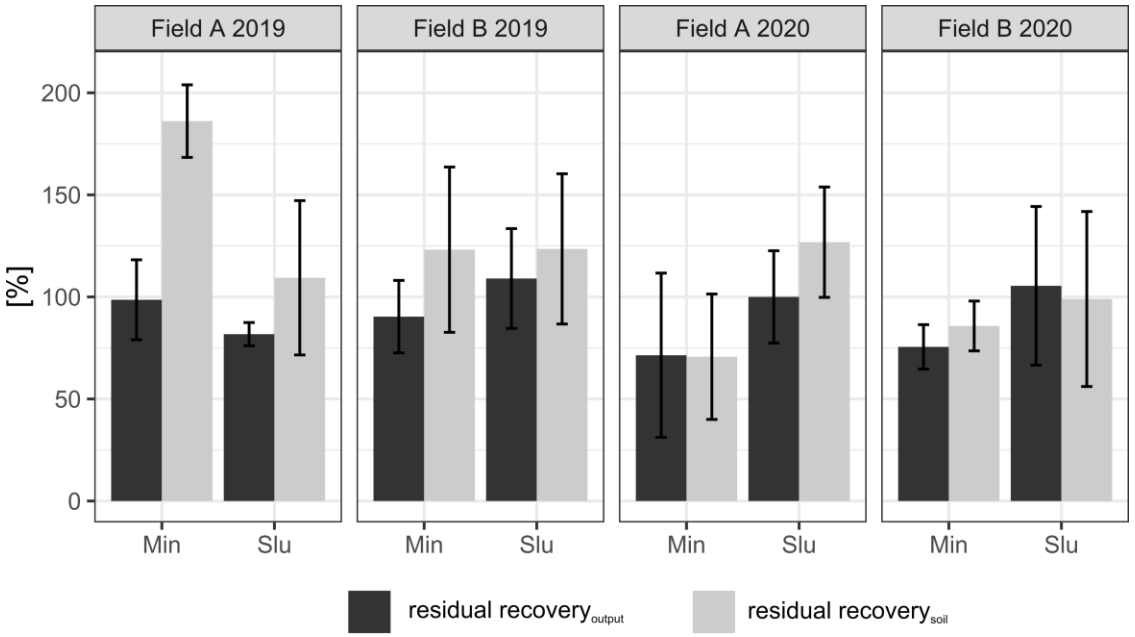
Supplementary Information Chapter 3

SI 3.1 Supplementary data on nitrate leaching measurements



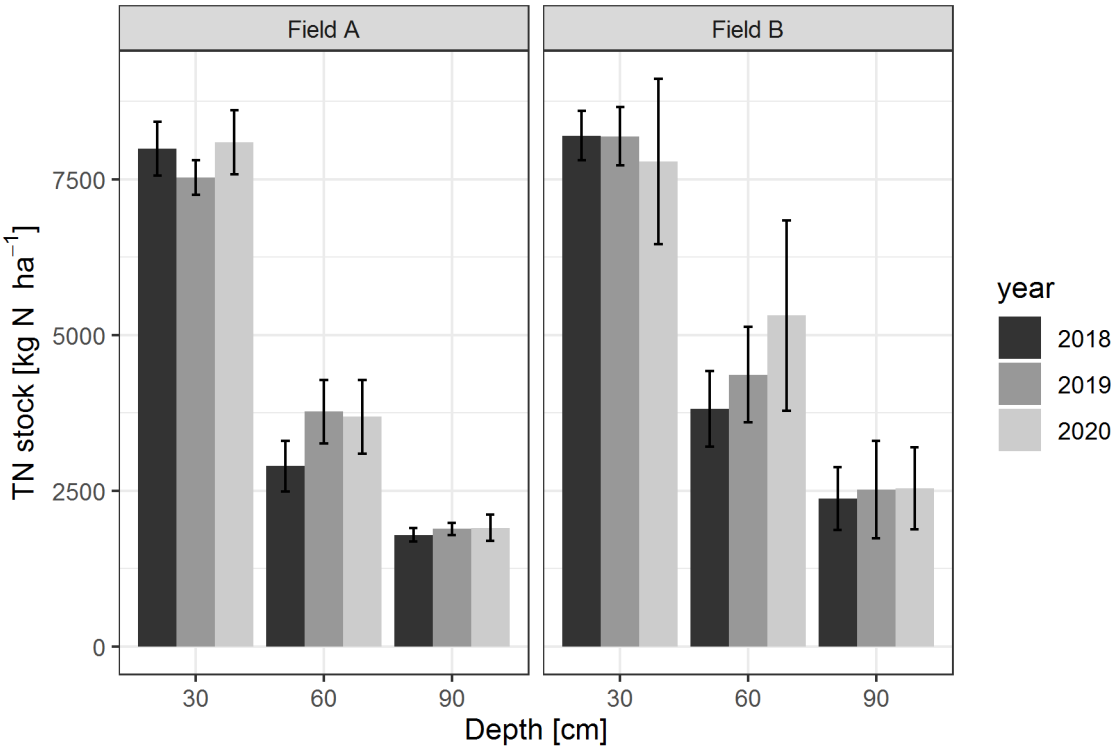
SI 3 Fig. 1: *Relative Ndff for leached nitrate at Field A (a – c) and Field B (d – f); especially for Field A in 2018 showing high data variability.*

SI 3.2 Residual recovery



SI 3 Fig. 2: *Residual recovery summed over plant uptake, leaching and soil (2019) or plant uptake, leaching, soil, stubble and roots (2020), obtained by two different approaches: residual recovery$_{output}$: calculation relative to originally applied ^{15}N fertilizer minus NH_3 emissions, NO_3 leaching and N uptake of aboveground biomass in the preceding year; residual recovery$_{soil}$: calculation relative to ^{15}N in soil (0 – 90 cm) in October of the preceding year*

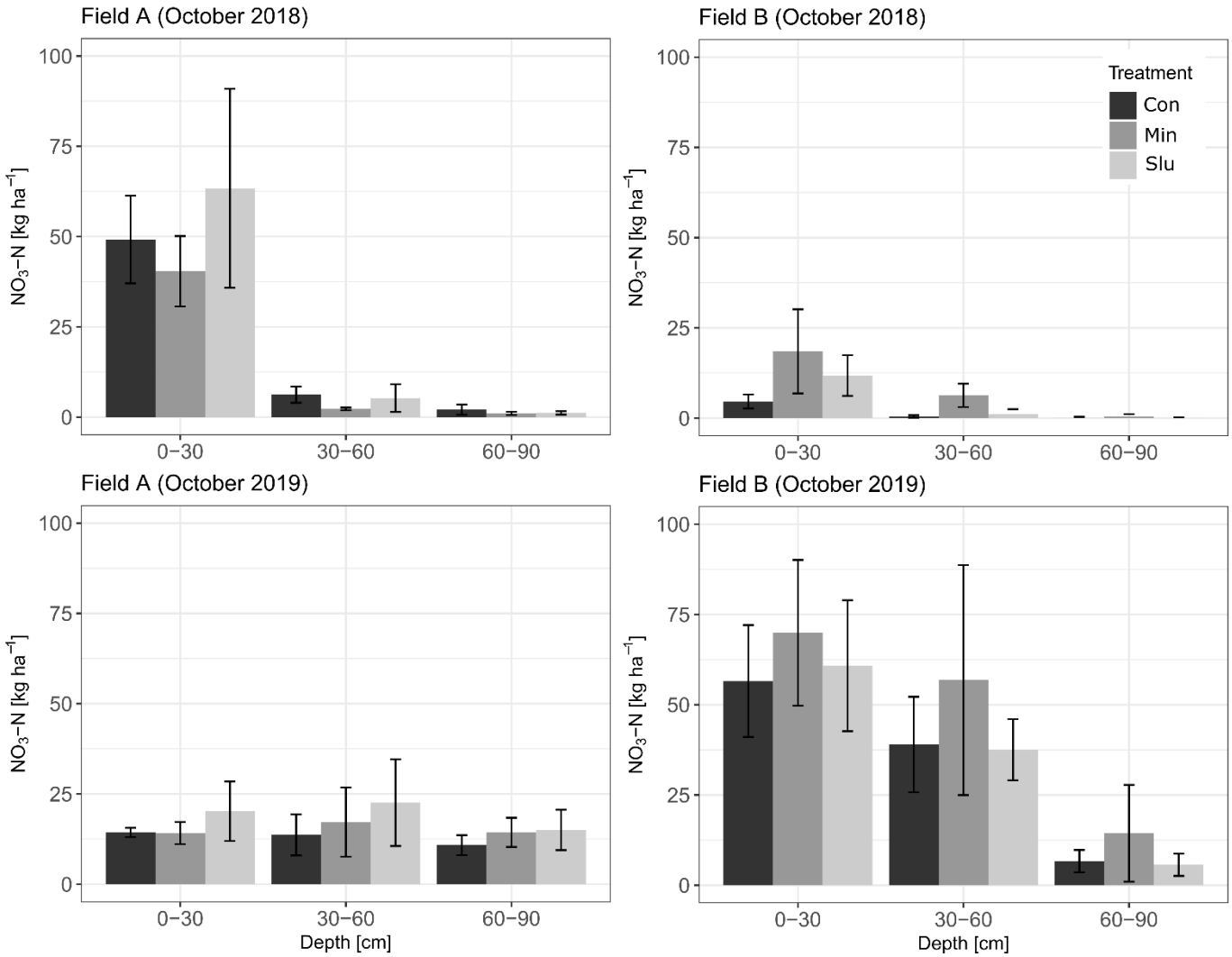
SI 3.3 Total N stocks in soil



SI 3 Fig. 3: *Total N (TN) stock variability in different depth layers (0 – 30 cm, 30 – 60 cm, 60 – 90 cm) over time. Data represents mean values ± standard deviation, n = 4*

Note: For 2018, soil corer broke at 80 cm. Therefore, data was upscaled to match the 60 – 90 cm depth layer in the other years.

SI 3.4 Nitrate levels in soil



SI 3 Fig. 4: *Nitrate levels [kg N ha^{-1}] in soil in October 2018 and 2019 at Field A (left) and Field B (right)*

SI 3.5 Details on field management

SI 3 Table 1: *Nutrient inputs and time points as well as timing of drilling, harvest and soil tillage over the duration of the experiment*

Field	Crop (Year)	Date	Cultivation	Input type	Nutrient input amount		
					N	P	K
					[kg ha^{-1}]		
Field A	Maize (2018)	2018-05-11	rotary band seeding of maize				
		2018-05-17	Fertilization	P (Landor P26)		23	
		2018-05-28	Fertilization	^{15}N Con/Min/Slu	0/36.8/60	7	75
		2018-07-02	Fertilization	Urea	69		
		2018-08-28	Harvest of maize				
	Winter wheat (2019)	2018-10-09	(manual) soil ploughing				
		2018-10-19	Drilling of winter wheat				
		2019-02-27	Fertilization	Nitrophos	60	13	
		2019-04-15	Fertilization	Urea	92		
		2019-07-22	Harvest of winter wheat				
		2019-08-17	(manual) soil tillage				
		2019-08-17	Drilling of grass-clover				
	Grass-clover (2019/ 2020)	2019-09-??	Fertilization	Cattle slurry	95	14	92
		2019-10-21	Cut1 of grass-clover				
		2020-03-17	Fertilization	Cattle slurry	95	14	92
		2020-04-16	Cut 2 of grass-clover				
		2020-04-23	Final sampling				
Field B	Grass-clover (2018/ 2019)	2018-05-21	Fertilization	^{15}N Con/Min/Slu	0/36.8/60	7	75
		2018-06-14	Cut1 of grass-clover				
		2018-06-19	Fertilization	^{15}N Con/Min/Slu	0/36.8/60	7	75
		2018-07-23	Cut 2 of grass-clover				
		2018-07-31	Fertilization	^{15}N Con/Min/Slu	0/36.8/60	7	75
		2018-09-18	Cut 3 of grass-clover				
		2018-09-25	Fertilization	^{15}N Con/Min/Slu	0/36.8/60	7	75
		2019-03-29	Fertilization	Cattle slurry	95	14	84
		2019-04-23	Cut4 of grass-clover				
		2019-05-12	Harvest of grass-clover				
	Maize (2019)	2019-05-17	rotary band seeding of maize				
		2019-05-23	Fertilization	NPK (15:15:15)	30	13	25
		2019-05-24	Fertilization	Cattle slurry	76	11	74
		2019-06-20	Fertilization	Urea	92		
		2019-09-10	Harvest of maize				
	Winter wheat (2020)	2019-10-17	(manual) soil tillage				
		2019-10-25	Drilling of winter wheat				

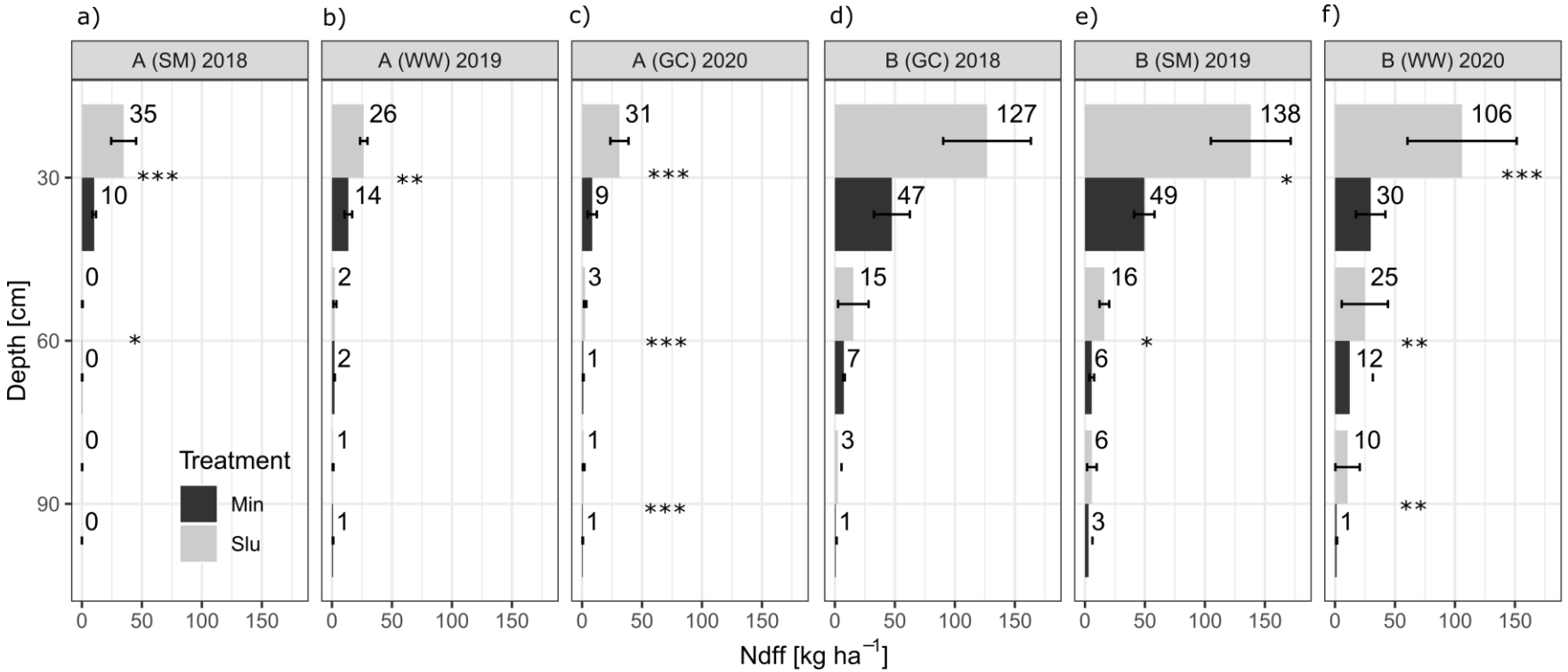
	2019-10-25	Fertilization	PK (20:30)		17	50
	2020-03-??	Fertilization	Nitrophos	40	9	
	2020-04-??	Fertilization	Urea	69		
	2020-07-10	Harvest of winter wheat				
	2020-07-22	Final sampling				

SI 3.6 Root and stubble biomass

SI 3 Table 2: *Biomass production, N uptake, N derived from fertilizer (Ndff) and recovery in roots and stubble relative to originally applied ^{15}N (final sampling in 2020)*

Field	Plant part	Treatment	Yield	N uptake	Ndff	Recovery
			dt ha^{-1}	kg N ha^{-1}		%
Field A (grass-clover)	roots	Con	27.6 ± 3.7	42.3 ± 8.0	-	-
		Min	26.1 ± 6.9	37.6 ± 9.0	0.22 ± 0.09	0.60 ± 0.25
		Slu	33.5 ± 2.6	48.1 ± 6.3	0.47 ± 0.09	0.79 ± 0.15
	stubble	Con	29.2 ± 1.9	42.3 ± 6.2	-	-
		Min	23.6 ± 3.3	34.0 ± 4.0	0.16 ± 0.04	0.45 ± 0.10
		Slu	30.0 ± 2.2	41.8 ± 6.9	0.37 ± 0.04	0.61 ± 0.07
Field B (winter wheat)	roots	Con	10.9 ± 1.7	11.6 ± 1.3	-	-
		Min	11.1 ± 1.9	11.8 ± 1.7	0.31 ± 0.12	0.21 ± 0.08
		Slu	11.2 ± 3.0	11.2 ± 2.7	0.41 ± 0.16	0.17 ± 0.06
	stubble	Con	10.3 ± 1.8	7.1 ± 1.6	-	-
		Min	9.0 ± 1.6	7.0 ± 1.4	0.01 ± 0.02	0.06 ± 0.01
		Slu	10.6 ± 1.5	7.0 ± 0.9	0.16 ± 0.02	0.07 ± 0.01

SI 3.7 Amounts of N derived from fertilizer in soil at different depth layers



SI 3 Fig. 5: *N derived from fertilizer (Ndff) in the three depth layers 0 – 30 cm, 30 – 60 cm and 60 – 90 cm over time for Field A (a – c) and for Field B (d – f). Samples in 2018 and 2019 were taken at the end of the vegetation period in mid-October, while sampling in 2020 took place upon harvest of the grass-clover in April (Field A) or upon harvest of winter wheat in July (Field B) SM = silage maize, WW = winter wheat, GC = grass-clover*
*mean ± standard deviation, n = 4, with * ($p < 0.05$), ** ($p < 0.01$), and ***($p < 0.001$)*

SI 3.8 Statistics on recovery of ^{15}N in soil

Field A

Differences between fertilizer treatments (Min, Slu) in depth translocation over the three years were assessed separately for the two fields using the following mixed effect linear model.

*recovery_mix_A <- lmer(log(recovery) ~Treatment * Depth + Depth * year + year * Treatment + (1|microplot) + (1|Block) + (1|microplot:year), data=FieldA)*

Log-transformed data was used to comply with the assumption of normal distribution and homoscedasticity of residuals.

Values below the detection limit (n = 6) were excluded from analysis.

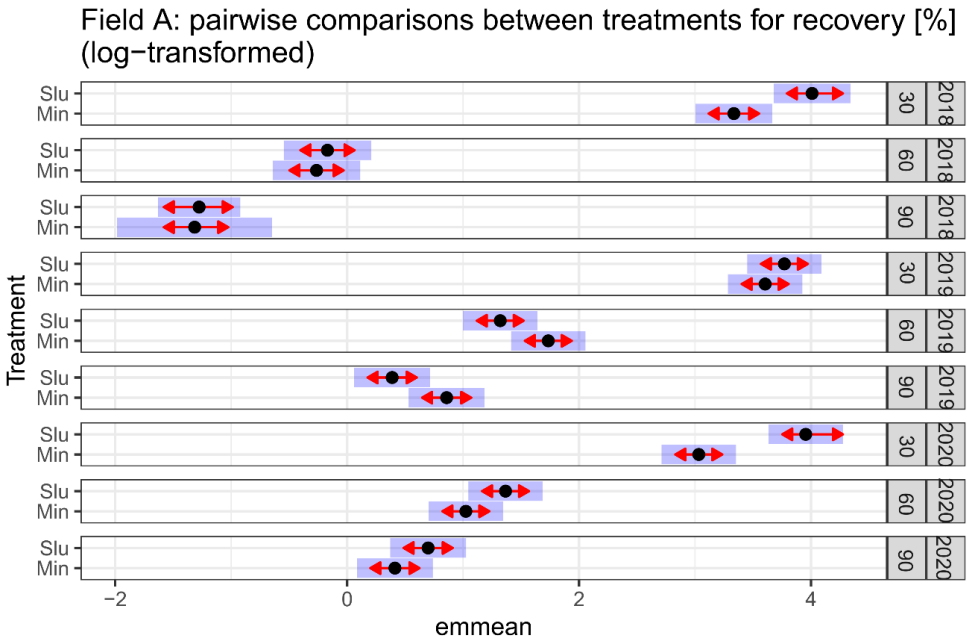
SI 3 Table 3: *ANOVA on ^{15}N recovery in soil (analysis on log-transformed data, negative values excluded) for Field A*

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr (>F)
Treatment	0.38	0.38	1	4.05	3.27	0.14
Depth	144.33	72.16	2	35.42	618.04	0.00
Year	11.43	5.72	2	14.12	48.81	0.00
Treatment:Depth	1.27	0.63	2	35.07	5.43	0.01
Depth:Year	9.18	2.3	4	35.31	19.66	0.00
Treatment:Year	1.44	0.72	2	14.36	6.15	0.01

All interactions as well as treatment, depth and year were highly significant.

Pairwise comparisons were performed using *emmeans*:

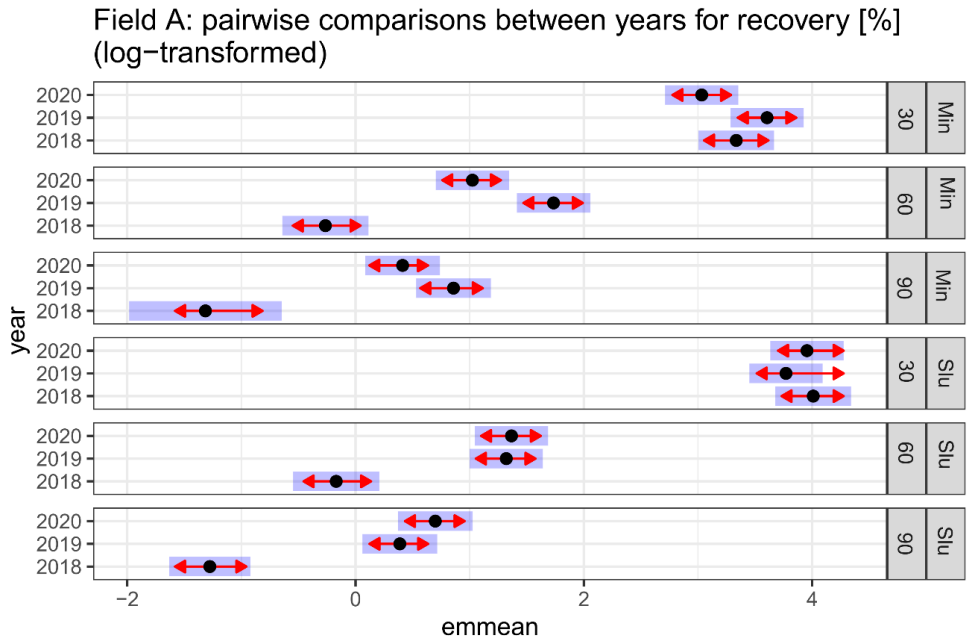
emmeans(recovery_mix_A, pairwise ~Treatment|year:Depth)



SI 3 Fig. 6: *Field A, pairwise comparisons between treatments for recovery [%] (log-transformed); numbers indicated depth (cm) and year; differences are significantly different ($p < 0.05$) when red arrows do not overlap.*

For comparisons over the years:

emmeans(recovery_mix_A, pairwise ~year | Depth:Treatment)



SI 3 Fig. 7: *Field A, pairwise comparisons between year for recovery [%] (log-transformed); differences are significantly different ($p < 0.05$) when red arrows do not overlap.*

Field B

For Field B, the same procedure as outlined above for Field A was followed.

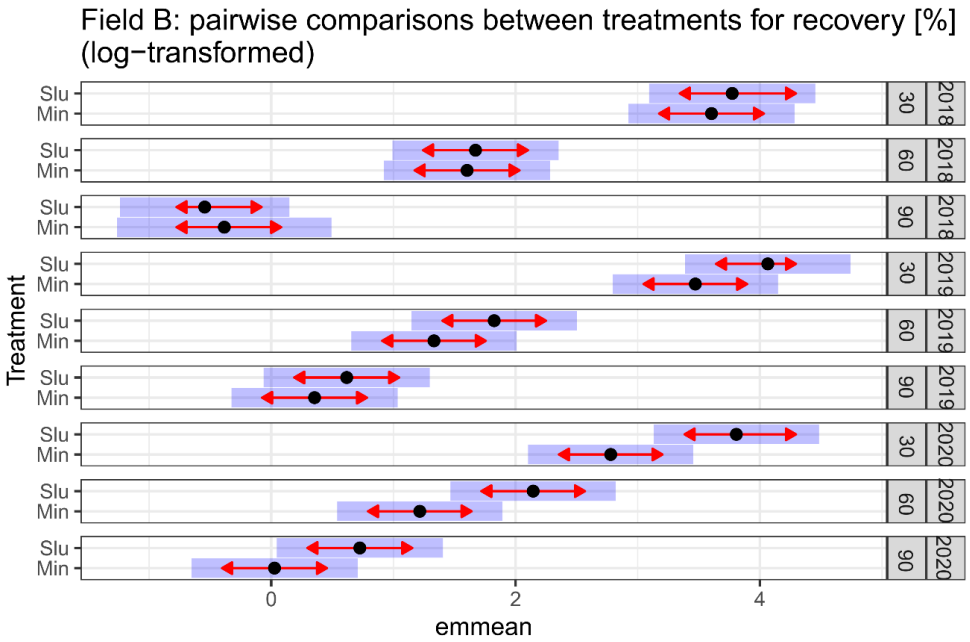
Values below the detection limit (n = 2) were excluded from analysis.

SI 3 Table 4: *ANOVA on ^{15}N recovery in soil (analysis on log-transformed data, negative values excluded) for Field B*

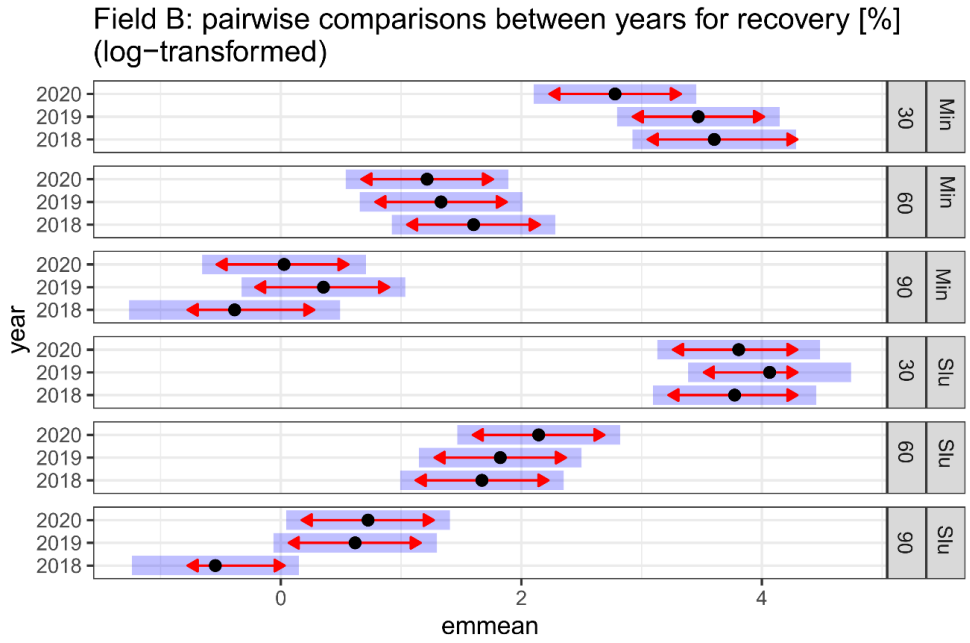
	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr (>F)
Treatment	2.65	2.65	1	3.04	5.19	0.11
Depth	133.78	66.89	2	38.88	130.94	0.00
Year	0.90	0.45	2	12.08	0.88	0.44
Treatment:Depth	0.31	0.16	2	38.88	0.30	0.74
Depth:Year	3.52	0.88	4	38.81	1.72	0.16
Treatment:Year	1.59	0.80	2	12.08	1.56	0.25

In contrast to Field A, none of the interactions was significant and only a significant effect of depth could be detected.

The results of pairwise comparisons are shown below.



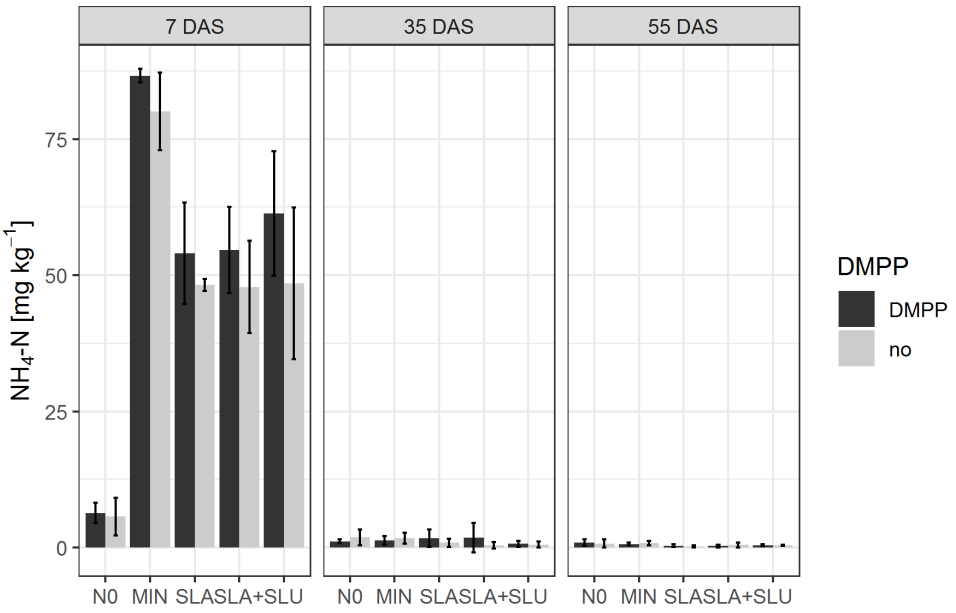
SI 3 Fig. 8: *Field B, pairwise comparisons between treatments for recovery [%] (log-transformed); numbers indicated depth (cm) and year; differences are significantly different ($p < 0.05$) when red arrows do not overlap.*



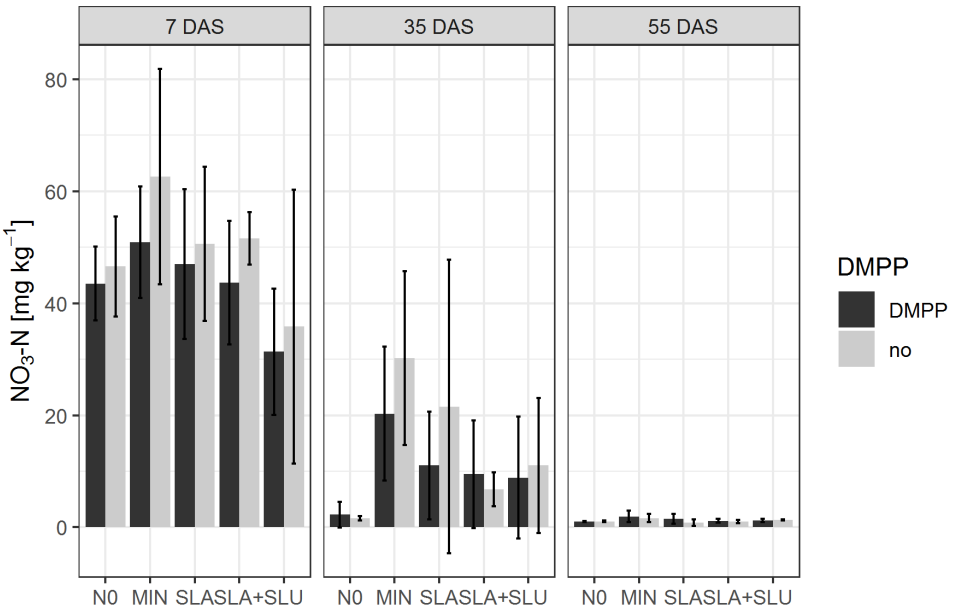
SI 3 Fig. 9: *Field B, pairwise comparisons between year for recovery [%] (log-transformed); differences are significantly different ($p < 0.05$) when red arrows do not overlap.*

Supplementary Information Chapter 4

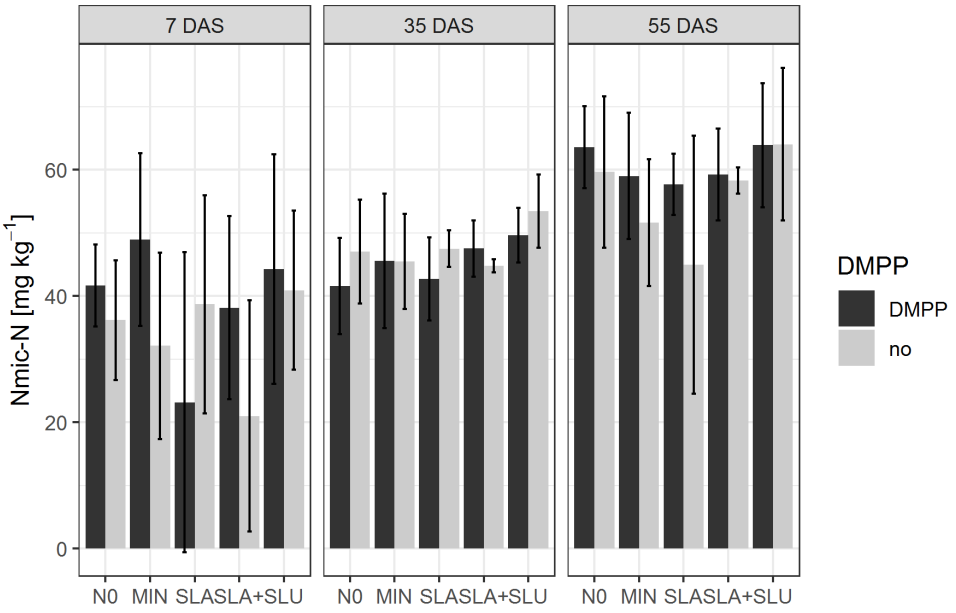
SI 4.1 Ammonium, nitrate and microbial N in soil



SI 4 Fig. 1: *Ammonium (NH_4^+) content in soil 7, 35 and 55 days after set-up (DAS) data represents mean ± standard deviation, n = 4*
N0 = no N fertilizer, MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry

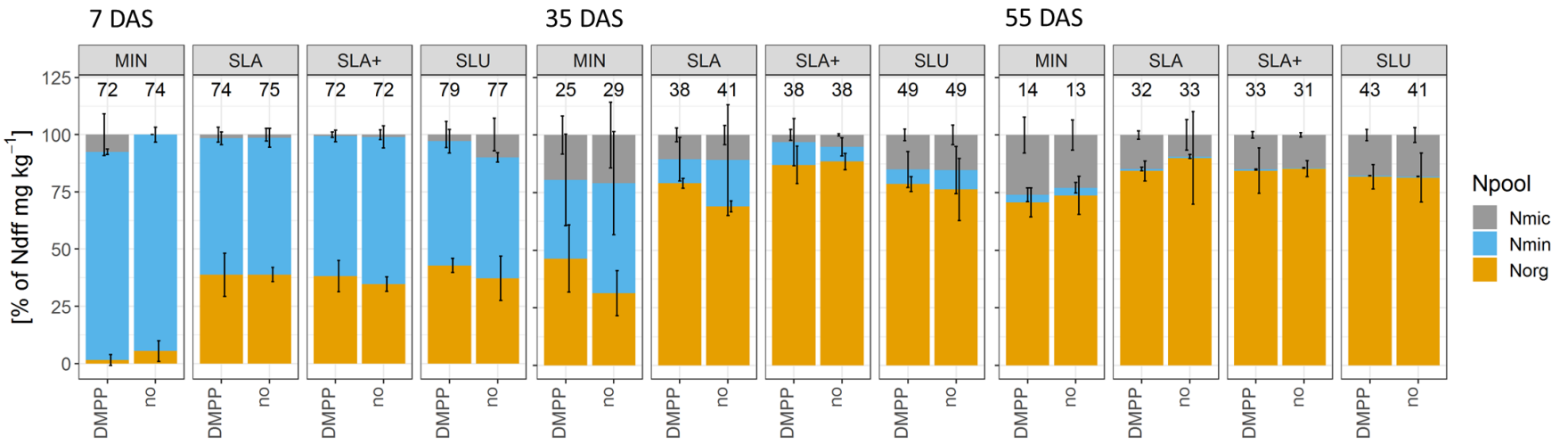


SI 4 Fig. 2: *Nitrate (NO_3^-) content in soil 7, 35 and 55 days after set-up (DAS) data represents mean ± standard deviation, n = 4*
N0 = no N fertilizer, MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry



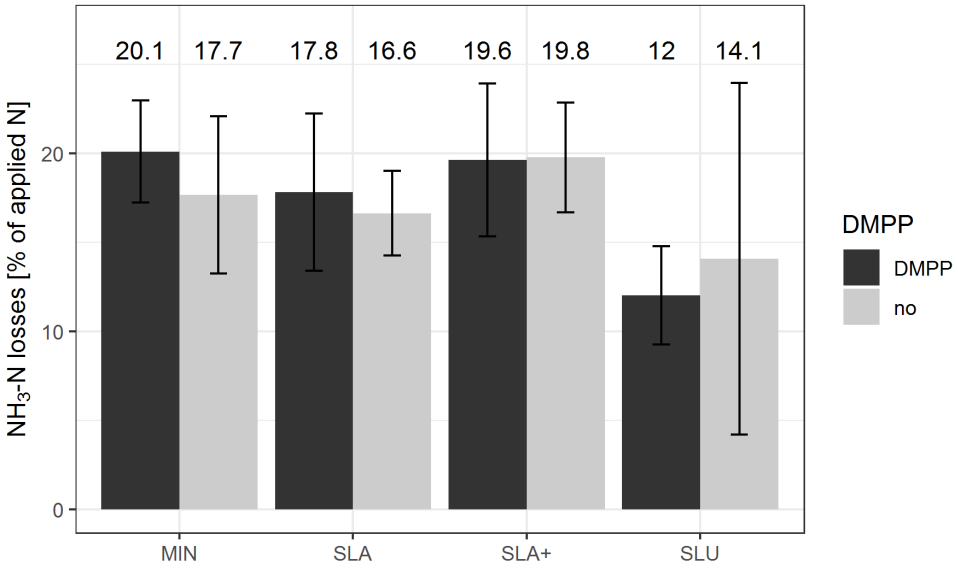
SI 4 Fig. 3: *Microbial N (Nmic) content in soil 7, 35 and 55 days after set-up (DAS) data represents mean ± standard deviation, n = 4*
N0 = no N fertilizer, MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry

SI 4.2 Relative distribution of residual 15N in soil



SI 4 Fig. 4: *Relative distribution of residual ^{15}N in soil at 7, 35 and 55 days after set-up (DAS). Numbers on top indicate absolute amounts of residual ^{15}N in soil [mg kg^{-1} soil]; data represents mean ± standard deviation, n = 4*
Nmic = microbial N, Nmin = mineral N (NH_4^+-N + NO_3^--N), Norg = non-microbial organic N
MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry
Note: Upon 7 DAS, (almost) no ^{15}N could be detected in Nmic, causing negative values for ^{15}N-Nmic. For graphical illustration and for calculation of ^{15}N-Norg, negative values were replaced by 0. Statistical analysis was only performed for the later time points (35 DAS and 55 DAS).

SI 4.3 Estimated NH_3 emissions



SI 4 Fig. 5: *Estimated NH_3-emissions as percentage of total fertilizer N added. Estimates are based on the assumption that the difference between ^{15}N recovery in soil at 7 days after set-up and the originally applied amount were lost via NH_3-volatilization; data represents mean ± standard deviation, n = 4; mean values also are indicated above.*
MIN = ^{15}N mineral fertilizer, SLA = ^{15}N anaerobically digested slurry, SLA+ = ^{15}N anaerobically digested slurry + biochar, SLU = ^{15}N cattle slurry

SI 4.4 Further analysis on cattle slurry and anaerobically digested cattle slurry

SI 4 Table 1: *Extensive characterization of ^{15}N cattle slurry (SLU) and ^{15}N anaerobically digested cattle slurry (SLA). Data obtained from bonalytic GmbH.*

		SLU	SLA
dry matter	%	3.3	2.7
organic dry matter	%	2.1	1.5
total N	g kg^{-1} DM	68.4	94.6
NH_4^+-N	g kg^{-1} DM	42.0	62.0
Ca	g kg^{-1} DM	14.0	20.5
K	g kg^{-1} DM	118.0	139.0
Mg	g kg^{-1} DM	5.6	9.2
Na	g kg^{-1} DM	4.5	6.1
P	g kg^{-1} DM	9.5	12.8
S	g kg^{-1} DM	7.1	4.5
pH	[-]	7.9	8.0
Volatile fatty acids (VFA)	g HAC L^{-1}	4.2	1.9
Total inorganic carbonate (TIC)	g CaCO3 L^{-1}	5.5	9.3
VFA/TIC	[-]	0.8	0.2
acetic acid	g L^{-1}	2.61	0.10
propionic acid	g L^{-1}	0.22	<0.03
butyric acid	g L^{-1}	0.09	<0.03
iso-butyric acid	g L^{-1}	0.07	<0.03
valeric acid	g L^{-1}	<0.03	<0.03
iso-valeric acid	g L^{-1}	0.09	<0.03
caproic acid	g L^{-1}	<0.03	<0.03
acetic acid equivalents (calculated)	g HAc_eq L^{-1}	2.95	0.11
Al	mg kg^{-1} DM	269.0	438.5
As	mg kg^{-1} DM	<15.0	<15.0
B	mg kg^{-1} DM	16.5	34.3
Ba	mg kg^{-1} DM	21.8	35.5
Cd	mg kg^{-1} DM	<0.2	<0.2
Co	mg kg^{-1} DM	0.6	0.8
Cr	mg kg^{-1} DM	2.1	2.9
Cu	mg kg^{-1} DM	21.2	29.8
Fe	mg kg^{-1} DM	496.0	873.0
Hg	mg kg^{-1} DM	<4.2	<4.2
Li	mg kg^{-1} DM	9.6	11.5
Mn	mg kg^{-1} DM	235.0	324.5
Mo	mg kg^{-1} DM	6.0	7.5

Nb	mg kg^{-1} DM	<0.3	<0.3
Ni	mg kg^{-1} DM	3.1	4.3
Pb	mg kg^{-1} DM	<0.9	1.4
Se	mg kg^{-1} DM	<15.0	<15.0
Si	mg kg^{-1} DM	225.0	266.5
Sn	mg kg^{-1} DM	<1.4	<1.4
Sr	mg kg^{-1} DM	39.0	44.7
V	mg kg^{-1} DM	0.8	1.3
Zn	mg kg^{-1} DM	86.4	147.5

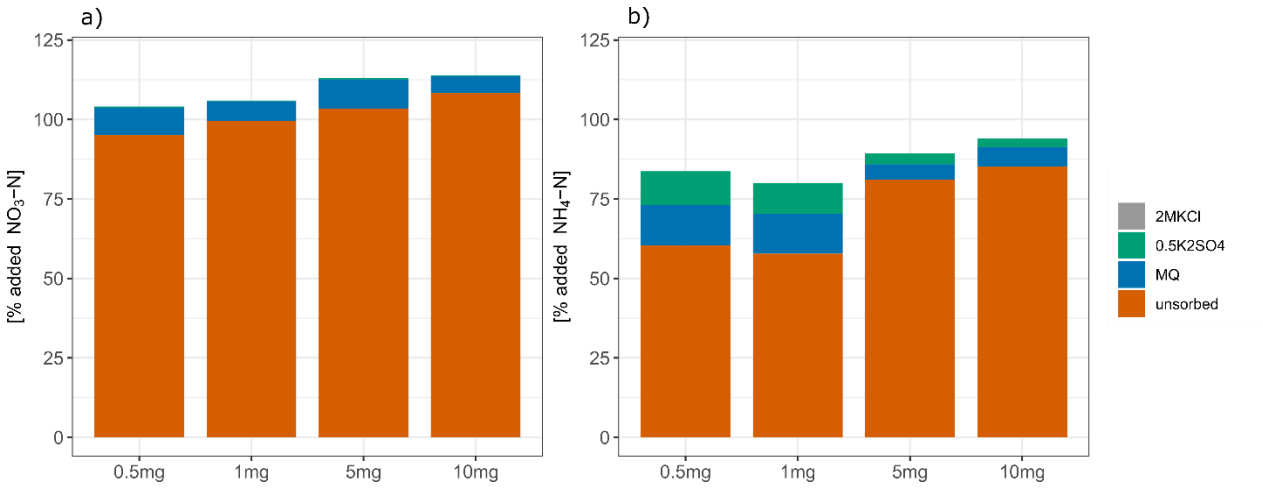
SI 4.5 Biochar batch sorption experiment

In order to test the ability of the biochar used in this experiment to sorb both NH_4^+ and NO_3^-, a batch sorption experiment was conducted. The protocol was adapted from Wang et al. (2015).

In short, 0.5 g of milled biochar were shaken with 40 mL of either KNO_3 or $(NH_4)_2SO_4$ solution at the following concentration range: 0, 0.5, 1, 5 and 10 mg N. Blanks (without biochar) were included as well. The experiment was conducted in triplicates.

After 16 hours, samples were centrifuged at 5500 rpm for 10 minutes. The supernatant was transferred to new falcon tubes and analysed for NH_4^+ and NO_3^- in order to quantify sorption to the biochar particles. By weighing the supernatant, we were able to correct for residual extractant volume that could not be removed without removing biochar particles. In order to test for the strength of the adsorption, biochar was extracted with milliQ water by shaking for 1 hour. Again, the supernatant was removed after centrifuging at 5500 rpm for 10 minutes. The extraction procedure was repeated twice with 0.5 M K_2SO_4 and once with 2 M KCl solution.

As it can be seen in **SI 4 Fig. 6**, biochar was not capable to sorb major amounts of NO_3^- and the adsorbed amount was directly extractable with milliQ water. In contrast, biochar effectively adsorbed NH_4^+ (40 % of added N at concentrations up to 1 mg NH_4^+-N per litre, up to 20 % at higher concentrations). Even with the highest molar salt solution, this NH_4^+ was not extractable any more.



SI 4 Fig. 6: *Sorption and desorption of nitrate (NO_3^-) (a) and ammonium (NH_4^+) (b) to biochar particles. unsorbed = fraction of added N that was not adsorbed to biochar particles; MQ = fraction that could be desorbed with milliQ water; 0.5M K_2SO_4 = fraction that could be desorbed by 0.5 M K_2SO_4 (2 extraction cycles); 2M KCl = fraction that could be desorbed by 2M KCl*