Nitrogen availability and retention in clayey steppe soils of semi-arid North Kazakhstan

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„Das Problem des Wissens besteht darin, dass es sehr viel mehr Bücher über Vögel gibt, die von Ornithologen geschrieben werden, als Bücher von Vögeln über Vögel und Bücher von Vögeln über Ornithologen.“

Nassim Nicholas Taleb

Abstract

Though nitrogen (N) is the most abundant element on earth, it is the least available for most organisms in terrestrial systems. Northern hemisphere steppe soils are globally very important as they store huge amounts of N as soil organic matter (SOM). Because of their potential high fertility, these soils are widely under intensive agricultural use which strongly affected nutrient availability. Especially in the semi-arid steppe soils of North Kazakhstan, which serve as a global bread basket, soils were unsustainable managed during the “Virgin Land Campaign”, resulting in soil degradation, low contents of mineral N and a decreasing productivity.

However, there is yet a lack of information on the N cycle and its availability in these clayey, semi-arid agricultural used steppe soils. Therefore this thesis aimed at (i) investigating the N availability and retention of N under the current common agricultural practice, and (ii) tested if with slight and cost-limited changes towards a climate adapted and more sustainable agricultural practice, N availability can be increased. Therefore, three experiments were conducted with soil samples from North Kazakhstan. The first laboratory study investigated the gross N mineralization and biotic and abiotic retention of fertilizer N in grassland and arable soil in spring time. In the second *in vitro* study, grassland and arable soils were subjected to different climatic scenarios to investigate the effect of climate change on the N availability (net N mineralization). And lastly, the third study aimed at testing the effect of fertilizer and tillage form on the plant-microorganism competition for available N in the field.

Our results suggest similar rates of gross N mineralization and immobilization which results in low net N mineralization and hence low natural N availability under the current agricultural practice. Land use, fertilization or changes in temperature and soil moisture did not affect N availability. A transition of fertilizer form did not enhance plant productivity and plant N uptake. But our results suggest that reduced soil tillage might be favorable over no tillage in these clayey and semi-arid soils. Contents of inorganic N forms are strongly limited

in these clay-rich semi-arid soils and attributed to strong abiotic N retention processes. Hence, competition for limited inorganic N in these soils is severe.

Nitrogen is effectively kept in the soil-plant system; however the availability of inorganic N is strongly limited by abiotic N retention processes, which jeopardize soil fertility. Nitrogen fertilization adapted to the specific characteristics of these clay-rich semi-arid soils is necessary to ensure productive and sustainable wheat production in this global bread basked in the future.

Keywords: Semi-arid, steppe soil, Kazakhstan, nitrogen, ^{15}N, isotope, land use, arable soil, grassland soil, plant-microbe competition, liquid fertilizer, granular fertilizer, soil organic matter, net mineralization, gross mineralization, climate change, N fixation, N immobilization

Zusammenfassung

Obwohl Stickstoff (N) das am häufigsten vorkommende Element auf der Erde ist, ist es für die meisten Organismen in terrestrischen Systemen das doch am wenigsten verfügbare. Steppen-Böden der nördlichen Hemisphäre spielen global eine sehr wichtige Rolle, da sie große Mengen N in Form organischer Bodensubstanz (SOM) speichern. Eben wegen ihrer hohen potentiellen Fruchtbarkeit sind diese Böden weitreichend unter intensiver landwirtschaftlicher Nutzung, welche die Verfügbarkeit von Nährelementen stark beeinflusst. Besonders die als globaler Brotkorb fungierenden semi-ariden Steppen-Böden Nord Kasachstans wurden während der „Neulandkampagne“ nicht nachhaltig bearbeitet, mit Bodendegradation und einer Verringerung der Produktivität als Folge.

Jedoch fehlen bisher weiter Informationen über den N Kreislauf und die N Verfügbarkeit in diese Ton-reichen, landwirtschaftlich genutzten semi-ariden Steppe-Böden. Daher zielt diese Arbeit darauf ab, (i) die Verfügbarkeit und Retention von N unter der aktuell üblichen landwirtschaftlichen Praxis zu untersuchen, und (ii) zu testen, ob mit geringfügigen und Kosten-beschränkten Änderungen hin zu einer Klima-adaptierten und nachhaltigeren landwirtschaftlichen Praxis, die N Verfügbarkeit gesteigert werden kann.

Hierzu wurden drei Experimente mit Böden von fünf Standorten in Nord Kasachstan durchgeführt. Die erste Labor-Studie untersuchte die brutto N Mineralisation und biotische sowie abiotische Retention von Dünger N im Frühjahr in Grassland- und Acker-Böden. Im zweiten *in vitro* Experiment wurden Grassland- und Acker-Böden verschiedenen Klima-Szenarien ausgesetzt um den Effekt des Klimawandels auf die N Verfügbarkeit zu untersuchen (netto N Mineralisation). Und schließlich wurde im dritten Experiment der Effekt von Dünger- und Bodenbearbeitungs-Form auf die Pflanze-Mikroorganismen Konkurrenz um verfügbares N im Feld getestet.

Unsere Ergebnisse zeigen ähnliche Raten für die brutto N Mineralisation und Immobilisation auf, welches in einer geringen netto N Mineralisation resultiert und lassen somit eine geringe natürliche N Verfügbarkeit unter der aktuell üblichen landwirtschaftlichen Praxis schließen. Landnutzung, Düngung, oder Veränderungen in Temperatur und

Bodenfeuchte hatten keinen Einfluss auf die Verfügbarkeit von N. Ein Umstieg der Düngerform erhöhte weder die Pflanzenproduktivität noch die N Aufnahme von Pflanzen. Unsere Ergebnisse lassen weiter vermuten, dass eine reduzierte keiner Bodenbearbeitung in diesen Ton-reichen semi-ariden Böden vorzuziehen sein könnte. Die Gehalte anorganischer N Formen in diesen tonreichen und semi-ariden Böden ist stark limitiert und kann der starken abiotischen Retention von N zugeordnet werden. Daher ist die Konkurrenz für das begrenzt verfügbare anorganische N in diesen Böden hoch.

Stickstoff wird effektiv im System Boden-Pflanze gehalten. Dennoch ist die Verfügbarkeit an anorganischen N für Pflanzen stark durch abiotische Retentions-Prozesse limitiert welche die Bodenfruchtbarkeit gefährden. Eine an die spezifischen Charakteristika dieser Ton-reichen, semi-ariden Böden adaptierte N Düngung ist notwendig um die Produktivität und Nachhaltigkeit der Weizen Produktion in diesem globalen Brotkorb in Zukunft aufrecht zu erhalten.

Stichworte: Semi-arid, Steppe Boden, Kasachstan, Stickstoff, ^{15}N, Isotope, Landnutzung, Ackerboden, Grasslandboden, Pflanze-Mikroorganismen Konkurrenz, Flüssigdünger, Granulatdünger, Organische Bodensubstanz, Netto Mineralisation, Brutto Mineralisation, Klimawandel, N Fixierung, N Immobilisation

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Table 2: Basic properties of study soils. Abbreviations: AS: arable soil; GS: grassland soil; TC: total carbon; IC: inorganic carbon, OC: organic carbon; fPOM and oPOM: free and occluded particulate organic matter; MAOM: mineral-associated organic matter; TN: total nitrogen; TP: total phosphorous; ON: organic nitrogen; initial N_{min}: $CaCl_2$ extractable NH_4-N plus NO_3-N before incubation. Given is the mean of n = 3 samples with the standard deviation in brackets. For SOM fractions n = 1. 71

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Abbreviations

μ	Mean
$\delta^{15}N$	Ratio of stable nitrogen isotopes ^{15}N to ^{14}N in a sample to that in air as reference
ANOVA	Analysis of variance
AR	Absolute ratio of mole fractions of ^{15}N in air
C	Carbon
CFE	Chloroform fumigation extraction
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
EA	Element analyzer
EO-P	Extractable organic phosphorous
fPOM	Free particulate organic matter
GC	Gas chromatography
IC	Inorganic carbon
ICP-OES	Inductively coupled plasma optical emission spectrometry
IPD	Isotope pool dilution
IRMS	Isotope ratio mass spectrometry
k	Rate constant
LOS	Losovoe
MAOM	Mineral-associated organic matter
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
N	Nitrogen
n	Number of samples
NdfF	Nitrogen derived from fertilizer
NdfS	Nitrogen derived from soil
NHI	Nitrogen harvest index
N_{min}	Mineralized nitrogen
OC	Organic carbon
OM	Organic matter
ON	Organic nitrogen
oPOM	Occluded particulate organic matter
P	Phosphorus
p	Probability value
PC	Principal component
PCA	Principal component analysis

pMC	Percent modern carbon
POM	Particulate organic matter
r	Correlation coefficient
R^2	Determination coefficient
SD	Standard deviation
SI	Supporting information
SOM	Soil organic matter
SPINMas	Sample preparation unit for inorganic nitrogen coupled to a quadrupole Mass spectrometer
t	Time
T	Temperature
TC	Total carbon
TN	Total nitrogen
TOC	Total organic carbon
Tuckey's HSD	Honest significance post-hoc test
WEOC	Water-extractable organic carbon
WETN	Water-extractable total nitrogen
YP	Yasnaya Polyana

1. General Introduction

1.1. The nitrogen cycle

Nitrogen (N) is a major nutrient for organisms and key element for life (Denk et al., 2017). In the pedosphere, e.g. the quality and productivity of plants (Barker and Pilbeam, 2015; Hooper and Johnson, 1999) as well as the growth and metabolism of soil microorganisms (Cui et al., 2018; Harder and Dijkhuizen, 1983) is largely controlled by the availability of N. However, N is often limited in natural and anthropogenic influenced terrestrial systems (Vitousek and Howarth, 1991).

Naturally, N may enter the soil via atmospheric deposition (Anderson and Downing, 2006), or N fixation by legumes (Liu et al., 2011) and some microorganisms (Denk et al., 2017) during which unreactive atmospheric N_2 is fixed in microbial or plant biomass. After biological fixation, N will get further transformed within the organism, or, if N compounds were excreted or deposited, become a part of the soil organic matter (SOM), which includes amongst others plant, animal or microbial residues and tissues, amino acids, amino sugars, peptides (Kögel-Knabner, 2002). During mineralization organic N (ON) compounds are transformed to inorganic reactive N compounds by enzymatic processes of heterotrophic microorganisms or plants (IAEA, 2001; Knicker, 2011; Murphy et al., 2003; Myrold and Bottomley, 2008; Risch et al., 2019). Hereby N becomes available to other plants and microorganisms. This decomposition process is often referred to as ammonification because ammonium is the primary product (IAEA, 2001; Murphy et al., 2003). Ammonium may accumulate in the soil or may directly be nitrified to nitrate (Murphy et al., 2003). Both these soluble inorganic N compounds are rather mobile in the soil (e.g. Huang et al., 2017; Mian et al., 2009) and hence, can remain or leave the system in various pathways. Nitrogen may get leached from the soil within the soil solution (van Groenigen et al., 2015), as output of biomass e.g. as harvest (Peckham and Gower, 2011), or as gaseous losses in form of NO_x, N_2O, NO during denitrification (van Groenigen et al., 2015), or as NH_3 if volatilized especially under alkaline conditions (Bouwman and Boumans, 2002).

Nitrogen compounds may also get retained in the soil by fixation and immobilization processes, or by physical protection (Knicker, 2011; Nieder et al., 2011). These N retention processes can be quite fast and may account for over 60 % of the applied N for microorganisms (Grace et al., 1993) and up to 59 % (Nieder et al., 2011) for clay minerals within a few days after N application. Fixation denotes to a very strong but reversible binding, as ammonium may be incorporated into the lattice of expandable clay minerals (Nieder et al., 2011). Immobilization refers to the stabilization of inorganic and organic N compounds by e.g. sorption on or incorporation into SOM (Knicker, 2011; Nommik, 1965) or pedogenic minerals (Sollins et al., 2006). These sorption processes can be weak or strong but are in both cases reversible (Knicker, 2011; Sollins et al., 2006). Nitrogen may also be immobilized by plant (Schimel and Bennett, 2004a; van Groenigen et al., 2015) or by microbial uptake and is then retained until excretion of solutes or the death (Hodge et al., 2000a).

Especially in N limited systems, microorganisms and plants compete for N (Kuzyakov and Xu, 2013; Schimel and Bennett, 2004a). Their larger surface area-to-volume ratio and rapid growth (Hodge et al., 2000a) are beneficial for microorganisms in the competition for N with plants. Moreover, N acquisition by plants is mostly limited to certain vegetative stages (Beathgen and Alley, 1989; Chen et al., 2014), whereas the N assimilation of microorganisms is not bound to certain growing stages. Accordingly, in grassland soils, it was shown that microorganisms cannot only assimilate >60 % of added N shortly after N application (Grace et al., 1993; Harrison et al., 2008; Hodge et al., 2000a), but also that the assimilation of inorganic N forms is several times faster than for plants (Jackson et al., 1989). Traditionally, it was believed that plants only assimilate inorganic N fractions, so that microbial mineralization controls plant N availability (Harrison et al., 2007; Schimel and Bennett, 2004a). However, it was found that plants also compete for ON compounds like amino acids and peptides (Chen et al., 2015; Dunn et al., 2006; Näsholm et al., 2009; Schimel and Bennett, 2004a), therefore partially bypassing the microbial dependence (Harrison et al., 2007; Hodge et al., 2000a; Schimel and Bennett, 2004a). This competition depends mainly on the extent of N limitation, with larger competition at lower N contents

(Harrison et al., 2007; Kuzyakov and Xu, 2013; Schimel and Bennett, 2004a), and on the microbial activity, which, if high, decreases the availability of ON to plants (Jones, 1999; Rutherford and Juma, 1992).

1.2. Nitrogen mineralization

Nitrogen mineralization is key to many of the above described processes and largely controls the availability of N to plants and microorganisms. However, one can differentiate into gross or net mineralization. The net mineralization is measured over longer time periods (weeks to months) and hence allows the evaluation of changes in N pools (Murphy et al., 2003). The gross mineralization in contrast is measured over short time intervals (hours to days) and therefore gives information about controlling processes of SOM decomposition (IAEA, 2001; Murphy et al., 2003). While in the net mineralization many co-depending processes (e.g. immobilization, fixation, nitrification, or denitrification) are included (Stark and Schimel, 2001), the gross mineralization is determined with smaller influence of these comprising effects and allows to determine process rates (IAEA, 2001; Murphy et al., 2003).

Naturally, N mineralization is affected by the availability of SOM, soil properties and ambient conditions like soil moisture or temperature. Stabilization processes by e.g. soil texture (Hassink, 1997) or mineral composition (Mikutta et al., 2019) can limit the availability of SOM (Gershenson et al., 2009; Qin et al., 2019). The C:N:P-ratio (P for phosphorus) of SOM is an indicator for the degradability of SOM (Hessen et al., 2004; Heuck and Spohn, 2016). Hereby, the most efficient SOM decomposition (the highest C use efficiency) is reached when its C:N:P stoichiometry matches the microbial demand (Chen et al., 2014; Hessen et al., 2004). Climatic conditions affect considerably the N mineralization in form of temperature (Dessureault-Rompré et al., 2010; Y. Liu et al., 2016; Zaman and Chang, 2004) and moisture (Hu et al., 2019; Paul et al., 2003) and have been studied intensively. For temperature, results generally found that higher temperature increased N mineralization (Zaman and Chang, 2004; Wang et al., 2006). Too wet and too dry soil moisture conditions,

however, both reduce the decomposition of SOM (Grierson et al., 1999; Hu et al., 2019; Moyano et al., 2013; Wang et al., 2006).

Anthropogenic impacts on the soil further constrain N mineralization. Land use might strongly impact N mineralization (Ihori et al., 1995; Yang et al., 2020)., e.g. in cropland N mineralization was found to be higher than abandoned cropland and native steppe soils (Ihori et al., 1995) and may depend on farm type and cropping time (Yang et al., 2020). In contrast to Ihori et al. (1995), higher gross N mineralization rates in grassland soils than in arable soils were attributed to different soil properties (Lang et al., 2016) and can be explained by smaller SOM contents in arable soils resulting in a reduced N mineralization (Booth et al., 2005a). Differences in soil properties as a consequence of different land uses, like ON, organic carbon (OC), water content (Ihori et al., 1995), SOM and bulk density (Yang et al., 2020), plant species (Jiang et al., 2011), or different microbial communities (Moreno et al., 2019), may thus further impact N mineralization (Sun et al., 2013). However, the impact of land use is not clear.

Coming along with arable land use, tillage may further strongly affect soil properties, for example soil OC contents (Logan et al., 1991) or the water retention (Bescanasa et al., 2006; Fabrizzi et al., 2005; Gozubuyuk et al., 2014), and there are many research papers and reviews available on this matter (Jug et al., 2019; Li et al., 2019). In a study on the C and N mineralization in the prairie and agricultural used soils, Ajwa et al. (1998) found that soil cultivation can affect mineralization, as type and distribution of mineralizable compounds changed between cultivation techniques. Also, the SOM quantity is influenced by soil management (Valboa et al., 2015; Yang et al., 2012) which is an important factor in the microbial respiration (Colman and Schimel, 2013) and therefore mineralization. Bonde et al. (1988) found that N mineralization changes with tillage intensity. While the advantages of conservation tillage (e.g. mini-till) over conservative tillage (plough) are well known (Baker et al., 2007; Derpsch et al., 2010; Kravchenko et al., 2012), the preference of either no-till or mini-till is not so clear. Scientific evidence deviate which tillage form increased yield most;

mostly equal or lower yields for no-till compared with mini-till are reported (Fabrizzi et al., 2005; Rieger et al., 2008; Tessier et al., 1990).

In arable fields, N is often artificially applied in increasing amounts with fertilization (Conant et al., 2013) to increase plant productivity. Generally, nutrient uptake can be increased by applying liquid instead of granular N fertilizer (Holloway et al., 2006, 2001). With liquid N application nutrients are more readily availability and their distribution in the soil matrix is favorable compared to their granular counterparts (Holloway et al., 2001; Pittawy et al., 2015). Moreover, a priming effect can occur with fertilization, resulting in an increased microbial N consumption (Blagodatskaya et al., 2007). Nitrogen addition stimulates N mineralization (Luo et al., 2019) but tend to be higher for lower applied N rates (Song et al., 2021). Lower N mineralization at high N rates are explained by a decreased microbial activity as a direct result of soil acidification (Li et al., 2010; Zhang et al., 2008).

As a consequence of these variable pathways and influencing factors, N is continuously transformed in the soil and its form and availability for plants and microorganisms is hence quite volatile. Yet, there is still only little information about the impact of the named parameters on the N availability in clay-rich agricultural used steppe zones under severe climatic conditions, which suffer from N limitation.

1.3. Kazakhstan as study region

According to the FAO, grasslands cover approximately 40 % of the terrestrial area and played a major role in human land expansion (Dixon et al., 2014; White et al., 2001). Fifty to 70 % of especially highly productive grasslands have already been converted to arable fields (Foley et al., 2011; Ramankutty et al., 2008; Suttie et al., 2005). Land conversion is also omnipresent in Kazakhstan, which was key to become one of the biggest exporters of wheat worldwide and hence a global bread basket (FAO, 2019; Feher and Fieldsend, 2019; Swinnen et al., 2017). Soils in this region are strongly adversely affected by an unique history of long term agricultural use with unsustainable land management since they were converted from grassland into cropland during the “Virgin Land Campaign”

(Russian “zelina”) under UdSSR’s head of state Nikita Khrushchev in 1954 (Mizina et al., 1999; Muratova and Terekhov, 2004; Yanai et al., 2005). This resulted in soil degradation and decreased productivity of croplands (as summarized by Kraemer et al., 2015; Takata et al., 2008) which may cause strong limitations in yields and quality of crops. Further limiting in this region are the severe semi-arid climatic conditions with rare summer precipitation (Kraemer et al., 2015; Muratova and Terekhov, 2004; Takata et al., 2008) and short vegetation periods of about 125 days from May to August (Muratova and Terekhov, 2004). But since 1901, for the Western Siberian Plain higher precipitation and an increase in temperature was observed and also projected for the future (Hu et al., 2017; Huang et al., 2014). Consequently, Kazakh agriculture in the semi-arid steppe might even profit from global climate change due to longer vegetation periods (Lioubimtseva and Henebry, 2009). Due to these ecological but also economic changing conditions in North Kazakhstan, an appropriate and adapted soil management is crucial in order to use limited resources more efficiently and to develop a more sustainable agriculture while keeping productivity and yields high, similarly as happened in the United States (Evenson et al., 1979).

Most of Kazakhstan’s agriculture is located in its north (Gramazow and Suleimenov, 2011) where potential fertile soils, especially Chernozems, are widely abundant. These soils are often characterized by high SOM and clay contents (Karbozova-Saljnikov et al., 2004; Saljnikov et al., 2013; Takata et al., 2007a). The current commonly used agricultural practice includes 3 to 5 years crop rotations with wheat as main crop, with on some farms occasionally including a fallow year for sanitary purposes, lentils, barley, or flax. Tillage forms are mostly conservative such as mini-till (5 to 7 cm deep tillage) and no-till, but sometimes still conventional (30 cm ploughing). Nitrogen fertilizer commonly in form of NH_4NO_3, or $(NH_4)_2SO_4$ are mostly applied in granular form simultaneously with seeding into the furrow. Fertilizer rates in the study region range from 0 to 80 kg N ha^{-1}, but in most cases are between 20 and 40 kg N ha^{-1}. However, yearly N, P, K fertilizer consumption in arable lands in Kazakhstan in 2018 was in general low with about 8 kg ha^{-1} (Swinnen et al., 2017; The World Bank Group, 2021). Because of the limited N fertilization, the N mineralization and fate

of N in soils is of special importance for the N supply to crop plants and further studies can help to understand and possibly improve N management in these regions.

1.4. Motivation and hypothesis

Up to now, the N cycle has been studied in numerous publications and the principal mechanisms are known (see section 1.2.). However, there is a lack of knowledge for the N cycle in the vulnerable, very clayey and severe semi-arid Kazakh steppe soils. This thesis investigates various sources, sinks and the fate of N in grassland and intensively agricultural used steppe soils. Hereby, the current agricultural practice was examined and tested, if changes in the current agricultural practices may keep the actual productivity while increasing sustainability.

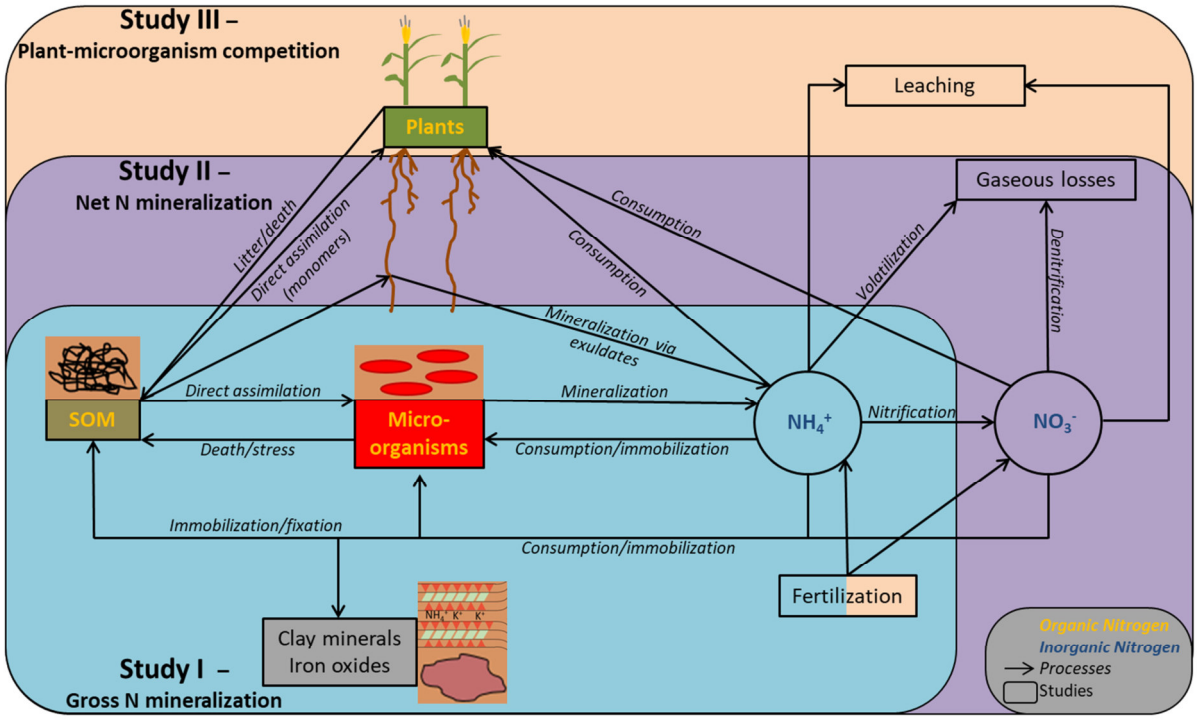

Figure 1: Schematically sketch of the main investigated processes of the N cycle and the structure of this work. Italic words describe processes. Main N-compounds if soluble or retained are given in boxes or circles, with organic N compounds are written in yellow, inorganic N compounds in blue, and not clearly assignable N compounds in black. The different studies of this work are indicated by colored boxes: Light blue for study I, purple for study II, and orange for study III including all investigated N compounds and processes. Soil organic matter is abbreviated as SOM. This figure is based on the new paradigm of N mineralization (Knicker, 2011; Murphy et al., 2003; Schimel and Bennett, 2004a).

This work comprises three individual studies introduced as different chapters (2. to 4.) and lead from small- to large-scale soil processes (Figure 1). Study I (chapter 2; "*Gross mineralization versus biotic and abiotic retention of nitrogen in steppe soils of North Kazakhstan"*) examines *in vitro* the effect of fertilization on the gross N mineralization on living topsoil of a grassland and arable soil via ^{15}N isotope pool dilution (IPD). Moreover, abiotic N fixation on SOM and by clay minerals was determined on sterilized soil. It was hypothesized that (H1.1.) gross N mineralization is higher in grassland soil than in arable soil because of higher SOM quality and quantity, (H1.2.) Nitrogen fertilization results in a higher gross N mineralization because of a higher microbial activity as a result of large amounts of easily available N, (H1.3.) Abiotic exceeds biotic N immobilization because of the short incubation time period.

The second *in vitro* study (chapter 3.; *"Sensitivity of carbon, nitrogen, and phosphorus mineralization in semi-arid steppe soils to temperature and moisture"*) investigates the effect of different climate scenarios on the net C, N, and P mineralization of SOM in grassland and arable soils at different sites and with different land use histories. Carbon, N, and P mineralization were linked to each other and mineralization products as well as the ^{14}C activity of released CO_2-C to SOM fractions. It was hypothesized that (H2.1.) Irrespective of moisture, the temperature response of SOM mineralization is higher for grassland soils than arable soils. (H2.2.) Increasing in soil moisture will further accelerate SOM decomposition beyond the expected temperature response. It was hypothesized that (H2.3.) the observed C and net N nutrient release can be linked to the SOM fraction's initial C:N:P element ratio.

Study III (chapter 4.; *"Competition of plants and microorganisms for added nitrogen in different fertilizer forms in a semi-arid climate during the vegetation period"*) deals with the *in situ* plant-microorganism competition for liquid and granular applied ^{15}N labeled fertilizer to arable soil under two different tillage systems during the vegetation period. Hereby, the effect of tillage and fertilizer form on the competition between microorganisms and plants for N were evaluated. It was hypothesized that (H3.1.) the use of liquid fertilizer can increase plant growth and N uptake in these semi-arid regions, regardless of the tillage form used, thus

increasing plant competition for N in the long run. Further, we assume that (H3.2.) the plant–microorganism competition occurring in semi-arid, clay-rich soils is more severe than that reported for more humid regions.

In the Synthesis (chapter 5.) the most important findings are summarized, put in context and are critically discussed. Chapter 5 is divided in two subsections. Section 5.1. focuses on the current N availability and processes, while impacts of changes in soil management on the N supply are discussed in section 5.2. The first part of section 5.1. highlights the N mineralization, and biotic and abiotic retention of N (5.1.1.). To the N mineralization and retention, mainly study I and II contribute whereas retention processes are covered by studies I, II, and III. The experiment on the gross N mineralization (study I) contributes to the "pure" processes involved in the N cycle and its availability. The experiment on the net N mineralization (study II) includes long-term side effects in the soil and gives changes in N pool size over the vegetation period, excluding plants and leaching. *In situ* study III includes plants and leaching processes in the N cycle and investigates the competition for N between plants and microorganisms over the vegetation period. Section 5.1.2. deals with the effects of land use and fertilization on the N availability. The effect of land use will be discussed with findings of study I and II; effects of fertilization are covered by studies I and III. In section 5.2.1., a slightly changed agricultural practice towards a more sustainable agriculture was tested to determine if the efficiency and N availability of the current agricultural practice in North Kazakhstan could be increased (study III). Section 5.2.2. investigates whether a change in temperature and soil moisture effects net N mineralization (study II).

Finally, in the Conclusion (chapter 6.) the study is critical evaluated (6.1.) and an outlook is given (6.2.).

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2. Study I

Gross mineralization *versus* biotic and abiotic retention of nitrogen in steppe soils of North Kazakhstan

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Abstract

Nitrogen (N) mineralization is a prerequisite for plant available N in soils and contributes to the soil fertility. Because of limited mineralization potential in soils, abiotic or biotic retention, N is often limited in terrestrial ecosystems. This study investigates the gross N mineralization as well as biotic and abiotic N retention processes by ^{15}N isotope pool dilution technique (IPD) over two days in adjacent arable and grassland soils with (60 kg N ha^{-1}) and without (0.25 kg N ha^{-1}) N fertilization, respectively, during simulated spring time when arable fields are normally fertilized. The aim of this study was to detect N transformation processes in fertilized clay-rich soils. Overall net N mineralization was low, ranging from -1 to 7 mg kg^{-1} d^{-1}, indicating similar high gross mineralization and consumption rates. Land use had no effect on the N mineralization, owing to low organic N contents and similar gross N mineralization and NH_4^+ consumption rates. Nitrogen fertilization did not stimulate microorganisms. A decreased quality in soil conditions with decreasing pH after fertilization or carbon limitation caused by abundant easily available N may have caused no effect of N fertilization on N mineralization. Therefore, biotic N immobilization was small but we observed large abiotic N retention processes (29 to 91 % of added fertilizer N for the fertilized and non-fertilized treatment) in these clay-rich soils. These results show the importance of abiotic N retention in natural and N fertilized clay-rich soils.

Keywords: ammonium sulfate, arable land, clay fixation, grassland, isotope pool dilution, land use, microbial biomass nitrogen, nitrogen fertilization, ^{15}N

Glossary:

Nitrogen retention: Comprises biotic and abiotic nitrogen retention processes like uptake/consumption by microorganisms, clay fixation, or sorption to soil organic matter, and/or reactive pedogenic minerals.

Nitrogen immobilization: Is the microbial consumption/uptake of nitrogen forms into their biomass.

Clay fixation: Is the incorporation of ammonium into the interlattice space of expandable clay minerals.

1. Introduction

Available nitrogen (N) forms are often considered as a limiting nutrients in terrestrial systems (Galloway et al., 2003; Vitousek and Howarth, 1991). In arable fields the knowledge about the natural N supply of the soil by soil organic matter (SOM) mineralization is important to estimate the available N contents and to calculate appropriate fertilization rates (Denk et al., 2017; Schimel and Bennett, 2004b).

Though, it has to be distinguished between net and gross mineralization. The net N mineralization gives information about the change in N pool size over a longer time interval (Murphy et al., 2003), hence the soil's ability to supply N to e.g. plants (Verchot et al., 2001). But it only gives little information about the individual processes and associated fluxes in soil N turnover (IAEA, 2001) as many co-depending processes (e.g. microbial immobilization, plant uptake, sorption to pedogenic minerals and SOM, clay fixation, nitrification, leaching with the soil solution, or volatilization) are involved (Stark and Schimel, 2001). By measuring the gross N mineralization in contrast, controlling parameters and processes of SOM decomposition can be determined with small NH_4^+ removal side effects (IAEA, 2001; Murphy et al., 2003). Hence, measuring the gross N mineralization allows to estimate mineralization rate and contents (Schimel and Bennett, 2004b). The gross N mineralization can be measured using the ^{15}N isotope pool dilution technique (IPD) (Braun et al., 2018; Davidson et al., 1991; Kirkham and Bartholomew, 1995, 1954). To determine the gross N mineralization, the product pool NH_4^+ is labelled with $^{15}NH_4^+$ (Braun et al., 2018), and by mineralization of unlabeled organic N (ON) compounds the $^{15}NH_4^+$ pool is getting diluted (IAEA, 2001; Murphy et al., 2003). The gross N mineralization is calculated from the change in NH_4^+ pool size along with the change of ^{15}N in this pool, which is both measured over time (Braun et al., 2018; IAEA, 2001; Murphy et al., 2003).

During the mineralization, mineralized NH_4^+ can be retained in the soil by various processes. For example, when arable soils are fertilized a priming effect with an increased microbial N consumption should be visible (Blagodatskaya et al., 2007). Microbial N consumption may account for over 60 % of the applied N after its addition (Grace et al., 1993). Another crucial point in the N mineralization is the abiotic N immobilization, especially in soils rich in SOM and clay (Nieder et al., 2011; Osborne, 1976). While binding of NH_4^+ on sorption sites of SOM (Nommik, 1965) is relatively small with less than 3 % of total abiotic N retention (Braun et al., 2018), up to 59 % of the applied NH_4^+ may be fixed into the interlattice of expandable 2:1 clay minerals (Kowalenko, 1978; Nieder et al., 2011). All these biotic and abiotic N retention processes can thus strongly impact the N availability of mineral N and fertilizer N in soils. The respective N retention was reported to vary greatly (35 to 95%) depending on the soil, as summarized by (Bengtsson et al., 2003).

Soils from north Kazakhstan are clay-rich (Karbozova-Saljnikov et al., 2004; Saljnikov et al., 2013; Takata et al., 2007a) and were often scarcely fertilized with N (Yanai et al., 2005; personal communications with farmers). The assessment of gross N mineralization in these agricultural soils is of special interest, firstly because of the decadal N mining in these largely unfertilized soils and, secondly due to a potential abiotic N immobilization caused by high SOM and particularly clay contents.

This incubation study aims at quantifying the gross N mineralization during spring time in an adjacent Kazakh semi-arid arable and grassland soil by using the IPD method. Hereby, the fertilization effect of $(NH_4)_2SO_4$ on the gross N mineralization was tested and biotic and abiotic retention of N were determined. We hypothesized the following: (i) Gross N mineralization is higher in grassland soil than in arable soil because of higher SOM quality and quantity. (ii) Nitrogen fertilization results in a higher gross N mineralization because of a higher microbial activity as a result of large amounts of easily available N. (iii) Abiotic exceeds biotic N immobilization because of the short incubation time period.

2. Material and Methods

2.1. Soil sampling and soil characterization

In 2019 0-10 cm soils were collected from a grassland soil (N 53°31.664, E 64°47.582) and an adjacent arable soil (N 53°31.603, E 64°47.629) near Kostanay, North Kazakhstan. The native vegetation is characterized as typical steppe. Mean annual precipitation amounts to 300 to 350 mm, and the mean annual temperature is 3.1 °C; in July during the vegetation phase of 20.9 °C (data from the University of Kostanay; (Fleck, 2020)). Soils are classified as Chernozems (IUSS Working Group WRB 2015, 2015). For our studies, we have chosen a grassland soil with *Stipa capillata* as dominating plant species which was never under agricultural use and adjacent soil that has been under arable land use since 1980. Currently it is under a wheat-wheat-flax-barley crop rotation with flax growing during the sampling campaign. It is under minimum tillage (tillage 5-6 cm deep) and has never been fertilized.

From each of the two land uses, we sampled the top 10 cm of the soil from four pits located at random on the fields and prepared a mixed sample. The soil was dried and <2 mm sieved. Texture was determined after pipette analysis (Köhn, 1928). Both soils were sand-rich (533 and 546 g kg^{-1} for grassland and arable soil), but contained also a large share of clay (276 and 282 g kg^{-1}; Table 1). The bulk density determined with cylindrical cores and the pH in water (1:4; *w:v*) are similar for both fields. Total carbon (TC) and total N (TN) were determined from milled samples using an elemental analyzer (vario ISOTOPE, Elementar Analysensysteme GmbH, Langenselbold, Germany). Total C and TN were significantly higher in the grassland soil (26 and 2.2 g kg^{-1}, respectively) than in the arable soil (21 and 1.8 g kg^{-1}, respectively).

Table 1: Basic soil properties of the grassland and the arable soil in 0-10 cm. For the pH, electrical conductivity, total C (TC), total N (TN), and TC:TN the mean of n = 3 samples with the standard deviation in brackets is given. Bulk density and texture data is taken from a Master of Science thesis (Fleck, 2020). Superscripted letters show statistic significant differences between both land uses as determined by t-test with Bonferroni correction.

Soil parameter	Unit	Grassland soil	Arable soil
pH (water)	-	6.6 (0.1) [a]	6.7 (0.1) [a]
Bulk density	g cm^{-3}	1.5	1.5
Electrical conductivity	µS cm^{-1}	45.7 (2.3) [a]	45.1 (3.5) [a]
Clay	g kg^{-1}	275.5	282.5
Silt	g kg^{-1}	191.8	171.7
Sand	g kg^{-1}	532.7	545.8
TC	g kg^{-1}	26.3 (1.3) [a]	20.5 (0.8) [b]
TN	g kg^{-1}	2.2 (0.1) [a]	1.8 (0.1) [b]
TC:TN	-	11.8 (0.2) [a]	11.5 (0.2) [b]

2.2. Experimental design

This experiment aims to quantify the gross N mineralization in spring time in the topsoil (0-10 cm) depending on fertilization and land use. Moreover, we distinguished between biotic and abiotic processes by using living and autoclaved soil samples (Figure 1). Isotope pool dilution was performed in two fertilization treatments for both land uses. One treatment received only 0.25 kg N ha^{-1} as $(^{15}NH_4)_2SO_4$ (9.5 at% ^{15}N excess) to simulate the unfertilized conditions. To determine fertilization effects, the other treatment received 60 kg N ha^{-1} $(^{15}NH_4)_2SO_4$. To determine biotic processes, all living soil samples were pre-incubated for 2 weeks. During the experiment, four consecutive measurements each 24 h of the concentration and ^{15}N abundance of NH_4^+, NO_3^-, and TN were taken 24 h before (-24 h) and 0.5, 24, and 48 h after tracer application, with 0 h being the time point of fertilizer application. On the first and fourth sampling time (-24 and 48 h) additionally microbial biomass carbon (MBC) and nitrogen (MBN), and abiotic NH_4^+ retention by clay minerals and/or humic substances was measured (Figure 1). Abiotic NH_4^+ retention was determined on autoclaved soils (Braun et al., 2018; Davidson et al., 1991). Each variant was performed in three replicates. The experimental design follows the basic suggestions proposed by IAEA (2001) and Murphy et al. (2003).

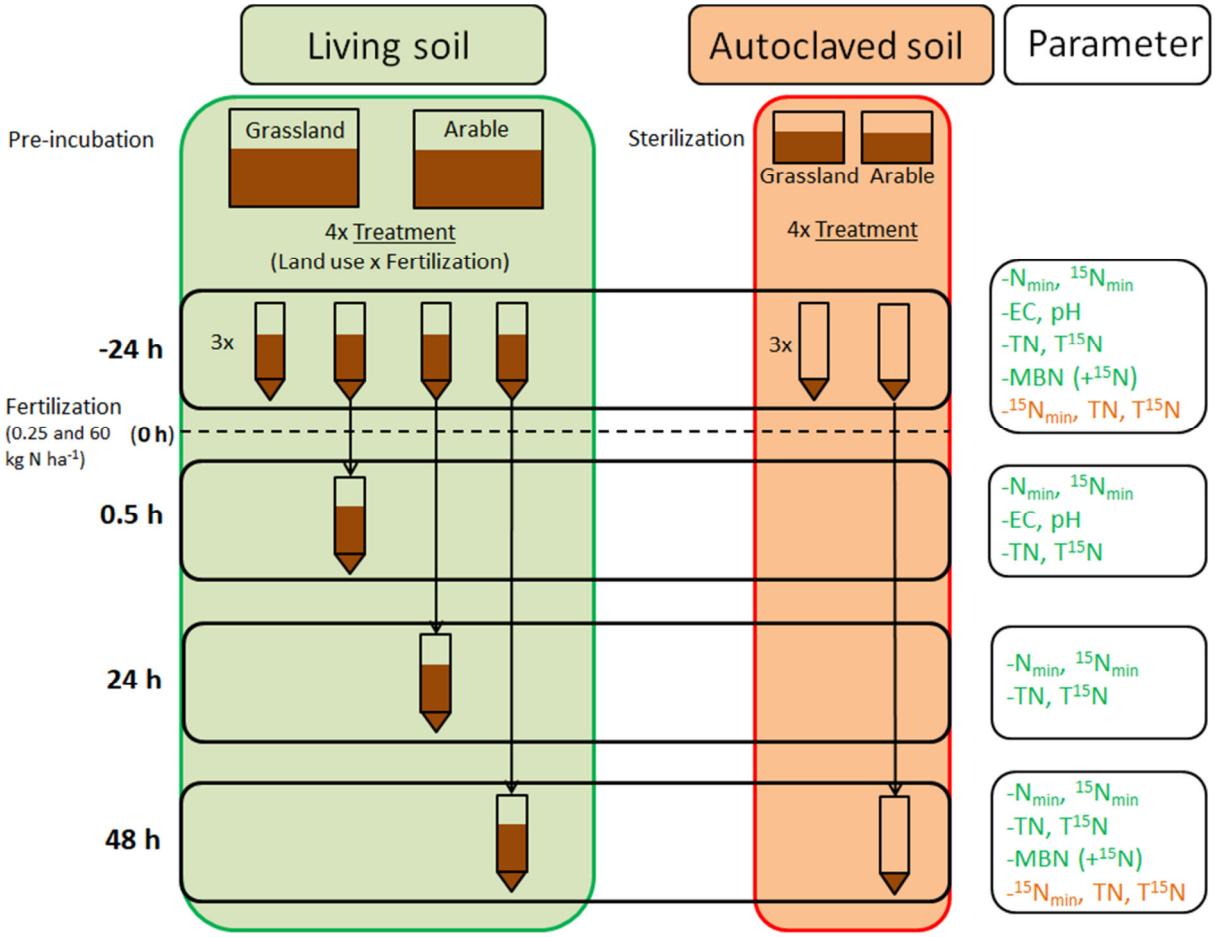

Figure 1: Schematic experimental design of this study for one treatment (land use x fertilization). Two variants were investigated: living soil (green box) to determine biotic processes and autoclaved soils (orange box) to determine abiotic retention of N. Each sampling time is given on the left site. Soil samples were pre-incubated (living) or autoclaved (sterilized) before the experiment. Then, soil samples were divided according to the treatments (fertilization or no fertilization) in triplicates for each sampling time point. ^{15}N labeled $(^{15}NH_4)_2SO_4$) (9.5 at% ^{15}N excess) was applied at 0 h. The shown flasks indicate the time until destructive sampling. Investigated parameters at each sampling are denoted on the right side in green letters for living soil and orange letters for autoclaved soils. Abbreviations are: EC: electrical conductivity, TN: total N, MBN: microbial biomass N.

2.3. Sample preparation

Soil samples were adjusted to 60 % water holding capacity in order to simulate wet conditions in spring time when usually fields are seeded and fertilized in Kazakhstan. For the gross mineralization experiment, soil samples were pre-incubated at 15 °C for two weeks in order to reactivate microorganisms. The chosen temperature equals the mean daily temperature in spring at seeding time in Kazakhstan (June). During pre-incubation the water content of the samples was readjusted if needed. Two days before $(^{15}NH_4)_2SO_4$ applications,

water contents were not adjusted anymore, so that the water content after application of liquid fertilizer could have been readjusted to approximately 60 % water holding capacity.

To determine the abiotic NH_4^+ retention, a part of the soil was not pre-incubated but autoclaved twice for 20 min at 121 °C (2 days before the experiment). After the first autoclavation, soil samples were incubated at 15 °C for one day to allow remaining microorganisms and fungi to germinate. Afterwards, samples were autoclaved a second time to completely sterilize the soil (Braun et al., 2018; Davidson et al., 1991). Samples were then allowed to cool to room temperature before the start of the experiment.

2.4. Isotope pool dilution experiment

The effect of fertilization on the gross N mineralization was tested on living and autoclaved soils. For the non-fertilized system a maximum of 20 % of the initial NH_4^+ pool was applied as 9.5 at% $(^{15}NH_4)_2SO_4$-solution in order to avoid stimulation of microorganisms but also ensuring sufficient ^{15}N-enrichment in the NH_4^+ pool (Braun et al., 2018; Davidson et al., 1991). Tracer solutions were prepared by mixing labeled $(NH_4)_2SO_4$ (99 at% ^{15}N) (Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA) with unlabeled $(NH_4)_2SO_4$ (Sigma-Aldrich, St. Louis, MO, USA). For adjusting the fertilization, 0.1 mL of 9.5 at% $(NH_4)_2SO_4$ 0.001 or rather 0.29 mmol $(NH_4)_2SO_4$ L^{-1} were applied per g fresh soil for the treatments without and with fertilization, respectively. Fertilizer applied by spreading the soil in a plastic bag, spraying approximately one quarter of the totally needed fertilizer solution on the whole soil and subsequently mixing the soil to achieve a homogeneous ^{15}N distribution within the soil. This procedure was repeated three times. This was done to achieve highest fertilizer distribution within the soil. 45 g (-24 h, and 48 h) and 14 g (0.5 h and 24 h) of fresh living and 6 g autoclaved soils were then transferred into 50 mL centrifuge tubes. The headspace was harmonized by cutting the flasks. Flasks containing living soil were closed with perforated parafilm to avoid evaporation but allow for respiration. Flasks for abiotic retention were closed with aluminum foil. The samples were then incubated at 15 °C in the darkness for the given incubation times. The incubation was stopped by destructive

sampling. For each parameter of interest (water content, TN, NH_4^+ and NO_3^-, MBC and MBN, pH and EC) an aliquot was taken.

2.5. Determination of ^{15}N-pools

Soil NH_4-N and NO_3-N contents and their ^{15}N abundance were determined by extraction of 4 g of soil at a ratio of 1:4 (*w:v*) in 12.5 mM $CaCl_2$ (VDLUFA, 2002). Extracts were shaken horizontally for 1.5 h at 120 rpm. Extracts were filtered through 0.45 µm ash-less cellulose acetate syringe filters (Berrytech, Grünwald, Germany). Mineral N extracts and the fertilizer solutions were frozen until the measurement via the SPINMas technique (sample preparation for inorganic nitrogen by quadrupole mass spectrometry; (Stange et al., 2007)) for their NH_4-N and NO_3-N contents as well as their ^{15}N abundance in at%. Hereby, NO_3^- is reduced at 85 °C under acidic conditions (37 % HCl) by a 0.1 M vanadium(III)chloride solution to NO which is subsequently measured. In a different measurement step, NH_4^+ is oxidized at 70 °C in an alkaline medium (0.1 M NaOH) with 0.156 M NaOBr to N_2. Both product gasses NO and N_2 are subsequently measured to determine the NO_3-N and NH_4-N content, respectively, as well as their ^{15}N abundance.

Total N and ^{15}N as $\delta^{15}N$ ratios were measured on 40 °C oven-dried, <2mm sieved and milled soil samples with an EA-IRMS (vario ISOTOPE coupled with an isoprime precisION, Elementar Analysensysteme GmbH, Langenselbold, Germany). Microbial biomass carbon and MBN were extracted by chloroform fumigation extraction (CFE) according to Brookes et al. (1985b) after a modified method by Müller and Fragstein und Niemsdorff (2006). This method uses a pre-extraction in order to remove excessive dissolved N and was chosen to gain more precise data about the possibly small ^{15}N contents in the microbial biomass. 15 g of fresh soil were weighted in PE-tubes (non-fumigated samples) and glass-tubes (fumigated samples). 60 mL 0.05 M K_2SO_4 were given to the soil and the samples were subsequently shaken for 30 min on an overhead-shaker. Afterwards samples were centrifuged at 4000 g for 5 min. Extracts were filtered through glasfiber filters (Whatman, GF6, Maidstone, UK) using a Büchner funnel. Filters were pre-rinsed with K_2SO_4.

Non-fumigated soil samples were then again extracted and treated in the same procedure using 0.5 M K_2SO_4. To the fumigated samples 300 µL ethanol-free chloroform were added directly to the sample and subsequently mixed with a glass rod. Samples were closed and incubated for 62 h. Afterwards samples were treated like the non-fumigated samples. All extracts were stored at -15 °C until the measurement.

Contents of MBN were determined after alkaline persulfate digestion modified after (Sollins, 1999). Persulfate digestion in solutions allows for the determination of TN in form of NO_3^-, as all N-compounds are oxidized during this reaction (Hood-Nowotny et al., 2010). All 0.5 M K_2SO_4 samples were measured for NO_3^-. Afterwards, 3 mL of each sample were filled into 50 mL centrifuge flasks and mixed with 1 mL 0.148 M potassium perchlorate solution and 0.1 mL 3 M NaOH solution in a ratio of 3:1:0.1 (*v:v:v*). Samples were then closed and reacted in the heating block for 4 h at 80 °C before their measurement for NO_3^- contents and ^{15}N abundance on the SPINMas. Microbial biomass carbon and MBN were additionally measured on a LiquiTOC (vario TOC cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) for their total organic carbon (TOC) and TN content.

To determine the abiotic N retention, the content and ^{15}N enrichment of mineral N forms and TN was determined in autoclaved soils in the same way as for living soils.

In any case, blanks without soil and tracer application were included in triplicates for each analysis to correct for ^{15}N backgrounds in flaks and solutions.

2.6. Data and statistical analysis

All data analyses were carried out in Excel (Microsoft) and R 3.6.3. (R Core Team, 2020). $\delta^{15}N$ ratios were transformed into at% as:

$$at\% = \frac{100 * AR * \frac{\delta^{15}N}{1000+1}}{1 + (AR * \frac{\delta^{15}N}{1000+1})} \quad (1)$$

where at% is the atomic percentage of ^{15}N, AR is the absolute ratio of mole fractions of ^{15}N in air of 0.0036764 (Coplen, 2002) and $\delta^{15}N$ is the ratio of ^{15}N to ^{14}N in a sample to that of air as a standard.

Afterwards, ^{15}N abundance was corrected for the background abundance and expressed as at% excess. The ^{15}N recovery was calculated based on masses as the total recovered ^{15}N pool divided by the amount of added ^{15}N.

For living soils, MBC and MBN were calculated as the difference of C and N contents of fumigated samples and the contents of non-fumigated samples, respectively.

Mineralization and consumption rates (Braun et al., 2018; Kaiser et al., 2011) were calculated as:

$$\text{Gross mineralization} = \frac{M_t - M_0}{t} * \frac{\ln(\frac{APE_0}{APE_t})}{\ln(\frac{M_t}{M_0})} \quad (2)$$

$$\text{Gross consumption} = gm - \frac{M_t - M_0}{t} \quad (3)$$

$$\text{Net mineralization} = \frac{M_t - M_0}{t} \quad (4)$$

where M_t the pool size of NH_4-N at time t, M_0 the initial NH_4-N and APE: atom percent excess of ^{15}N in sample minus an unlabeled control.

For sterilized soils, total abiotic NH_4^+ retention was determined by the difference of the $^{15}NH_4$-N content at 0.5 h and 48 h.

To test for significant differences between land use and fertilization we used the aov-function in R to perform a two-way analysis of variance (ANOVA). Interactions were allowed. Groups were compared using the Tukey's honest significant post-hoc test (HSD). Parameters were tested if they meet the ANOVA assumptions. If this was not the case, the data was log-transformed or the non-parametric Kruskal-Wallis test was performed (supplementary Table S5). Significant differences between treatments before fertilization or between sampling time points were tested by Student t-test with Bonferroni correction (supplementary Table S1 and S2). Statistical differences are reported at a significance level of $p<0.05$. Statistic significant different groups are presented as small letters in tables and figures.

3. Results

3.1. ^{15}N recovery

Unfortunately, we were not able to determine total ^{15}N recovery in MBN as the measurement of TN and the ^{15}N abundance on the SPINMas by alkaline persulfate digestion was not successful (supplementary Figure S1).

0.5 h after fertilizer addition, high recoveries of ^{15}N in mineral N forms were observed for the treatments receiving N fertilization (75 to 98 %), whereas ^{15}N recoveries without N fertilization were only about 14 % (Table 2). Here, ^{15}N recovery was significantly affected by fertilization with higher recoveries under N fertilization than no fertilization. After 48 h, 60 to 108 % (N fertilization) of applied $^{15}NH_4$-N, and 12 to 16 % (no fertilization) were recovered as NH_4-N and NO_3-N (Table 2). ^{15}N recovery after 48 h of incubation was significantly higher under N fertilization and grassland soils compared to the no fertilization and arable soil.

For all treatments of living soils the recovery of ^{15}N in NH_4-N tended to slightly decreased with time, indicating slow losses of applied fertilizer N (Figure 2). At all sampling time points, ^{15}N abundance in NH_4-N was significantly higher for treatments receiving N fertilization. The ^{15}N abundance in NH_4-N after 48 h was strongly negatively correlated to the difference in MBC (MBC content after 48 h subtracted by the MBN content at -24 h) ($r=-0.65$, $p=0.03$), MBN ($r=-0.68$, $p=0.03$) and the difference in MBN ($r=-0.72$, $p=0.00$) (supplementary Table S4). ^{15}N abundance in NO_3^- tended to increase with time (Figure 2), showing small nitrification of applied $^{15}NH_4$-N to $^{15}NO_3$-N especially in the highly fertilized soils ($p<0.05$). After 0.5 and 24 h, higher ^{15}N abundance in NO_3-N for arable soils was observed. At all sampling time points, N fertilization showed significantly higher ^{15}N abundance in NO_3-N. 24 h after fertilization ^{15}N abundance in NO_3-N moderately negatively correlated with NO_3-N contents ($r=-0.62$, $p=0.03$), whereas a positive correlation was found for NH_4-N contents ($r=0.63$, $p=0.03$) and ^{15}N abundance in NH_4^+ ($r=0.91$, $p=0.00$) (supplementary Table S3). After 48 h, $^{15}NO_3$-N contents were strongly positively correlated the NH_4-N content ($r=0.90$, $p=0.00$).

Table 2: Recovery of ^{15}N (%) in mineral N forms from applied ^{15}N in living soils 0.5 and 48 h after fertilizer application. Given are the means of n = 3 samples with the standard deviation in brackets. Superscripted letters indicate statistical significant differences between treatments (land use x fertilization; ANOVA) at a specific sampling time. Note that the recovery after 24 h was not calculated, as the measurement of NO_3-N contents was compromised.

Land Use	Fertilization (kg N ha^{-1})	0.5 h	48 h
Grassland	0.25	14 (2) ^{a}	16 (5) ^{a}
Grassland	60	78 (4) ^{b}	88 (6) ^{b}
Arable	0.25	14 (3) ^{a}	12 (7) ^{a}
Arable	60	82 (28) ^{b}	60 (18) ^{b}

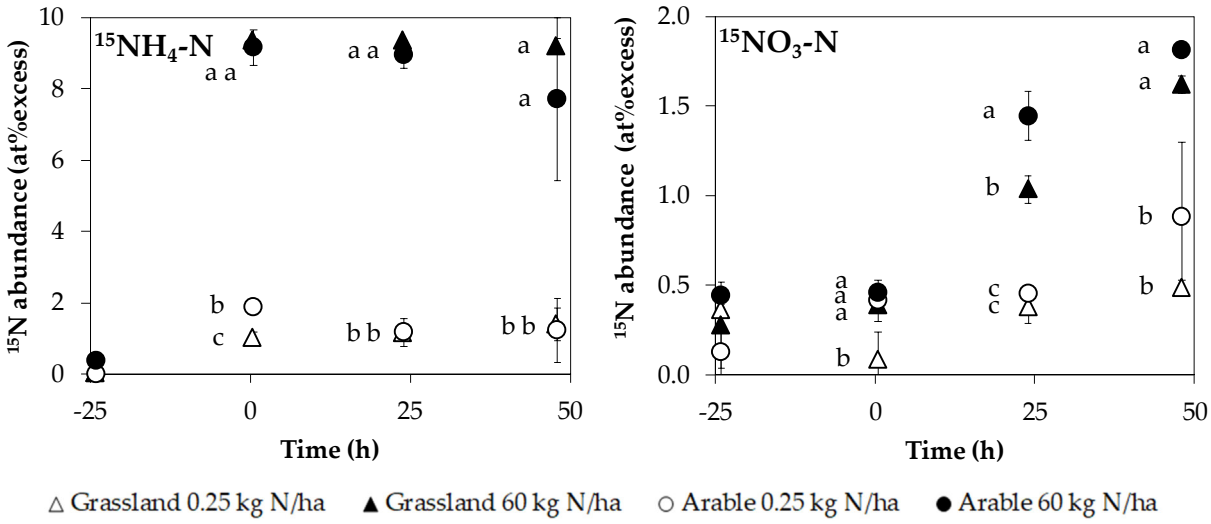

Figure 2: Abundance of ^{15}N-NH_4^+ and ^{15}N-NO_3^- over the incubation time period. Fertilizer was applied at 0 h. Symbols give the mean of n = 3 samples and error bars represent the standard deviation. Superscripted letters indicate statistical significant differences between treatments (land use x fertilization; ANOVA) at a specific sampling time.

In sterile soils, about 8 and 71 % of applied NH_4-^{15}N were recovered in mineral N forms without and with N fertilization, respectively, 48 h after fertilizer addition (Table 3). ^{15}N recovery in sterile soils was significantly higher under high than no fertilization but did not differ between land uses. We assumed that NH_3 volatilization is insignificant for these soils at pH 5.9 to 6.6. Hence, about 91 and 29 % of the added fertilizer was abiotically immobilized in the soil at no and at fertilization, respectively (Table 3). Abiotic retention of applied N was significantly different for both fertilization treatments, but did not differ between land use types.

Table 3: Estimate of abiotic $^{15}NH_4^+$ retention of as determined in sterilized soil samples after 48 h of incubation. Given is the mean of n = 3 samples and the standard deviation in brackets. Superscripted letters indicate statistical significant differences between treatments (land use x fertilization; ANOVA) at a specific sampling time.

Land Use	Fertilisation (kg N ha^{-1})	Recovery ^{15}N (%)	Retention (%)	Retention (µmol g^{-1})
Grassland	0.25	9 (1) [a]	91 (1) [a]	0.01 (0.00) [a]
Grassland	60	71 (2) [b]	29 (2) [b]	0.24 (0.01) [b]
Arable	0.25	8 (0) [a]	92 (0) [a]	0.02 (0.00) [a]
Arable	60	71 (1) [b]	29 (1) [b]	0.24 (0.01) [b]

3.2. Nitrogen fractions in living soils

In contrast to no fertilization, high fertilization had a significant effect on basic soil properties as the EC significantly increased from 44.4 ± 2.5 (mean ± SD) to 893 ± 21.2 µS cm^{-1} and the pH decreased from 6.6 ± 0.0 to 5.9 ± 0.0. Ammonium-N and NO_3-N contents before fertilization were similar for all soils ($p<0.05$). After fertilization, NH_4-N contents at no fertilization were not affected, whereas for N fertilization NH_4-N significantly increased (Figure 3). In general NH_4-N contents did not vary between 0.5 to 24 and 24 to 48 h after fertilization. Ammonium-N contents 48 h after fertilization correlated negatively with the difference in MBC ($r=-0.61$, $p=0.05$). For both fertilization treatments, NO_3-N contents increased significantly directly after fertilization for all treatments. In general, NO_3-N contents tended to slightly increase over the following 48 h (Figure 3). After 48 h NO_3-N negatively correlated with MBC ($r=-0.93$, $p=0.00$) and the difference in MBC ($r=-0.77$, $r=0.03$) and the difference in MBN ($r=-0.73$, $p=0.04$).

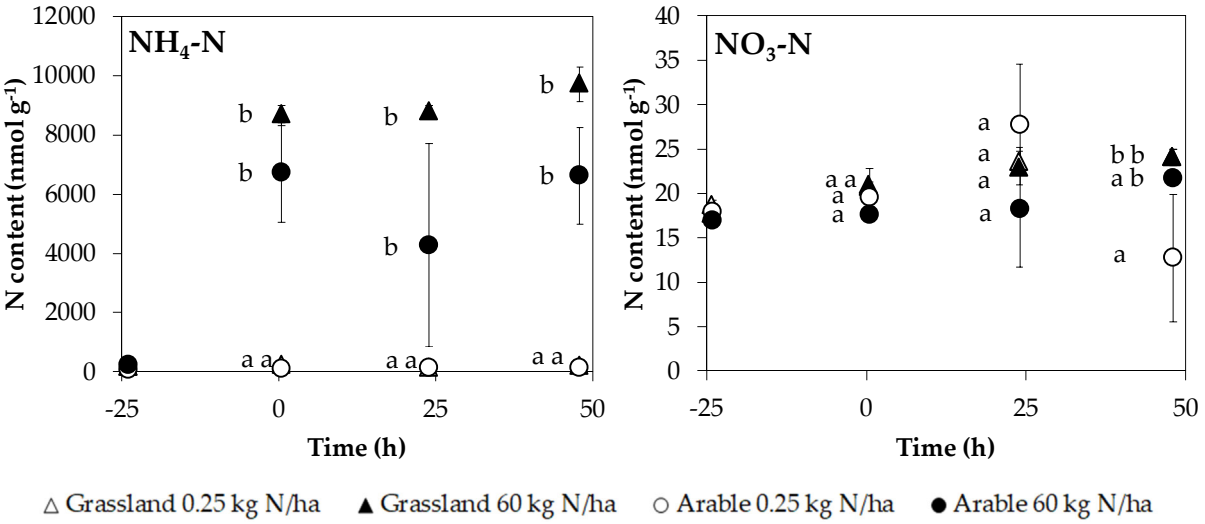

Figure 3: Contents of NH_4-N and NO_3-N, over the incubation period. Given is the mean of n = 3 samples. Error bars represent the standard deviation. Superscripted letters indicate statistical significant differences between treatments (land use x fertilization; ANOVA) at a specific sampling time. (Note that to meet ANOVA assumptions, NH_4-N contents at 24 and 48 h had to be log transformed.)

Before fertilization, MBC was significantly higher under grassland compared to arable soils (Figure 4). In the fertilized soils, MBC tended to remain constant. For no fertilization MBC tended to slightly increase during the 72 h between sampling of microbial biomass (both not significantly; Figure 4). Fertilization did not affect MBC contents, but MBC was significantly higher under grassland than arable soils after 48 h. Microbial biomass N before fertilization was significantly higher under grassland than arable soil. 48 h after fertilization, microbial immobilized N tended to increase for no fertilization but tended to decrease for N fertilization (Figure 4). The difference in MBN between -24 and 48 h positively correlated to the pH (fertilization effect; r=0.66, p=0.04). Before fertilization, MBC:MBN tended to be smaller in grassland than arable soils. 48 h after fertilization MBC:MBN tended to increase in all treatments (Figure 4).

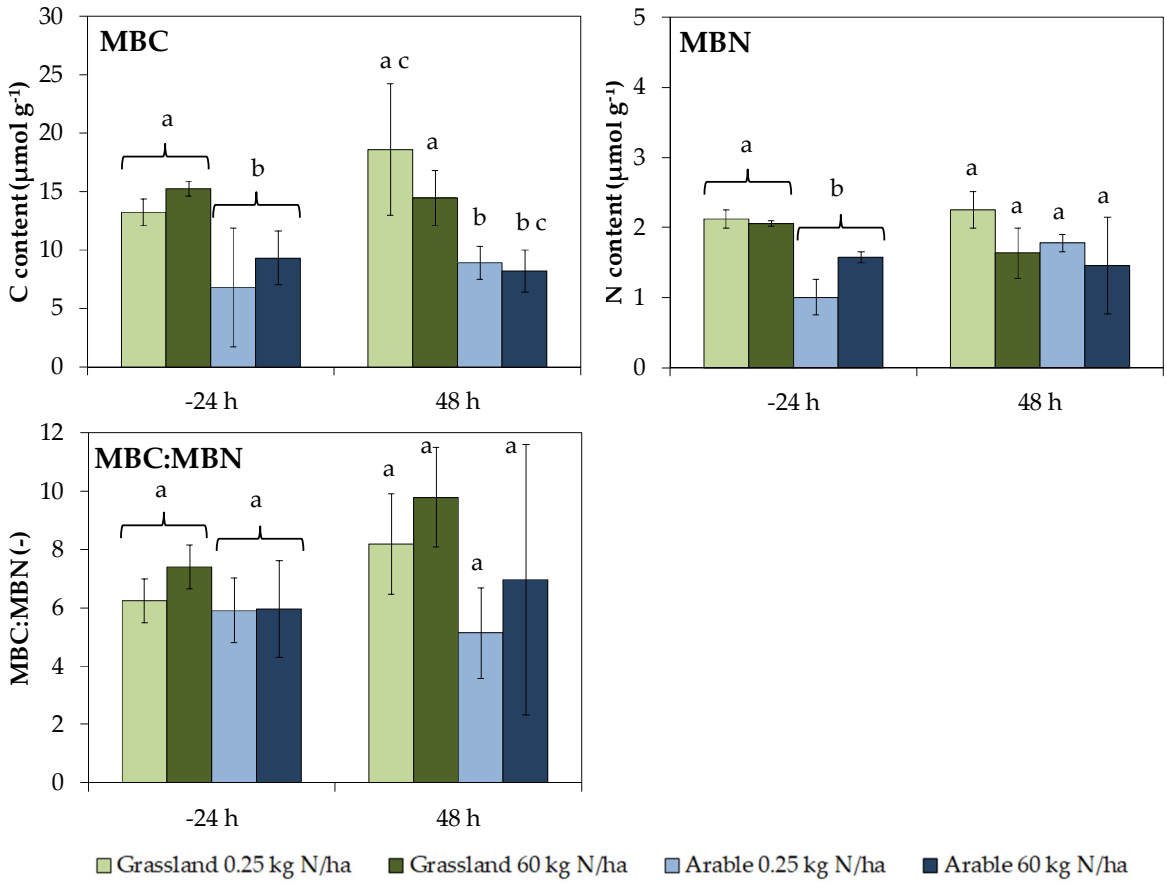

Figure 4: Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass C:N ratios 24 h before and 48 h after fertilizer application. Bars represent the mean of n = 3 and error bars represent the standard deviation. Superscripted letters at -24 h represent statistical significant differences between land uses (t-test). Superscripted letters at 48 h indicate statistical significant differences between treatments (land use x fertilization; ANOVA).

Net mineralization is the difference of gross mineralization and NH_4^+ consumption. Positive net mineralization rates indicate higher NH_4^+ production than consumption (Table 4). After 24 h, gross mineralization and consumption rates were small and did not differ between land use and fertilization. Low consumption rates indicate small nitrification. For all treatments gross mineralization rates are similar to the NH_4^+ consumption rates, resulting in small net mineralization rates. Highest net mineralization rates were observed after 48 h, ranging between -1 to 7 mg kg^{-1} d^{-1} (equivalent to -2 and 22 nmol g^{-1} h^{-1}). Here, gross mineralization (0 to 11 mg kg^{-1} d^{-1} equivalent to -1 to 33 nmol g^{-1} h^{-1}) and consumption rates (-6 to 12 mg kg^{-1} d^{-1} equivalent to -19 to 36 nmol g^{-1} h^{-1}) were not significantly affected by neither fertilization nor land use. Net and gross mineralization and gross consumption rates were not correlated to any tested soil parameter.

Table 4: Net mineralization, gross mineralization and gross NH_4^+ consumption rates in mg kg^{-1} d^{-1} 24 and 48 h after fertilizer application. Given is the mean of n = 3 samples and the standard deviation in brackets. No superscripted letters are given to indicate significant differences as the non-parametric Kruskal-Wallis test was performed (no interactions). Results did not show differences between neither fertilization, nor between land uses.

Time (h)	Land Use	Fertilization (kg N ha^{-1})	Net mineralization rate	Gross mineralization rate	Gross consumption rate
24	Grassland	0.25	-1.4 (0.6)	-0.2 (0.1)	1.2 (0.5)
	Grassland	60	1.8 (5.5)	0.1 (0.5)	-1.7 (5.1)
	Arable	0.25	0.3 (1.4)	1.1 (1.4)	0.8 (0.4)
	Arable	60	-1.2 (0.6)	-1.1 (2.0)	0.1 (1.4)
48	Grassland	0.25	-0.3 (0.2)	-0.4 (0.4)	-0.1 (0.3)
	Grassland	60	7.4 (6.2)	1.2 (1.7)	-6.2 (5.3)
	Arable	0.25	0.3 (0.2)	0.7 (1.1)	0.5 (0.9)
	Arable	60	-0.7 (18.2)	11.4 (20.2)	12.1 (28.8)

4. Discussion

4.1. Effect of land use on nitrogen mineralization

Different land use may result in different activity and diversity of the microbial community (Moreno et al., 2019) and thus having an impact on N mineralization (Sun et al., 2013). We hypothesized that because of the better SOM quality and quantity under grassland soils (Cambardella and Elliott, 1992), N mineralization under grassland soil would exceed N mineralization under arable soil.

During this short term incubation experiment net and gross N mineralization rates did not vary between grassland and arable soils (Table 4), hence our hypothesis must be rejected. In contrast to our study, under wetter conditions higher gross N mineralization rates were observed in grassland soils than in arable soils and attributed to different soil properties like the (water soluble) soil organic carbon content (Lang et al., 2016). In fact, SOM depletion in arable soils may reduce N mineralization (Booth et al., 2005a). Low ON contents of 2.2 ± 0.1 g kg^{-1} and 1.8 ± 0.1 g kg^{-1} for grassland and arable soil, respectively, (data not shown) of the tested soils in our study may limit overall N mineralization. However, literature reports are not consistent as net N mineralization may also be higher in arable than in grassland soils due to a slower N turnover in grassland soils (Ihori et al., 1995). Also Schimel (1986) stated that land use did not affect gross N mineralization. In our study NH_4-N and NO_3-N contents

did not vary over the incubation time period (Figure 3), indicating small net N mineralization. Observed net N mineralization rates (Table 4; -0.4 to 7.4 mg kg^{-1} d^{-1}) are comparable to -0.1 to 1.0 and 1.2 to 3.3 mg kg^{-1} d^{-1} reported for temperate grassland soils in Inner Mongolia (Liu et al., 2010) and adjacent arable and grassland soils in North Dakota, USA (Schimel, 1986). Gross mineralization rates (Table 4; -0.4 to 11.4 mg kg^{-1} d^{-1}) are also comparable to semi-arid grassland soils in Australia (Cookson et al., 2006) and adjacent crop and grassland soils in the US (Schimel, 1986) with values of 1.7 to 13 and 5.5 to 6.7 mg kg^{-1} d^{-1}, respectively. Low ON contents may cause the negligible effect of land use resulting in similar gross NH_4^+ consumption rates and gross N mineralization and, hence, the small net mineralization in our study.

4.2. Effect of the nitrogen fertilization on the nitrogen mineralization

Generally, unfertilized grassland soils are N limited. Appropriate N fertilization increases the available N pool in the soil. Thus, we hypothesized that applied N may stimulate microbial activity, resulting in higher gross N mineralization rates, as microorganisms were supplied with easily available fertilizer N.

We did not find significant higher net or gross N mineralization rates with or without N fertilization (Table 4). For the N fertilization treatments, ^{15}N recoveries in mineral N fractions (Table 2) was high and the MBN contents stable over time (Figure 4) which disagrees with assumed stimulation of microorganisms by fertilizer application. Similarly to our results, Sun et al. (2013) observed no effect on N cycling parameters after N addition. However generally, gross N mineralization rates were reported to increase with both low and high N additions (Carpenter-Boggs et al., 2000; Lu et al., 2021; Song et al., 2021). Hence, in our study, N mineralization was constrained.

Without N fertilization in our study, small ^{15}N recovery (Table 2) corresponds well with the high abiotic retention (Table 3) and the trend of increasing MBN over 48 h of incubation (Figure 4). Hence, at low concentrations NH_4^+ was largely immobilized directly after N application (Grace et al., 1993; Nieder et al., 2011). Assuming the same happens in living

soils, added NH_4^+ would therefore partly protected from nitrification, ultimately resulting in small net N mineralization (Table 4). At N fertilization, the NH_4-^{15}N abundance correlated negatively to MBN and MBC contents, indicating that for microorganisms toxic amounts of N may have been applied. This is further underlined by the increasing EC and decreasing pH directly after high N fertilization. Similar fertilization effects were also described for grassland soils (Hao et al., 2020; Li et al., 2010; Treseder, 2008; Zhang et al., 2008), where e.g. the pH was decreased from 6.6 to 5.4 after addition of 20 g N m^{-2} (Li et al., 2010). A low pH can negatively influence the microbial activity in unfertilized soils (Kemmitt et al., 2006; Rousk et al., 2009). In a N mineralization study on grassland soils, N addition at a rate of 30 kg N ha^{-1} did also not affect N mineralization (Hao et al., 2020). Alternatively, N fertilization might also have induced C limitation to microorganisms leading as well to a reduced microbial biomass (Zaman et al., 1999; Zhang et al., 2008).

Consequently, we must reject our second hypothesis. Without N fertilization, N was rapidly retained by abiotic processes, hence making the added NH_4^+ inaccessible for microorganisms and keeps the *status quo*. In contrast to the assumed enhanced mineralization under moderate N fertilization of 60 kg N ha^{-1}, fertilization did neither stimulate microbial activity nor affect the N mineralization. Carbon limitation or fertilizer induced declined soil conditions for microorganisms may be the cause of constant N mineralization after fertilization.

4.3. Biotic and abiotic nitrogen retention

^{15}N labeling experiments often indicate initial rapid NH_4^+ consumption attributed to both biotic N immobilization (Jones et al., 2013) and abiotic retention (Davidson et al., 1991). However, the relative contribution of biotic and abiotic N retention apparently depends on the soil and on experimental conditions. We hypothesized that abiotic N retention exceeds the biotic process as the short time period observed favors abiotic retention.

In living soils, we observed no significant microbial N immobilization for both fertilization treatments (Figure 4). Small biotic N immobilization at N fertilization fits to small

amounts of microbial immobilized fertilizer N in North Kazakh arable soils with a fertilization dose of 20 kg N ha^{-1} ammonium nitrate (Koch et al., 2021). Small microbial NH_4^+ immobilization at no fertilization (Figure 4) fits well to a meta study of field observations, that under low N addition microbial NH_4^+ consumption is inhibited (Song et al., 2021). However, the same study found that high N addition (>55 kg N ha^{-1}) increased microbial N immobilization (Song et al., 2021). Unfortunately, the meta-study (Song et al., 2021) did not include soil properties, which may strongly affect N retention processes as shown in the Introduction.

Abiotic N retention in % of the applied amount without living competition in sterile soils was significantly higher in not fertilized treatments but absolute N retention was higher under high N fertilization (Table 3). At N fertilization equivalent to 60 kg N ha^{-1} 29 % abiotic N retention after 48 h (Table 3) fits well to the 37 % reported for temperate grassland soils 24 h after ^{15}N application (Braun et al., 2018). At no N fertilization, the small amount of added NH_4^+ to sterile soils were probably instantly abiotically fixed after application (91 %; Table 3) (Russow et al., 2008), as there was no biotic competition for N but abundant sorption sites at pedogenic minerals (Nieder et al., 2011) or SOM (Nommik, 1965). Interlattice fixation of added NH_4^+ in expandable clay minerals may be rapid (Nieder et al., 2011) and a great sink (Allison and Roller, 1955; Scherer et al., 2014). Additionally, the soil organic carbon content is an important parameter for N immobilization (Barrett and Burke, 2000), especially the light fraction SOM (Compton and Boone, 2002). But after all, our result suggest, that the applied N with fertilization is rapidly immobilized by especially abiotic N retention processes. Hence, hypothesis three can be accepted, as abiotic exceeded biotic N retention.

It should be noted that high observed N retention in IPD experiments is often attributed to the stimulation of biotic consumption rates by added NH_4^+ so that immobilization rates are probably overestimated (Booth et al., 2005a; Davidson et al., 1991). Possibly, autoclavation could also cause enhanced N retention by an increased proportion of sorption sites as observed in experiments on CO_2 sorption in autoclaved soils (personal communication with C. F. Stange). However, this study shows that in clay-rich and N-poor

soils, added N did not stimulate microbial activity, but N is largely and rapidly retained by abiotic processes. Consequently, the N availability after fertilization, but also its natural supply by mineralization is reduced. This result will help to adjust N fertilization management in these clay-rich soils in North Kazakhstan to ensure future productive agriculture.

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Supplementary Material

Figures

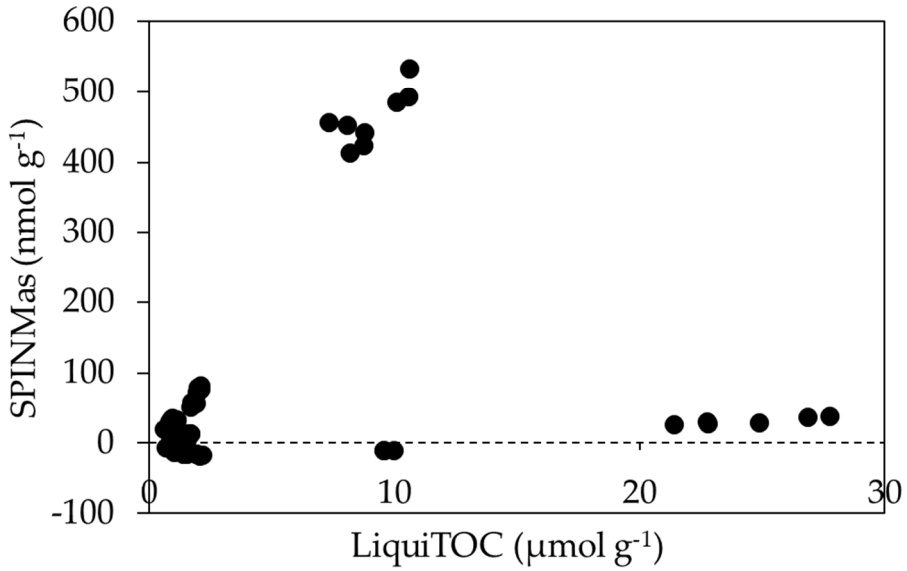

Figure S1: Total nitrogen (TN) measured on the LiquiTOC in 0.5 M K_2SO_4 and on the SPINMas in 0.5 K_2SO_4 after persulfate digestion. Note the different units on the x- and y-axis.

Tables

Table S1: p-values derived from Student t-tests with Bonferroni-correction on the effect of land use on basic soil parameters before incubation (top) and the effect of fertilizer application (bottom) on selected soil parameters. Significant differences are marked by bold numbers. Fertilization 0 (before fertilization), 0.25 (no N fertilization treatment), and 60 (N fertilization treatment) are given in kg N ha^{-1}. Abbreviations are: AS: arable soil, GS: grassland soil, EC: electrical conductivity; TC: total carbon; TN: total nitrogen; MBN and MBC: microbial biomass nitrogen and carbon, respectively.

Time Point	Parameter	Land use		Fertilization	
		Comparision	p-value	Comparision	p-value
-24 h	pH	AS-GS	0.60		
	EC	AS-GS	0.75		
	TC	AS-GS	**0.00**		
	TN	AS-GS	**0.00**		
	C:N	AS-GS	**0.02**		
	MBN	AS-GS	**0.00**		
	NH_4-N	AS-GS	0.96		
	NO_3-N	AS-GS	0.11		
	MBC	AS-GS	**0.00**		
	MBC:MBN	AS-GS	0.20		
-24 and 0.5 h	EC			0-0.25	0.93
				0-60	**0.00**
	pH			0-0.25	0.55
				0-60	**0.00**
	NH_4-N			0-0.25	1.00
				0-60	**0.00**
	NO_3-N			0-0.25	**0.01**
				0-60	**0.05**

Table S2: p-values derived from Student t-tests with Bonferroni-correction on the effect of time on the ^{15}N recovery in mineral N forms, NH_4-N and NO_3-N contents. Significant differences are marked by bold numbers. Comparisons 0.5, 24, and 48 are given in hours.

Land use	Fertilization (kg N ha^{-1})	Recovery		NH_4-N		NO_3-N	
		Comparision	p-value	Comparision	p-value	Comparision	p-value
Grassland	0.25	0.5-24	0.73	0.5-24	**0.00**	0.5-24	0.20
		24-48	0.30	24-48	0.05	24-48	1.00
	60	0.5-24	1.00	0.5-24	1.00	0.5-24	**0.04**
		24-48	0.14	24-48	0.10	24-48	0.22
Arable	0.25	0.5-24	0.84	0.5-24	1.00	0.5-24	0.62
		24-48	1.00	24-48	1.00	24-48	0.15
	60	0.5-24	0.78	0.5-24	0.77	0.5-24	
		24-48	1.00	24-48	0.82	24-48	

Table S3: Correlation matrix of selected parameters 24 h after fertilization. The upper triangle gives the correlation coefficient r, the lower triangle the p-value. Significant correlations are marked by bold numbers. The unit of NH_4-N and NO_3-N is in nmol g^{-1}, mineralization and consumption rates in nmol g^{-1} h^{-1}, and NH_4-^{15}N and NO_3-^{15}N in at% excess.

	Content NH_4-N	Content NO_3-N	Net mineralization	Gross mineralization	Gross NH_4 consumption	NH_4-^{15}N abundance	NO_3-^{15}N abundance
Content NH_4-N		-0.29	0.35	0.03	-0.37	**0.85**	**0.63**
Content NO_3-N	0.35		0.00	0.18	0.08	-0.45	**-0.62**
Net mineralization	0.29	1.00		0.42	**-0.91**	0.21	0.17
Gross mineralization	0.93	0.61	0.20		0.00	-0.36	-0.43
Gross NH_4 consumption	0.26	0.82	**0.00**	1.00		-0.39	-0.39
NH_4-^{15}N abundance	**0.00**	0.14	0.54	0.28	0.24		**0.91**
NO_3-^{15}N abundance	**0.03**	**0.03**	0.61	0.18	0.24	**0.00**	

Table S4: Correlation matrix of selected parameters 48 h after fertilization. The upper triangle gives the correlation coefficient r, the lower triangle the p-value. Significant correlations are marked by bold numbers. Abbreviations are: electrical conductivity (EC), microbial biomass carbon and nitrogen (MBC and MBN). The difference (diff) is calculated as the contents at 48 h subtracted by the content of -24 h. The unit of EC is µS cm^{-1}, MBC and MBN in nmol g^{-1}, NH_4-N and NO_3-N in nmol g^{-1}, rates in nmol g^{-1} h^{-1}, and NH_4- and NO_3-^{15}N in at% excess.

	pH	Eh	Content MBC	diffMBC	MBN:MBC	Content MBN	diffMBN	Content NH_4-N	Content NO_3-N	Net mineralization	Gross mineralization	Gross NH_4 consumption	Abundance NH_4-^{15}N	Abundance NO_3-^{15}N
pH		**-0.98**	0.29	0.58	-0.37	0.50	**0.66**	**-0.93**	-0.42	-0.20	-0.33	-0.11	**-0.92**	**-0.89**
Eh	**0.00**		-0.23	-0.57	0.34	-0.58	-0.62	**0.94**	0.37	0.17	0.32	0.12	**0.95**	**0.93**
Content MBC	0.36	0.47		**0.76**	0.50	-0.16	0.50	-0.37	**-0.93**	-0.38	0.18	0.36	-0.37	0.09
diffMBC	0.06	0.07	**0.01**		0.05	0.58	0.57	**-0.61**	**-0.77**	-0.44	-0.05	0.24	**-0.65**	-0.17
MBN:MBC	0.29	0.34	0.14	0.90		-0.30	0.05	0.30	-0.36	0.14	0.53	0.29	0.14	0.53
Content MBN	0.14	0.08	0.65	0.10	0.47		0.57	-0.52	0.16	-0.64	0.17	0.43	**-0.68**	-0.55
diffMBN	**0.04**	0.06	0.14	0.11	0.90	0.09		-0.63	**-0.73**	-0.56	0.26	0.46	**-0.72**	-0.43
Content NH_4-N	**0.00**	**0.00**	0.23	**0.05**	0.40	0.12	0.05		0.38	0.40	0.28	-0.05	**0.92**	**0.90**
Content NO_3-N	0.26	0.33	**0.00**	**0.03**	0.43	0.71	**0.04**	0.31		0.20	-0.02	-0.09	0.47	0.01
Net mineralization	0.53	0.61	0.23	0.17	0.69	0.05	0.09	0.20	0.61		-0.14	**-0.72**	0.25	0.52
Gross mineralization	0.30	0.30	0.57	0.87	0.12	0.64	0.48	0.37	0.96	0.66		**0.79**	0.03	0.52
Gross NH_4 consumption	0.74	0.70	0.25	0.48	0.41	0.21	0.18	0.88	0.81	**0.01**	**0.00**		-0.13	0.26
Abundance NH_4-^{15}N	**0.00**	**0.00**	0.24	**0.03**	0.71	**0.03**	**0.02**	**0.00**	0.20	0.44	0.92	0.68		**0.80**
Abundance NO_3-^{15}N	**0.00**	**0.00**	0.82	0.68	0.22	0.16	0.29	**0.00**	0.99	0.15	0.15	0.50	**0.01**	

Table S5: Results of 2-way-ANOVA (A-S) and Kruskal-Wallis (T-Z) test for the influence of fertilization, land use and their interactions for various soil parameters for the sampling times 0.5 h (A-D, R), 24 h (E-H, T, X-Z) and 48 h (I-Q, S, U-W) after fertilizer application. Units are nmol g^{-1} (MBC, MBN, NH_4-N, NO_3-N, abiotic retention), nmol g^{-1} h^{-1} (net and gross mineralization rate, gross consumption rate), at%excess (NH_4-^{15}N, NO_3-^{15}N), and % (biotic and abiotic recovery, abiotic retention). Interactions (fertilization x land use) are only shown if they were significant.

NO_3-^{15}N 0.5 h **A**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	0.0986	0.0986	9.73	2.10E-02	*
Land use	1	0.096	0.096	9.47	0.022	*
Fertilization : Land use	1	0.0398	0.0398	3.93	0.095	.
Residuals	6	0.0608	0.0101			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-0.199	-0.354	-0.0428	0.021	*
Grassland - Arable	-0.2	-0.359	-0.041	0.022	*
Grassland:0.25 - Arable:60	-0.3728	-0.691	-0.0547	0.026	*
Grassland:0.25 - Arable:0.25	-0.3288	-0.647	-0.0107	0.044	*
Grassland:0.25 - Grassland:60	-0.3017	-0.586	-0.0171	0.039	*

NH_4-^{15}N 0.5 h **B**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	182.9	182.9	2330.29	3.80E-11	***
Land use	1	0.3	0.3	3.77	0.088	.
Fertilization : Land use	1	0.8	0.8	10.47	0.012	*
Residuals	8	0.6	0.1			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-7.81	-8.18	-7.44	0	***
Arable:0.25 - Arable:60	-7.285	-8.018	-6.553	0	***
Grassland:0.25 - Arable:60	-8.123	-8.855	-7.39	0	***
Grassland:60 - Arable:0.25	7.495	6.762	8.227	0	***
Grassland:0.25 - Arable:0.25	-0.838	-1.57	-0.105	0.26	*
Grassland:0.25 - Grassland:60	-8.332	-9.065	-7.6	0	***

NO_3-N 0.5 h **C**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	1.04	1.04	0.75	4.19E-01	
Land use	1	12.94	12.94	9.36	0.022	*
Fertilization : Land use	1	2.89	2.89	2.09	0.198	
Residuals	6	8.3	1.38			

Comparison	diff	lwr	upr	p adj	Significance
Grassland - Arable	2.32	0.465	4.18	0.022	*

NH_4-N 0.5 h **D**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	8.36	8.36	1776.97	1.10E-10	***
Land use	1	0.12	0.12	24.77	0.0011	**
Fertilization : Land use	1	0.02	0.02	3.87	0.0846	.
Residuals	8	0.04	0			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-1.67	-1.76	-1.58	0	***
Grassland - Arable	0.197	0.106	0.288	0.001	**
Arable:0.25 - Arable:60	-1.747	-1.9262	-1.568	0	***
Grassland:0.25 - Arable:60	-1.472	-1.6512	-1.293	0	***
Grassland:60 - Arable:0.25	1.866	1.6867	2.045	0	***
Grassland:0.25 - Arable:0.25	0.275	0.0957	0.454	0.005	**
Grassland:0.25 - Grassland:60	-1.591	-1.7703	-1.412	0	***

NH_4-N 24 h **E**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	7.92	7.92	78.07	2.10E-05	***
Land use	1	0.19	0.19	1.89	0.21	
Fertilization : Land use	1	0.16	0.16	1.58	0.24	
Residuals	8	0.81	0.1			

log transformed

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-1.62	-2.05	-1.2	0	***
Arable:0.25 - Arable:60	-1.393	-2.226	-0.56	0.003	***
Grassland:0.25 - Arable:60	-1.372	-2.204	-0.539	0.003	***
Grassland:60 - Arable:0.25	1.877	1.045	2.71	0	***
Grassland:0.25 - Grassland:60	-1.856	-2.689	-1.023	0	***

NO_3-N 24 h **F**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	79.3	79.3	3.49	9.90E-02	.
Land use	1	0.2	0.2	0.01	0.932	
Fertilization : Land use	1	59	59	2.6	0.146	
Residuals	8	181.6	22.7			

NO_3-^{15}N 24 h **G**

	D.f.	Sum Sq.	Mean Sq.	F value	3.30E-07	Significance
Fertilization	1	2.025	2.025	233.73	1.90E-03	***
Land use	1	0.178	0.178	20.52	0.0019	**
Fertilization : Land use	1	0.084	0.084	9.68	0.0144	*
Residuals	8	0.069	0.009			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-0.822	-0.946	-0.698	0	***
Grassland - Arable	-0.243	-0.367	-0.119	0.002	**
Arable:0.25 - Arable:60	-0.989	-1.232	-0.745	0	***
Grassland:60 - Arable:60	-0.411	-0.654	-0.167	0.003	***
Grassland:0.25 - Arable:60	-1.065	-1.308	-0.822	0	***
Grassland:60 - Arable:0.25	0.5782	0.335	0.822	0	***
Grassland:0.25 - Grassland:60	-0.654	-0.898	-0.411	0	***

NH_4-^{15}N 24 h **H**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	191.9	191.9	2231.93	4.50E-11	***
Land use	1	0.1	0.1	1.26	0.29	
Fertilization : Land use	1	0.1	0.1	1.55	0.25	
Residuals	8	0.7	0.1			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-8	-8.39	-7.61	0	***
Arable:0.25 - Arable:60	-7.786	-8.553	-7.019	0	***
Grassland:0.25 - Arable:60	-7.807	-8.573	-7.04	0	***
Grassland:60 - Arable:0.25	8.187	7.42	8.954	0	***
Grassland:0.25 - Grassland:60	-8.208	-8.974	-7.441	0	***

NH_4-N 48 h **I**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	44.8	44.8	1740.16	1.20E-10	***
Land use	1	0.2	0.2	8.16	0.021	*
Fertilization : Land use	1	0.1	0.1	2.22	0.174	
Residuals	8	0.2	0			

log transformed

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-3.86	-4.08	-3.65	0	***
Grassland - Arable	0.265	0.051	0.478	0.021	*
Arable:0.25 - Arable:60	-3.726	-4.1458	-3.307	0	***
Grassland:0.25 - Arable:60	-3.6	-4.0193	-3.18	0	***
Grassland:60 - Arable:0.25	4.129	3.7094	4.549	0	***
Grassland:0.25 - Grassland:60	-4.002	-4.4219	-3.583	0	***

NO_3-N 48 h **J**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	34.1	34.1	3.13	1.40E-01	
Land use	1	122.4	122.4	11.24	0.02	*
Fertilization : Land use	1	37.4	37.4	3.43	0.12	
Residuals	5	54.5	10.9			

Comparison	diff	lwr	upr	p adj	Significance
Grassland - Arable	7.73	1.73	13.7	0.021	*
Grassland:60 - Arable:0.25	11.344	0.227	22.46	0.046	*
Grassland:0.25 - Arable:0.25	11.384	0.268	22.5	0.046	*

Abiotic retention 48 h **K**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	11598	11598	8212.45	2.50E-13	***
Land use	1	2	2	1.13	0.32	
Fertilization : Land use	1	1	1	1.03	0.34	
Residuals	8	11	1			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	62.2	60.6	63.8	0	***
Arable:0.25 - Arable:60	62.873	59.77	65.98	0	***
Grassland:0.25 - Arable:60	61.4477	58.34	64.55	0	***
Grassland:60 - Arable:0.25	-62.9049	-66.01	-59.8	0	***
Grassland:0.25 - Grassland:60	61.4796	58.37	64.59	0	***

NH_4-^{15}N 48 h **L**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	152.8	152.8	97.67	9.30E-06	***
Land use	1	2	2	1.3	0.29	
Fertilization : Land use	1	1.3	1.3	0.81	0.39	
Residuals	8	12.5	1.6			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-7.14	-8.8	-5.47	0	***
Arable:0.25 - Arable:60	-6.487	-9.76	-3.22	0.001	***
Grassland:0.25 - Arable:60	-6.315	-9.59	-3.04	0.001	***
Grassland:60 - Arable:0.25	7.959	4.69	11.23	0	***
Grassland:0.25 - Grassland:60	-7.786	-11.06	-4.52	0	***

NO_3-^{15}N 48 h **M**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	2.316	2.316	63.78	5.00E-04	***
Land use	1	0.198	0.198	5.45	0.0669	.
Fertilization : Land use	1	0.019	0.019	0.52	0.5044	
Residuals	5	0.182	0.036			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-1.02	-1.35	-0.692	0	***
Arable:0.25 - Arable:60	-0.929	-1.7901	-0.0679	0.038	*
Graaland:0.25 - Arable:60	-1.325	-2.1368	-0.5131	0.007	**
Grassland:60 - Arable:0.25	0.735	0.0929	1.3765	0.03	*
Grassland:0.25 - Grassland:60	-1.131	-1.7047	-0.5566	0.003	**

MBC 48 h **N**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	1.80E+07	18000000	1.68	2.31E-01	
Land use	1	1.91E+08	191000000	17.85	0.0029	**
Fertilization : Land use	1	8.75E+06	8750000	0.82	0.392	
Residuals	8	5.88E+07	1.07E+07			

Comparison	diff	lwr	upr	p adj	Significance
Grassland - Arable	-7974	-12327	-3621	0.003	*
Grassland:60 - Arable:0.25	-10423	-18971	-1874	0.019	*
Grassland:0.25 - Arable:0.25	-9682	-18231	-1134	0.028	*

MBN 48 h **O**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	613616	613616	4.16	8.80E-02	.
Land use	1	257882	257882	1.75	0.234	
Fertilization : Land use	1	50008	50008	0.34	0.582	
Residuals	6	885375	147563			

MBC:MBN 48 h **P**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	7.2	7.2	1.22	3.10E-01	
Land use	1	20.7	20.7	3.5	0.11	
Fertilization : Land use	1	0	0	0.01	0.94	
Residuals	6	35.6	5.93			

Recovery sterile 48 h = Retention sterile soils 48 h **Q**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	11598	11598	8212.45	2.50E-13	***
Land use	1	2	2	1.13	0.32	
Fertilization : Land use	1	1	1	1.03	0.34	
Residuals	8	11	1			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-62.2	-63.8	-60.6	0	***
Arable:0.25 - Arable:60	-62.873	-65.98	-59.77	0	***
Grassland:0.25 - Arable:60	-61.448	-64.55	-58.34	0	***
Grassland:60 - Arable:0.25	62.905	59.8	66.01	0	***
Grassland:0.25 - Grassland:60	-61.480	-64.59	-58.37	0	***

Recovery living 0.5 h **R**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	12961	12961	64.92	4.10E-05	***
Land use	1	12	12	0.06	0.81	
Fertilization : Land use	1	13	13	0.07	0.8	
Residuals	8	1597	200			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-65.7	-84.5	-46.9	0	***
Arable:0.25 - Arable:60	-67.8374	-104.8	-30.9	0.002	***
Grassland:0.25 - Arable:60	-67.7532	-104.7	-30.8	0.002	***
Grassland:60 - Arable:0.25	63.7035	26.8	100.6	0.002	***
Grassland:0.25 - Grassland:60	-63.6193	-100.6	-26.7	0.003	***

Recovery living 48 h **S**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	Significance
Fertilization	1	10733	10733	95.25	1.00E-05	***
Land use	1	724	724	6.43	0.035	*
Fertilization : Land use	1	416	416	3.69	0.091	.
Residuals	8	901	113			

Comparison	diff	lwr	upr	p adj	Significance
Fertilization 0.5 - 60	-59.8	-73.9	-45.7	0	***
Grassland - Arable	15.5	1.4	29.7	0.035	*
Arable:0.25 - Arable:60	-48.03	-75.791	-20.3	0.002	**
Grassland:0.25 - Arable:60	-44.28	-72.032	-16.5	0.004	**
Grassland:60 - Arable:0.25	75.35	47.595	103.1	0	***
Grassland:0.25 - Grassland:60	-71.59	-99.349	-43.8	0	***

Recovery living 24 h **T**

	D.f.	chi squared	p value	Significance
Fertilization	1	4	0.04	*
Land use	1	1	0.3	

Kruskal-Wallis

Net mineralization rate 48 h **U**

	D.f.	chi squared	p value	Significance
Fertilization	1	3	0.1	
Land use	1	0.03	0.9	

Kruskal-Wallis

Gross mineralization rate 48 h **V**

	D.f.	chi squared	p value	Significance
Fertilization	1	2	0.1	
Land use	1	0.9	0.3	

Kruskal-Wallis

Gross consumption rate 48 h **W**

	D.f.	chi squared	p value	Significance
Fertilization	1	0.2	0.6	
Land use	1	2	0.1	

Kruskal-Wallis

Net mineralisation rate 24 h				**X**
	D.f.	chi squared	p value	Significance
Fertilization	1	0	1	
Land use	1	0.5	0.5	

Kruskal-Wallis

Gross mineralization rate 24 h				**Y**
	D.f.	chi squared	p value	Significance
Fertilization	1	0.3	0.6	
Land use	1	1	0.3	

Kruskal-Wallis

Gross consumption rate 24 h				**Z**
	D.f.	chi squared	p value	Significance
Fertilization	1	0.5	0.5	
Land use	1	0.1	0.7	

Kruskal-Wallis

3. Study II

Sensitivity of carbon, nitrogen, and phosphorus mineralization in semi-arid steppe soils to temperature and moisture

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Contribution: This manuscript bases on a joint-study with Alexey Prays of the Martin-Luther University of Halle. We were equally involved in the experimental design, performing the laboratory experiments incl. sample preparation and measurements, data evaluation and presentation, and writing of the manuscript.

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Abstract

Semi-arid steppe soils are currently threatened by more extreme climatic conditions, likely accelerating the loss of soil organic matter (SOM). While many studies addressed temperature and moisture effects on carbon (C) cycling, there is scarce information on nitrogen (N) and phosphorus (P) mineralization, and their linkage to C mineralization. Here, we incubated topsoils under grassland and arable use at different temperatures and matric potentials to quantify their effects on C, N, and P mineralization. Net mineralized C, N, and P and the ^{14}C activity of released CO_2 were related to SOM fractions as revealed by density fractionation. The cumulative CO_2-C evolution ranged from 4.1 to 62.7 mg g^{-1} organic C (OC) and was higher in grassland than arable soil in line with larger contents of free particulate organic matter (fPOM). Radiocarbon analyses suggest fPOM as primary source for mineralized C. Carbon mineralization and temperature sensitivity increased (mean Q_{10} of 2.5) at higher temperature, but were not affected by matric potential. Net mineralization of N and P ranged between -28.1 (i.e. immobilization) and 10.5 mg g^{-1} organic N (ON) and -1.9 to 1.4 mg P mg^{-1} Olsen-extractable organic P (Olsen OP), respectively. Higher temperature increased P but not N mineralization, whereas the moisture effect on C, N, and P cycling was small, probably due to clayey texture. Overall results suggest sensitivity of C cycling to temperature in the studied semi-arid steppe soils, irrespective of land use, while N and P cycling were apparently less affected. This suggests poor future natural nutrient supply by SOM mineralization in semi-arid steppe soils of North Kazakhstan.

Keywords: organic matter, land use, ^{14}C, decomposition, Q_{10}, particulate organic matter, mineral-associated organic matter

1. Introduction

Steppe soils cover vast areas of the terrestrial Earth surface, especially of the northern hemisphere. Their most striking pedogenic feature is the accumulation of large stocks of soil organic matter (SOM) (Parton et al., 1995; Scurlock and Hall, 1998), which support important ecological functions, such as nutrient and water storage and resistance to erosion. Due to their

high fertility, steppe soils are widely used as arable land and form the basis of a large share of World's staple food production (Swinnen et al., 2017). Since their functions and services are closely linked to SOM, these soils are highly vulnerable to disturbances affecting its turnover. Unsustainable soil management and climate change might change plant residue input and SOM decomposition and, hence, jeopardize the soils' functions. After extensive conversion of grassland to arable fields during the "Virgin Land Campaign" (Russian: "zelina", 1954-1963), Kazakh semi-arid steppe soils have been subjected to insufficient fertilization and poor management for decades (Mizina et al., 1999; Yanai et al., 2005), which resulted in a strong decrease in productivity (Takata et al., 2008).

The turnover of SOM is key to soil fertility (Chen et al., 2018; Paustian et al., 2016; Thiessen et al., 2013). With SOM decomposition, nutrients such as nitrogen (N) and phosphorus (P) are mineralized and made available to plants. Numerous studies addressed the sensitivity of SOM fraction degradation and related element cycling to temperature (Davidson and Janssens, 2006; Moinet and Millard, 2020; von Lützow and Kögel-Knabner, 2009) or soil moisture (Hu et al., 2019; Kadono et al., 2008; Liu et al., 2016). However, results were not always consistent. Many studies indicate that higher temperature causes higher C, N, and P mineralization in various ecosystems around the world (Grierson et al., 1999; Zaman and Chang, 2004; Davidson and Janssens, 2006; Wang et al., 2006). In contrast, other studies report either no or only short-term effects for increased temperature, especially at low temperature regimes (Giardina and Ryan, 2000; Nadelhoffer et al., 1991; Wang et al., 2006). Increasing temperature is generally assumed to result in lower temperature sensitivity (Q_{10}), allowing particularly a higher decomposition rate of more stable SOM (Davidson and Janssens, 2006) by increasing microbial biomass and enzymatic activities (Franzluebbers, 2020; Wallenstein et al., 2011).

Soil moisture likewise is another factor controlling the chemical and biological decomposition of SOM (Grierson et al., 1999; Hu et al., 2019; Moyano et al., 2013; Wang et al., 2006), as too dry or wet soil conditions suppress SOM decomposition (Moyano et al., 2013). Under wet conditions, pores are water-filled and hence oxygen supply is limiting

microbial respiration (Franzluebbers, 1999). Drought causes decreased microbial respiration as low water contents limit the supply of soluble substrates (Skopp et al. 1990) and cells have to maintain their osmotic equilibrium (Schimel et al., 2007).

Mineralization of SOM is not only constrained by temperature and soil moisture but also by chemical and physical stabilization processes that decrease SOM availability (Gershenson et al., 2009; Qin et al., 2019). Hence, SOM availability has been linked to soil texture (Hassink, 1997) and mineral composition (Mikutta et al., 2019), as well as to soil aggregation (Six et al., 2002b, 2002a). Free or occluded particulate organic matter (fPOM and oPOM, respectively) not attached to minerals is supposed to represent a labile fraction with fast turnover whereas mineral-associated OM (MAOM) is considered more stable with slower turnover rates (Kleber et al., 2015; Lavallee et al., 2020; von Lützow et al., 2006). In semi-arid steppe soils, MAOM contributes most to SOM under both, natural vegetation and arable management, with larger shares of POM under natural vegetation (Bischoff et al., 2016). While the limited availability of MAOM increases its temperature sensitivity, POM has been shown to be more sensitive to temperature (Benbi et al., 2014; Rocci et al., 2021). This in turn shows a stronger POM mineralization than MAOM destabilization under elevated temperatures (Lavallee et al., 2020). These differences may explain the minor mineralization of old SOM upon warming (Briones et al., 2021). Hence, soils containing larger shares of MAOM should react less sensitive to increasing soil temperatures than those harboring more POM not attached to minerals.

The quality of SOM and, hence, its degradability can be indicated by its stoichiometric ratio of C to its nutrients (Hessen et al., 2004; Heuck and Spohn, 2016). Carbon:N:P:S ratios of SOM around the world have been reviewed by Tipping et al. (2016) and investigations linking C:N:P ratios of SOM to mineralization have been conducted in various environments (e.g. Heuck and Spohn, 2016; Gan et al., 2020). Accordingly, decomposition of SOM is most efficient when its C:N:P ratio meets the demand of microorganisms (Chen et al., 2014; Hessen et al., 2004). In cases where enough nutrients are available, C, N, and P mineralization are likely to be coupled (Gan et al., 2020; Manzoni et al., 2008). If nutrients are depleted while

much C is available, C, N, and P mineralization becomes decoupled (Gan et al., 2020) with the excessive C being respired by microorganisms to meet their nutrient demands ("nutrient mining"; Gan et al., 2020; Heuck and Spohn, 2016).

For the West Siberian Plain more humid conditions and an increase in temperature were observed over the past years and projected for the future (Hu et al., 2017; Huang et al., 2014). Due to the projected seasonal climatic shifts, North Kazakh semi-arid steppe systems might even be a "winner" of climatic changes due to longer vegetation periods (Lioubimtseva and Henebry, 2009). However, there is a lack of empirical data on the influence of moisture and temperature on the C, N, and P mineralization in combination in soils in semi-arid regions.

Therefore, our main objective was to test how changes in temperature and soil moisture affect C, N, and P mineralization and their coupling in semi-arid steppe soils under different land uses. We carried out an incubation experiment with topsoil material of grassland and arable soils in order to test the following three hypotheses: (i) Irrespective of moisture, the temperature response of SOM mineralization is higher for grassland soils than arable soils. (ii) Increasing in soil moisture will further accelerate SOM decomposition beyond the expected temperature response. (iii) The observed C and net nutrient release can be linked to the SOM fraction's initial C:N:P element ratio. For testing the hypotheses, we incubated the soils for 126 days at 15°C and 25°C and at matric potentials of pF 2.5 and pF 3.5, respectively. We monitored CO_2, $^{14}CO_2$, total, inorganic, organic, and extractable C, N, and P at various time points and related the net mineralization of C, N, and P to contents of POM and MAOM fractions as estimated by density fractionation.

2. Material and Methods

2.1. Site description, soil sampling, and sample characterization

Soil samples were collected in the steppe zone of North Kazakhstan under grassland and arable management close to the villages Yasnaya Polyana, Zhaltir, and Losovoe in May 2018 (Figure 1; Table 1). Soils were classified as Chernozems (Yasnaya Polyana) and Kastanozems (Zhaltir, Losovoe) (Table 1; IUSS Working Group WRB 2015 (2015)). The

grassland soils were under virgin natural vegetation, whereas arable soils were under conservational tillage of the region continuously for 60 (Yasnaya Polyana), 25 (Zhaltir), and 15 years (Losovoe), and fertilized in the past years (Table 1). Six topsoil samples (0-10 cm) were taken randomly from each field to account for spatial variability. All samples were oven-dried at 40°C, sieved to <2 mm, pooled and homogenized.

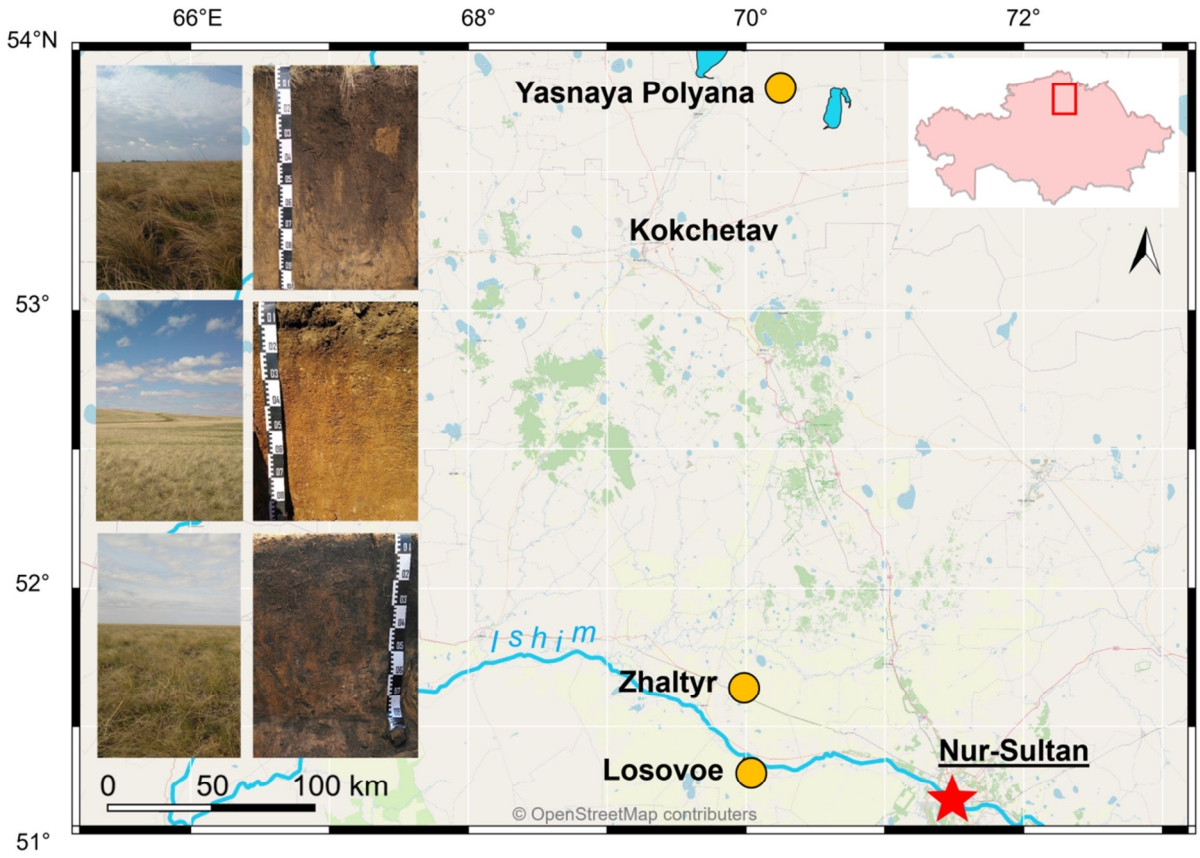

Figure 1: Study region in North Kazakhstan with the sampling sites Yasnaya Polyana in the north, Zhaltir, and Losovoe in the south. On the left, each photo image depicts landscape and soil under natural steppe at Yasnaya Polyana (top), Zhaltir (center), Losovoe (bottom). The map is based on ©OpenStreetMap contributors, for copyright see www.openstreetmap.org/copyright.

Table 1: Location and characteristics of study sites. Coordinates are given in WGS84. Soil samples were classified according IUSS Working Group WRB (2015). Abbreviations: MAT: mean annual temperature; MAP: mean annual precipitation; AS: arable soil; GS: grassland soil. Arable soils were fertilized with urea ammonium nitrate combined with ammonium phosphate at Yasnaya Polyana, with ammonium nitrate at Zhaltir, and with ammonium phosphate at Losovoe.

Site	Coordinates (longitude N, latitude E)	Soil type	MAP (mm)	MAT (°C)	Land use	Tillage	Fertilization N, P (kg ha^{-1})
Yasnaya Polyana	55.037222, 71.244167	Chernozem	326	1.9	AS	harrow	80, 40
Yasnaya Polyana	53.958611, 70.335278	Chernozem	326	1.9	GS	none	none
Zhaltir	51.567500, 70.075833	Kastanozem	298	1.9	AS	harrow	20, 0
Zhaltir	51.633056, 70.056667	Kastanozem	298	1.9	GS	none	none
Losovoe	51.185833, 70.070556	Kastanozem	292	3.0	AS	harrow	30, 20
Losovoe	51.291389, 70.081944	Kastanozem	292	3.0	GS	none	none

Soil texture was determined by the pipette analysis after Köhn (1928). Total C (TC) and N (TN) were analyzed by dry combustion using an elemental analyzer (Vario Max Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). Inorganic carbon (IC) was analyzed by treating ground 200 mg samples with 50 ml 2 M HCl at 50°C and subsequent detection of the released CO_2 (soliTIC modul interfaced to Vario Max Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). Organic carbon (OC) was calculated as the difference of TC and IC. Available inorganic N (NH_4-N and NO_3-N) were extracted with 0.0125 M $CaCl_2$ according to VDLUFA (2002) and analyzed photometrically with a continuous flow analyzer (SAN^{Plus}, Skalar Analytical B.V., Breda, Netherlands). Organic nitrogen (ON) was calculated as the difference of TN and inorganic N. We are aware that calculating ON in this way omits the fixed ammonium contents in the clay interlattice. Plant-available P was determined according to Olsen et al. (1954) and solutions were measured for total P by inductively coupled plasma optical emission spectrometry (ICP-OES; Ultima 2, Horiba Jobin-Yvon, Longjumeau, France) and photometrically for PO_4-P (SAN^{plus}). Extractable organic P

(EO-P) was calculated as the difference of total P and PO_4-P. Contents of all C, N, and P fractions are reported on soil dry mass basis (105°C).

Density fractionation of soils with sodium polytungstate solution (density 1.6 g cm^{-3}) was used to isolate fPOM, oPOM and MAOM (Golchin et al., 1995 as modified by Surey et al., 2020). Each sample was fractionated in duplicates. All SOM fractions were freeze-dried, weighted, and ground for TC/TN and IC analysis. Average recovery of soil mass during density fraction was 95 ± 1% (mean ± standard deviation); that of OC and TN was 93 ± 3% and 86 ± 8%, respectively. Total P concentrations of fPOM and oPOM fractions were determined by microwave-assisted digestion of 50 mg aliquots in mixtures of 4 ml concentrated HNO_3, 1 ml H_2O_2 (30%), and 1 ml deionized water for 45 minutes (MARS 6, CEM, Kamp-Lintfort, Germany). Solutions were allowed to settle for 2 hours, and then filtered through 0.45-µm syringe filters (Millex, Merck, Darmstadt, Germany) before analysis by ICP-OES. Total P concentrations of MAOM fractions were determined by sequential wave length-dispersive X-ray fluorescence spectroscopy (S8 Tiger Series 2, Bruker AXS, Karlsruhe, Germany) using fused beads prepared with 1 g sample aliquots ashed at 1000°C.

2.2. Soil incubation

Soils were incubated for 126 days using combinations of temperatures (15°C and 25°C) and matric potentials (pF 2.5 and 3.5). The incubation period represents the typical vegetation period in northern Kazakhstan and 15°C the current daily mean temperature during the vegetation phase (Merkel, 2020). The higher temperature of 25°C was selected because it sets a reasonable range of possible increases of the mean summer temperature. The matric potential of pF 2.5 simulates a sufficient water supply during the vegetation period, where water is held in pores with equivalent diameters of <10 µm. In contrast, the matric potential of pF 3.5 reflects dry conditions at the end of the vegetation period. For each treatment and sampling point, 10 g of each soil was weighted in triplicates into glass flasks and the moisture was adjusted by spraying deionized water homogeneously on the sample. Flasks were subsequently closed with polyethylene wool to minimize evaporation but allow for gas

exchange. Samples were then incubated in a climate chamber at 15°C or 25°C. The moisture content of all samples was checked every three days and readjusted if necessary.

2.3. Determination of C, N, and P fractions

During incubation, CO_2 emission was measured at days 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 18, 21, 42, 56, 70, 84, 98, 112, and 126 after starting the incubation. Twenty-four hours before each gas sampling, flasks were flushed with ambient air until the volume of the headspace was replaced at minimum three times. Then, all flasks were sealed with polytetrafluoroethylene silicone caps for 24 hours before an 18-ml gas volume was sampled with a gas-tight syringe and transferred into a 12-ml pre-evacuated exetainer vial. Headspace CO_2 concentrations were measured with a gas chromatography system using a HP 7890B GC as basis (Chromtech, Bad Camberg, Germany) and corrected for control samples without soil. Soil samples were destructively sampled at days 1, 3, 7, 11, 21, 70, and 126 after starting the incubation, and analyzed for C, N, and P. Analyses of TC, IC, OC, as well as TN, ON, NH_4-N, and NO_3-N were carried out as described above. Changes in P fractions, i.e. Olsen-P, PO_4-P, and EO-P, were determined at days 1 and 126.

To identify the prime source of emitted CO_2 (fPOM, oPOM, MAOM), ^{14}C was analyzed in SOM fractions and in CO_2 sampled at days 14 and 126. For that, the incubation was run as described above but using larger soil mass (30 g) and 1-l incubation flasks in order to obtain a minimal amount of 0.5 mg CO_2-C for ^{14}C analysis. Gas samples and SOM fractions were analyzed for ^{14}C activities at the Jena Radiocarbon Laboratory (Germany) by accelerator mass spectrometry (Steinhof et al., 2004). Data were analyzed after Steinhof (2013) and reported as percent modern carbon (pMC).

2.4. Data analysis and statistical evaluation

As flushing and sampling were separated by 24 hours, CO_2 emissions give a daily rate expressed as mg g^{-1} initial OC day^{-1}. The CO_2-C emission between sampling days was interpolated using a cubic spline function. Cumulative CO_2-C mineralization was then

calculated over the entire incubation period and the C loss was derived from fitting a one-pool first order decay function (Gentsch et al., 2018; La Scala Jr et al., 2009) to decomposition data using equation 1,

$$C_t = C_0 \times e^{-kt} \quad (1)$$

where C_0 is the amount of C available for decomposition, C_t the for decomposition available C at any time t, and k the decay constant. To determine the temperature sensitivity of the C mineralization, Q_{10} was calculated by equation 2 (Kirschbaum, 1995),

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T2-T1}} \quad (2)$$

where k_2 and k_1 are the decay constants at 25°C and 15°C and T_2 and T_1 are the temperatures 25 and 15°C, respectively. For the Q_{10} and k calculations, days 1-6 were removed to avoid inaccuracy caused by initial high decomposition and mineralization after rewetting the soil ("Birch effect"; Birch, 1958; Jarvis et al., 2007).

Net N mineralization over the whole incubation period was calculated from the difference of inorganic N content at day 126 (N_{t126}) and at the beginning of the incubation (day 1, N_{t1}) using equation 3:

$$\text{net N mineralization} = (NH_4\text{-}N_{t126} + NO_3\text{-}N_{t126}) - (NH_4\text{-}N_{t1} + NO_3\text{-}N_{t1}) \quad (3)$$

Net mineralized N was then normalized to the ON content (mg N g^{-1} ON). Negative N mineralization, i.e. negative numbers, indicate N immobilization.

Net P mineralization was calculated as the difference of PO_4-P at day 126 (P_{t126}) and day 1 (P_{t1}) using equation 4, and then normalized to EO-P.

$$\text{net P mineralization} = PO_4\text{-}P_{t126} - PO_4\text{-}P_{t1} \quad (4)$$

As for N, negative values indicate P immobilization.

Pairwise t-test with Bonferroni correction was performed on basic soil parameters to compare sites and land uses. Analysis of variance (ANOVA) was performed on the C, N, and P mineralization data and Q_{10} for each site in order to examine whether these parameters were significantly different among treatments (2-way ANOVA) and land uses (1-way ANOVA). ANOVA assumptions were tested and data was log transformed if necessary. If ANOVA assumptions were not met after log-transformation the non-parametric Kruskal-Wallis test was

performed (Q_{10}). Groups were compared using the Tuckey's honest significance post-hoc test (HSD) and statistical differences reported at a significance level of $p<0.05$. ANOVA results are available in the Supplementary Material. Principal component analysis (PCA) was performed with the R package factoextra (Kassambra and Mundt, 2020) to identify influencing parameters in the C, N, and P mineralization. All data were prepared and analyzed using Excel (Microsoft) and R (R Core Team, 2020, version 3.6.3). Figures were prepared using the R package ggplot2 (Wickham, 2016). No data on gas samples were available for incubation day 56 and 112 due to analytical problems.

3. Results

3.1. Basic soil properties

The study soils belonged to the silty clay and clay loam textural classes, with clay contents ranging from 363 to 506 g kg^{-1} (Table 2) and varying considerably between sites ($p<0.05$). The OC contents were between 20.8 and 49.7 g kg^{-1}, with larger values for grassland soils than arable soils ($p<0.05$) and decreasing in the order Yasnaya Polyana > Zhaltir ≥ Losovoe ($p<0.05$; Table 2). Most of the OC was with the MAOM fraction (87 to 94%), while fPOM and oPOM held 3 to 11% and 1 to 5% of the bulk soil OC, respectively. Free POM-C tends to be higher under grassland soils than arable soils, while for oPOM-C and MAOM-C no clear trends were observable. Organic N contents ranged from 1.9 to 4.4 g kg^{-1}, decreasing in the order Yasnaya Polyana > Zhaltir ≥ Losovoe ($p<0.05$), and being higher for grassland soils than for arable soils ($p<0.05$). Initial contents of NO_3-N and NH_4-N ranged between 20.6 and 144.5 mg kg^{-1}, and were not related to land use. Yasnaya Polyana grassland soil and arable soil, and Zhaltir grassland soil showed high initial mineralized N contents of >70 mg kg^{-1}, whereas contents for the other sites were less than 33 mg kg^{-1}. Contents in Olsen-P ranged from 7.9 to 20.8 mg kg^{-1}, with PO_4-P contributing 2.4 to 15.8 mg kg^{-1}, and EO-P 2.7 to 5.0 mg kg^{-1}. Arable soils were dominated by PO_4-P ($p<0.05$), which accounted for about 70% of the Olsen-P, while the native steppe soils (with exception of the ZHA site) contained 1.3 to 1.8-times more EO-P than PO_4-P ($p<0.05$).

Table 2: Basic properties of study soils. Abbreviations: AS: arable soil; GS: grassland soil; TC: total carbon; IC: inorganic carbon, OC: organic carbon; fPOM and oPOM: free and occluded particulate organic matter; MAOM: mineral-associated organic matter; TN: total nitrogen; TP: total phosphorous; ON: organic nitrogen; initial N_{min}: $CaCl_2$ extractable NH_4-N plus NO_3-N before incubation. Given is the mean of n = 3 samples with the standard deviation in brackets. For SOM fractions n = 1.

Parameter	Unit	Yasnaya Polyana		Zhaltir		Losovoe	
		AS	GS	AS	GS	AS	GS
Sand	g kg^{-1}	175.4	147.4	297.4	300.8	143.8	260.4
Silt	g kg^{-1}	319.0	359.1	364.5	291.1	352.0	377.0
Clay	g kg^{-1}	505.6	493.5	338.0	408.1	504.3	362.6
Clay mineralogy		Smectite + Vermiculite		Smectite		Smectite	
TC	g kg^{-1}	34.7 (0.7)	49.7 (1.6)	21.9 (0.9)	25.5 (11.0)	26.2 (8.3)	27.3 (6.2)
IC	g kg^{-1}	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.5 (0.0)	0.0 (0.0)
OC	g kg^{-1}	34.7 (0.7)	49.7 (1.6)	21.9 (0.9)	25.5 (11.0)	20.8 (8.3)	27.3 (6.2)
C:N bulk soil	g C g^{-1} N	11.1 (0.7)	11.2 (0.4)	10.8 (0.5)	10.8 (0.3)	12.4 (0.9)	10.8 (0.3)
TN	g kg^{-1}	3.1 (0.2)	4.4 (0.2)	2.0 (0.1)	2.4 (1.0)	2.2 (0.9)	2.5 (0.6)
ON	g kg^{-1}	2.9 (0.2)	4.4 (0.2)	1.9 (0.1)	2.2 (1.0)	2.1 (0.9)	2.5 (0.6)
initial N_{min}	mg kg^{-1}	144.5 (55.4)	32.6 (2.8)	71.5 (45.7)	124.9 (52.9)	26.6 (2.4)	20.6 (3.8)
Olsen-P	mg kg^{-1}	20.8 (5.0)	7.9 (1.8)	15.0 (2.5)	15.9 (2.5)	8.8 (1.3)	6.8 (1.4)
PO_4-P	mg kg^{-1}	15.8 (6.3)	3.4 (0.7)	10.6 (1.5)	8.9 (1.5)	6.1 (0.8)	2.4 (0.5)
EO-P	mg kg^{-1}	5.0 (3.8)	4.6 (1.3)	4.5 (1.2)	6.9 (1.1)	2.7 (0.6)	4.4 (0.9)
fPOM-N	% bulk TN	2.1	4.3	3.7	8.4	1.7	4.5
fPOM-C	% bulk OC	3.4	4.8	5.3	11.0	2.6	6.7
fPOM-TP	g kg^{-1}	3.8	4.8	4.1	4.4	5.1	5.8
oPOM-N	% bulk TN	4.0	1.1	2.3	1.5	3.1	2.8
oPOM-C	% bulk OC	5.2	1.4	2.8	2.4	3.4	4.1
oPOM-TP	g kg^{-1}	6.2	4.3	7.3	4.4	7.7	6.6
MAOM-N	% bulk TN	93.9	95.9	94.0	90.1	95.2	92.7
MAOM-C	% bulk OC	91.4	93.8	91.9	86.6	94.0	89.2
MAOM-TP	g kg^{-1}	0.7	0.7	0.6	0.7	0.6	0.5

3.2. Carbon mineralization

After initial large CO_2 production during the first 6 days of incubation (Birch-effect), the CO_2 evolution rates declined exponentially over time for all soil samples, with a maximum CO_2 release rate during the first two weeks (Supplementary Figure S1). The cumulative CO_2-C emission over the incubation period ranged from 4.1 to 62.8 mg CO_2-C g^{-1} OC (Figure 2). Cumulative C mineralization was highest (but differences were not significant) at Zhaltir, while Yasnaya Polyana and Losovoe showed similar lower cumulative C mineralization. For Yasnaya Polyana and Losovoe, grassland soils had significantly higher cumulative C mineralization than arable soils ($p<0.05$; Supplementary Table S2). Principal component (PC) analysis suggests that the difference can be mainly attributed to variations between sites in clay and sand, the relative contribution of fPOM-C and MAOM-C to bulk OC, and the OC:ON ratio of fPOM, with less influence of climatic factors (Figure 3). For Yasnaya Polyana, the proportion of fPOM-C and MAOM-C to bulk OC, and EO-P (PC1), and for Zhaltir and Losovoe especially the OC:ON ratio of bulk soil, and the proportion of fPOM-C and MAOM-C to bulk OC (PC2) were likely determinants controlling overall C mineralization (Figure 3). Together both PCs explained more than 56% of the variability. Of the variables under PC 1 and 2, the relative contribution of oPOM-C to bulk OC, the OC:ON ratio of fPOM, and the clay content were negatively correlated with the cumulative C mineralization ($r=-0.41$ to -0.43, $p<0.05$; Supplementary Table S4). In contrast, the relative contribution of fPOM-C to bulk OC, the OC:ON ratio of oPOM, and the sand content were positively correlated with the cumulative C mineralization ($r=0.42$ to 0.50, $p<0.05$; Supplementary Table S4).

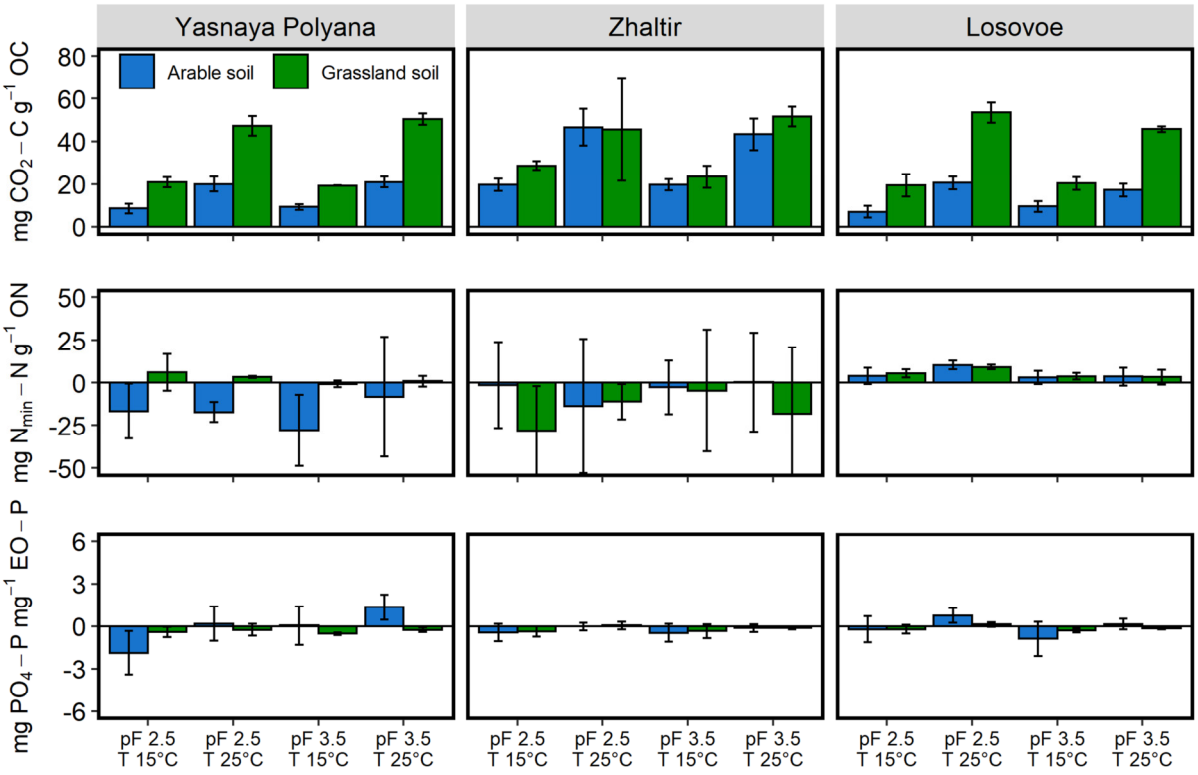

Figure 2: Cumulative carbon (C) mineralization (top row), net nitrogen (N) mineralization (middle row), and net phosphorous (P) mineralization (bottom row) after 126-day incubation of arable (blue) and grassland soils (green) at the three study sites. Mineralized C, N, and P were normalized to organic C (OC), organic N (ON), and extractable organic P (EO-P) of the respective soil samples. Treatments are given on the x-axis in the bottom row. Data are presented as means (n = 3) and standard deviation.

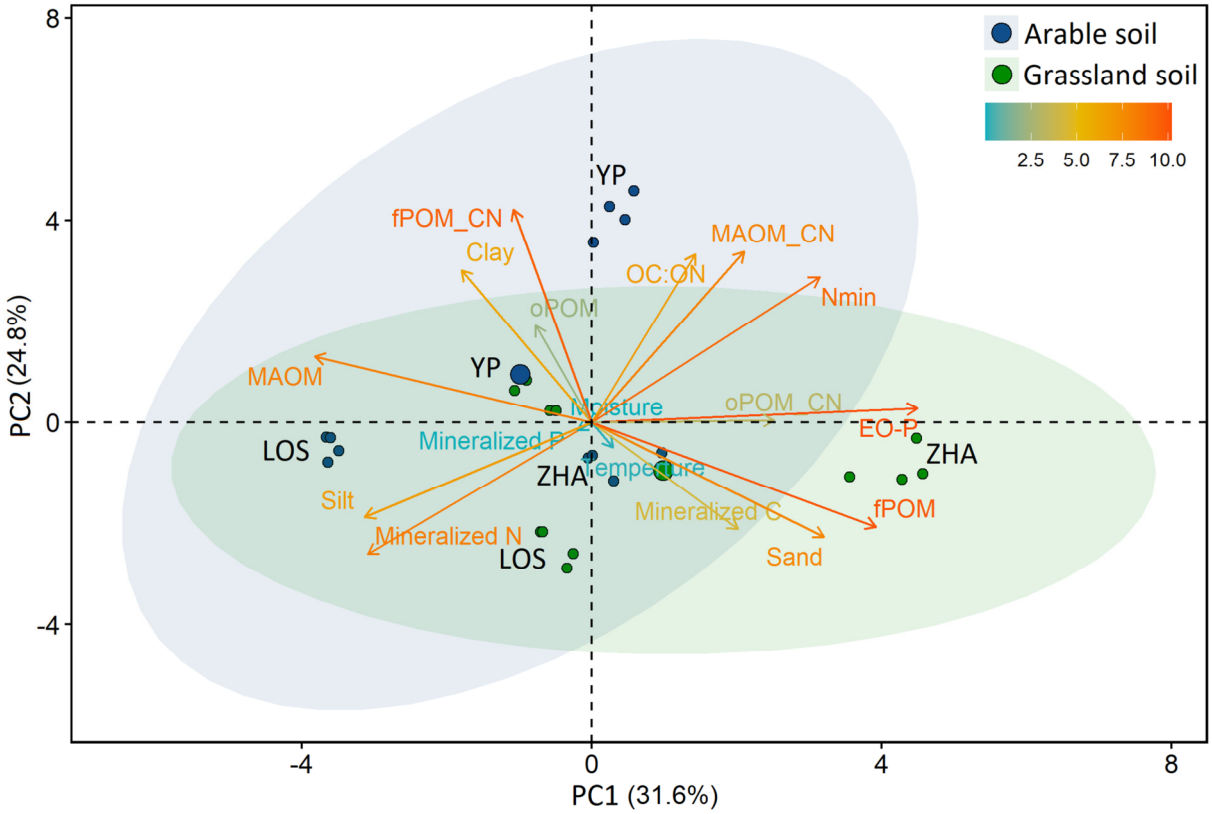

Figure 3: Biplots derived from principal component analysis (PCA) for each site. Colored symbols indicate land use: blue points refer to arable soil, green points to grassland soils. Arrows indicate contributing factors, and the strength of contribution is indicated by color; it increase from blue over yellow to red. Factors tested were: OC:ON of SOM fractions and bulk soil, initial mineral N (N_{min}), extractable organic P (EO-P), proportion of MAOM-C and POM-C and MAOM-C to bulk OC, sand, silt, clay, and the mineralized C, N, or P. Ellipses are 95 % confidence ellipses. Abbreviations are: YP: Yasnaya Polyana; ZHA: Zhaltir; LOS: Losovoe.

Higher temperature increased cumulative C mineralization significantly ($p<0.05$; Figure 2), whereas matric potential had no significant effect at any site (Figure 2, Supplementary Table S1). The Q_{10} values were similar for all sites and for both land use types and ranged from of 1.7 to 4.8 (Figure 5; mean 2.5). At Losovoe Q_{10} was affected by the matric potential but not at Yasnaya Polyana and Zhaltir (Supplementary Table S3).

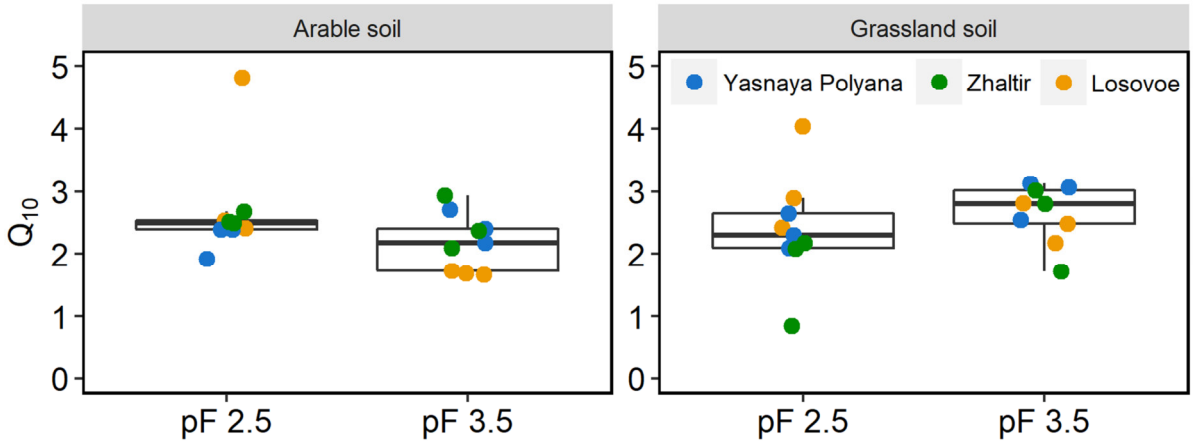

Figure 4: Q_{10} values of arable soils (left) and grassland soils (right) with individual sites being indicated by colored points. For the matric potentials pF 2.5 and pF 3.5, boxplots give the Q_{10} values of all samples.

3.3. Nitrogen mineralization

Mineralized N increased rapidly within the first 11 days, followed by slow and steady decrease over the next 114 days (Supplementary Figure S2). In general, there was net immobilization of N for arable soils from Yasnaya Polyana and grassland soils as well as arable soils from Zhaltir after 126 days of incubation, ranging between -1.4 and -28.1 mg g^{-1} ON (Figure 2). All other samples showed net N mineralization (0.9 to 10.5 mg g^{-1} ON). At Yasnaya Polyana, significantly more N was mineralized or consumed in arable soils than in grassland soils ($p<0.05$); N mineralization did not show significant differences between land uses at Zhaltir and Losovoe. Principal component analysis suggests that the magnitude of net N mineralization can be attributed to contents of silt and initial mineral N (possibly fertilizer-N), bulk soil OC:ON ratios, and OC:ON ratios of MAOM (Figure 3). Contents of initial mineral N, silt, and OC:ON ratios of MAOM showed the strongest correlations with net N mineralization ($r=-0.93$, 0.74, -0.64, $p<0.05$; Supplementary Table S5). Moderate correlations with net N mineralization were found for EO-P ($r=-0.40$, $p<0.05$), soil OC:ON ratios ($r=-0.47$, $p<0.05$), and the proportion of MAOM-C in bulk OC ($r=0.44$, $p<0.05$). No correlation was observed between net N mineralization and cumulative C mineralization. Temperature had no significant impact on net N mineralization (Figures 2, 3). Similar to C mineralization, matric potential neither affected N mineralization nor were there any interactions of matric potential with temperature

at Yasnaya Polyana and Zhaltir. In Losovoe, the lower matric potential led to significantly higher net N mineralization.

3.4. Phosphorus mineralization

In most samples there was no net P mineralization; normalized to EO-P the immobilization ranged from -1.9 to -0.1 mg PO_4-P mg^{-1} EO-P. A net P mineralization was only observed in some samples, mostly for Losovoe (0.1 to 1.4 mg PO_4-P mg^{-1} EO-P). Land use affected P mineralization significantly only at Losovoe ($p<0.05$), being higher for arable soils than for grassland soils. Net P mineralization or immobilization was not correlated to any other soil variable. In contrast, temperature and net P mineralization were positively correlated (Figure 3); the temperature effect was weak and not significant at Zhaltir and Losovoe but stronger at the northern Yasnaya Polyana site ($p<0.05$). Matric potential affected net P mineralization at Yasnaya Polyana but had no effect at Zhaltir and Losovoe. We did not observe interactions of matric potential and temperature on net P mineralization (Supplementary Table S1). Neither did we find correlations between P mineralization with C and net N mineralization.

3.5. Potential source of mineralized soil organic matter

The ^{14}C contents of fPOM, oPOM, MAOM, and bulk soil were in similar ranges for all sites and land uses (horizontal lines in Figure 5). Bulk soil exhibited ^{14}C contents of on average 91 pMC at all sites, usually arable soils had slightly lower ^{14}C contents than grassland soils (Figure 5). Highest mean contents were found for fPOM (102 ± 6 pMC), indicating the youngest SOM fraction. Occluded POM showed slightly lower mean values (97 ± 3 pMC) but lowest values were observed for MAOM (87 ± 4 pMC), confirming the stabilization of SOM by interactions with mineral surfaces. The $^{14}CO_2$ emission was measured at the beginning and the end of incubation (circles in Figure 5). All sites showed a similar $^{14}CO_2$ release pattern: on day 14, mean $^{14}CO_2$ contents were 101 ± 0 pMC (mean ± SD) for all sites and land uses, with the ^{14}C contents of grassland soils being slightly higher than for arable soils. At the end of

incubation, ^{14}C contents increased at all sites to 106 ± 0 and 102 ± 0 pMC for grassland soils and arable soils, respectively. This suggests that the contribution of bomb-derived C to respired CO_2, and thus of decadal cycling carbon, increases with incubation time, possibly as a result of the depletion of a more recently fixed active OC fraction. Apart from that general pattern, we did not observe a significant mobilization of old carbon by the applied temperature and moisture treatments. In general, the $^{14}CO_2$ activities fitted well the ^{14}C signature of fPOM but the lower contents at Losovoe grassland soils suggested partial mineralization of older MAOM and/or oPOM at the beginning of the incubation, possibly as a result of rewetting.

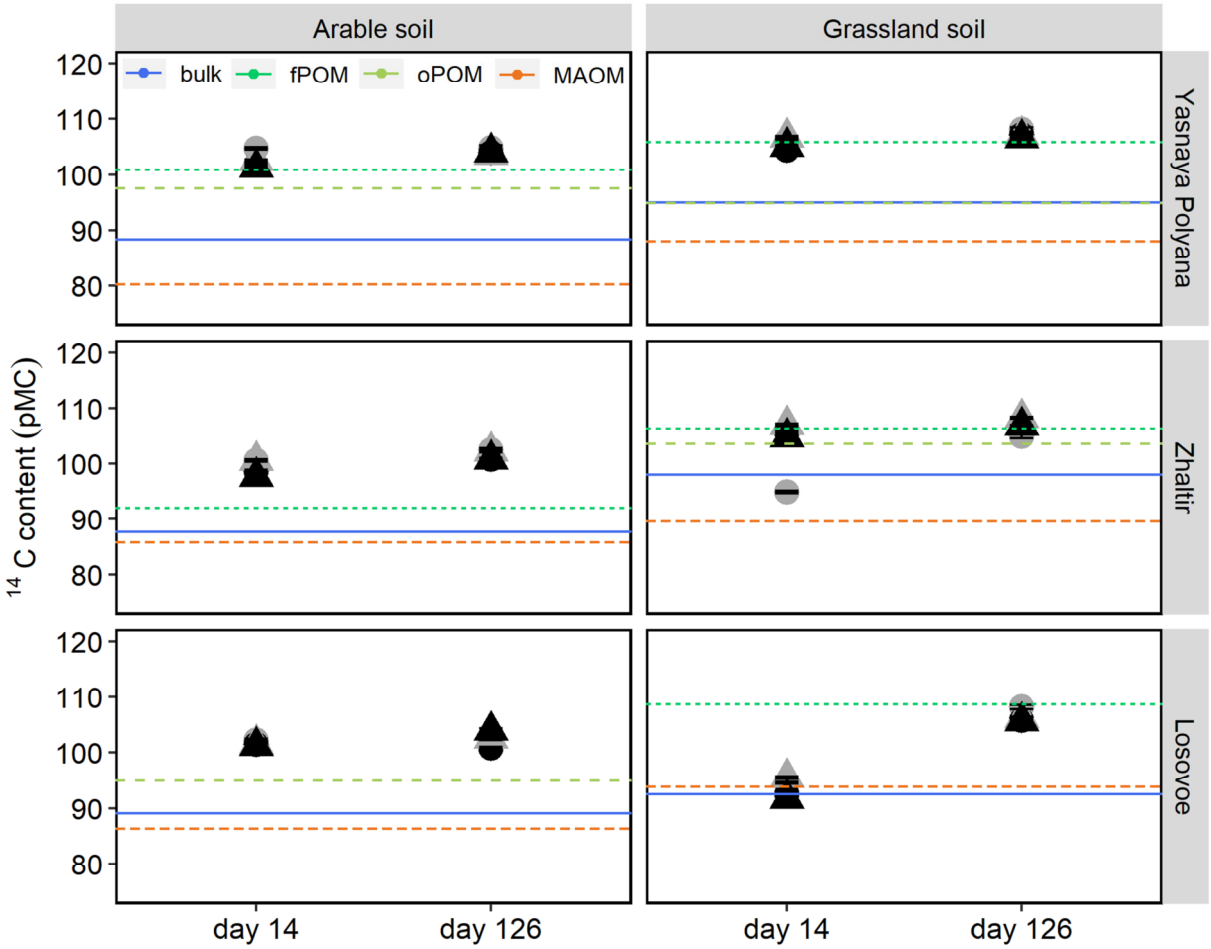

Figure 5: ^{14}C content of bulk soils and SOM fractions along with ^{14}C content of CO_2 emitted during the incubation of different treatments. Colored lines indicate the ^{14}C of free particulate organic matter (fPOM), occluded particulate organic matter (oPOM), mineral-associated organic matter (MAOM), and bulk soil. ^{14}C content of CO_2 of different treatments is represented by following symbols and colors: matric potential pF 2.5 (●) and pF 3.5 (▲), and temperature 25°C (grey) and 15°C (black) at day 14 and 126 of incubation. Data are presented as means (n = 3) and standard deviation. fPOM and oPOM fractions of Zhaltir (arable soil) and Losovoe (arable and grassland soil) are missing as too little material was available for ^{14}C analysis.

4. Discussion

4.1. General mineralization patterns

Cumulative C mineralization ranged from 4.1 to 62.8 mg CO_2-C g^{-1} of OC, of which 21 to 59% were mineralized due to the initial high mineralization during the first week of incubation (Birch effect). Net nutrient mineralization in these clay-rich soils (36 to 52% clay; Table 2) ranged between -28.1 to 10.5 mg g^{-1} ON, and -1.9 to 1.4 mg P mg^{-1} EO-P, with negative values showing immobilization (Figure 2). These values were small in comparison to values of potentially mineralizable C and N reported in other studies of northern Kazakhstan, where potentially mineralizable C and N was derived from fitting curves of C and N mineralization: Incubation studies for 70 to 133 days at 30°C and 50 to 60% water holding capacity revealed 5.8 and 12.8% of potentially mineralizable C (Karbozova-Saljnikov et al., 2004; Yanai et al., 2005; Takata et al., 2007; Kadono, Funakawa and Kosaki, 2008) and 9.4 and 13.7% of potentially mineralizable N in cropland and grassland soils (26 and 28% clay), respectively (Kadono et al., 2008). Also calculations based on data by Karbozova-Saljnikov et al. (2004) suggest as much as 3.2 to 7.9% of potential mineralizable N in cropland soils. However, these potentially mineralizable C and N values were fitted and not actual mineralized values as in our case. The high clay contents of our study soils may not only explain the relatively low C mineralization due to formation of mineral-organic associations (Sarkar et al., 2018), but likely contribute to the low net N mineralization. As expandable clay minerals are abundant (Table 2), partial interlattice fixation of NH_4^+ may have reduced the detection of the mineralized N (Allison and Roller, 1955; Scherer et al., 2014). Besides fixation by clays, the strong decline of mineralized N after the initial flush (Supplementary Figure S2) may also point at strong microbial immobilization (Geisseler et al., 2009). Values above unity for net P mineralization normalized to EO-P show that the P mineralization was larger than the EO-P fraction, indicating that also non-extractable P was microbial accessible.

4.2. Temperature and matric potential effect on mineralization under different land use

We hypothesized that the prognosed increase in soil moisture further accelerates SOM decomposition beyond the expected temperature response. In accordance with our second hypothesis, temperature strongly impacted C and less P mineralization (Figures 2, 4) while the effect of matric potential was generally minor for C, N, and P mineralization. The lacking effect of matric potential contrasts previous studies in semi-arid regions, reporting C and N mineralization increase with increasing water contents (Mi et al., 2015; Wang et al., 2006). But in accordance with our observations also Rocci et al. (2021) showed in a meta-analysis, that increased soil moisture had no effect on soil OC pools. No information on moisture effects on net P mineralization in semi-arid climate is available in literature, but if P is mineralized alongside C, as suggested by Spohn and Kuzyakov (2013), higher net P mineralization would be expected at higher matric potential. The negligible effect of matric potential in our study could be caused by the predominance of small pores in these clayey soils (Table 2). While the tested matric potentials (pF 2.5 and 3.5) reflect either wetter or drier conditions during the vegetation period, the difference in matric potentials may not be associated with large differences in absolute water contents, which ranged between 2.1 to 6.5 Vol%. These differences may be too small for triggering different microbial activities. Moyano et al. (2012) suggested that mineralization processes are more affected by differences in water tension than water content. But only few studies investigated the response of SOM mineralization to variable water tensions in Kazakh soils. Here, we show that, despite of huge changes in water tension, water contents in these clayey soils change only slightly and that these differences had little effect on mineralization.

The observed temperature sensitivity of C mineralization (Figure 4) corresponded well with literature in semi-arid to temperate arable and grassland soils (Briones et al., 2021; Funakawa et al., 2006; Ghimire et al., 2019), generally reporting higher C mineralization under elevated temperatures. Grassland soils contain larger contents of labile POM than arable soils (Table 2). Due to the similar quality of POM, Q_{10} values are similar (Figure 4), and the increase in C mineralization upon increased temperature may therefore basically a function of the soils'

POM content. Nitrogen mineralization was not significantly impacted by temperature at any site (Figure 2). This result is in contrast to findings that N mineralization increases with increasing temperature in grassland soils (Risch et al., 2019; Wang et al., 2006). The lacking temperature effect of N mineralization might be attributed to concurrent processes, such as immobilization by microorganisms and NH_4^+ fixation by clay minerals. The possible sorption of mineralization products calls for consideration of the soil mineral composition when assessing the temperature and moisture response of C, N, and P turnover in soils. Net P mineralization was less sensitive to temperature than C mineralization. This is in line with results of an incubation study of Prairie soils, showing little temperature effect on P mineralization for temperatures up to 30°C (Thompson and Black, 1948). Mineralized P can be immediately taken up by microorganisms (Bünemann et al., 2012) or sorbed by minerals (Gérard, 2016; McGechan and Lewis, 2002). These processes can result in low apparent net P mineralization, and thus, little apparent temperature sensitivity of P mineralization.

In summary, we cannot accept our second hypotheses that the temperature response of mineralization is accelerated by increased matric potential. An increase in temperature did only enhance C mineralization, while the matric potential apparently did not affect SOM mineralization in these clay-rich soils. Site specific factors, like the clay contents or quality of POM were stronger factors controlling SOM decomposition than temperature and matric potential.

4.3. Role of POM in SOM mineralization

We hypothesized that land use strongly affects mineralization, with less C, N and P mineralization in arable soils because of plant removal during harvest and subsequent depletion of SOM, especially of fresh litter-derived POM. In turn, POM was more available in grassland soils, resulting in higher C mineralization rates during incubation. In accordance with our first hypothesis, the effect of temperature on C mineralization was less pronounced for arable soils than for grassland soils (Figures 2, 5). These results are in line with observations of Ghimire et al. (2019) who observed a stronger increase in cumulative C mineralization at

elevated temperatures under grassland soils compared to arable soils. Carbon mineralization in the studied soils was linked to the decomposition of recent OM, indicating fPOM as primary source (Figures 3, 4; Table 2). Grassland soils contained 1.0 to 2.3-fold more POM-C than arable soils (Table 2), thus explaining the higher C mineralization rate in grassland soils than in arable soils (Figure 2). We did also not find evidence that considerably more MAOM-C was mineralized at higher temperature. Our results are in line with observations about SOM decomposition showing not only a higher lability but also a higher temperature sensitivity of POM than MAOM in various ecosystems (Benbi et al., 2014). In a simulation study on climate warming over a period of one decade, also Briones et al. (2021) did not find evidence for the mineralization of SOM with large mean residence time. Predominant C mineralization from the fPOM fraction suggests that also N and P mainly originated from this source. Due to the POM's wider C:N and C:P ratios, relatively small amounts of N and P would be released. In conclusion, we can confirm our first hypothesis that land use significantly affects the temperature sensitivity of SOM and C mineralization via controlling the content of labile plant residues.

4.4. Interaction of C, N, and P mineralization

We hypothesized that the net nutrient release from mineral soil is linked to the C:N:P ratio of SOM fractions. Above we have shown that overall C mineralization in the steppe soils is largely controlled by the fPOM fraction, suggesting that also parts of N mineralization and likely also of P release depends on this SOM fraction. However, we did not observe any evidence of coupling or direct correlation between C, N, and P mineralization. Assuming that CO_2-C evolution would only originate from fPOM as suggested by PCA (Figure 3) and radio-carbon dating (Figure 5), on average 59 ± 32% of fPOM-C would have been mineralized during the incubation. Neglecting the initial incubation phase (Birch effect), the C_{min}:N_{min} ratio during the days 7-126 deviated greatly and ranged from -1721 to 552 depending on treatment and site. Assuming an OC:ON ratio of fPOM of 20, based on the amount of mineralized C the estimated potential N release would have ranged between 158 and 1633 mg N g^{-1} ON. The

observed net N mineralization, however, ranged only between -28 and 11 mg g^{-1} ON (Figure 2), thus pointing at a large extent of microbial N uptake and/or N fixation by clay minerals.

We further showed, that temperature increased the mineralization of C and partly also of P, but not of N (Figures 2, 5). This observation can likely be explained by the different behavior of the mineralization products during incubation. While the CO_2 as the product of C mineralization was recovered in the headspace of the incubation flasks, NH_4^+ and PO_4^{2-} released by N and P mineralization can be immobilized by microorganisms or sorbed by minerals. Moreover, the extraction of mineralized N in 0.01 M $CaCl_2$ likely is not complete (Motavalli et al., 1995) and, similarly, Klotzbücher et al. (2019) showed, that over 90% of freshly mineral-sorbed PO_4^{2-} was not extractable in 0.5 M $NaHCO_3$. These methodological difficulties render it difficult to determine the coupling of C, N, and P mineralization and product ratios on basis of extractions.

5. Implications

Our study clearly shows that the release of CO_2 and mineral N and P of Chernozems and Kastanozems in North Kazakhstan is predominately linked to contents of fPOM. The ratios of released elements determined by classical methods, however, were not related to the elemental ratios of the SOM fractions, showing that the stoichiometry concept is not applicable to mineralization experiments with mineral soils with abundant reactive mineral phases present. For C mineralization, our results suggest enhanced SOM turnover with the predicted warming. This distinct temperature dependence was not found for net N and P mineralization. It appears that possible temperature dependence might have been obscured by microbial N and P uptake and/or sorption processes. The projected higher precipitation, here tested as higher matric potentials, does not seem to strongly alter mineralization in these clay-rich soils as differences in water contents were small and might not support strong changes in microbial activities. Nevertheless, our results imply that managed steppe soils might respond to increasing warming with increasing C losses, which ultimately requires climate-adapted soil management practices to mitigate decline in soil quality.

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Conflicts of Interest: The authors have no relevant financial or non-financial interests to disclose.

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Supplementary Material

Figures

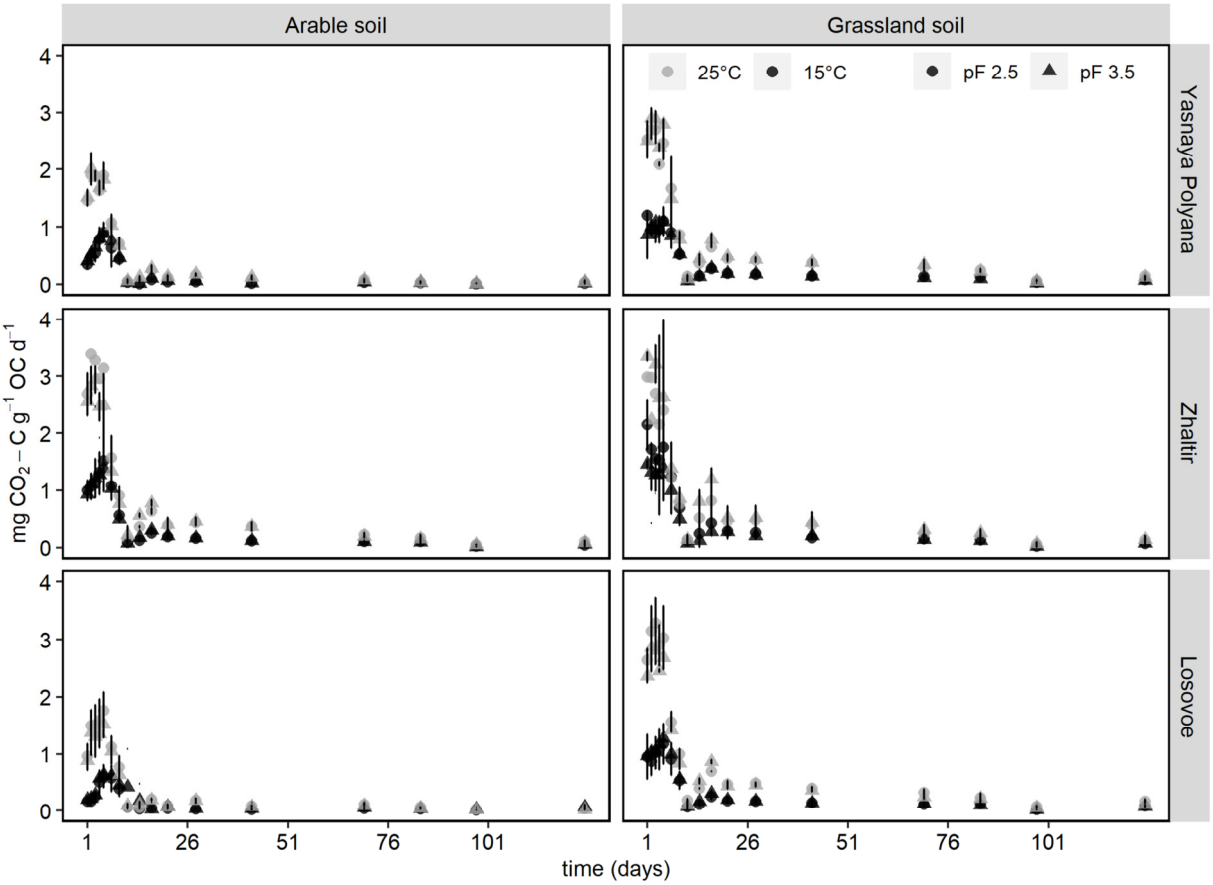

Figure S1: CO_2-C emission normalized to soil organic carbon during the incubation. Samples are split in land use (left to right) and sites (top to bottom). Circles (●) represent matric potential of pF 2.5, rectangles (▲) matric potential of pF 3.5. Each point represents the mean of n = 3, error bars show the corresponding standard deviation. Grey colors indicate the incubation temperature of 25°C and black colors that of 15°C. No data available for day 54 and 112 due to analytical problems.

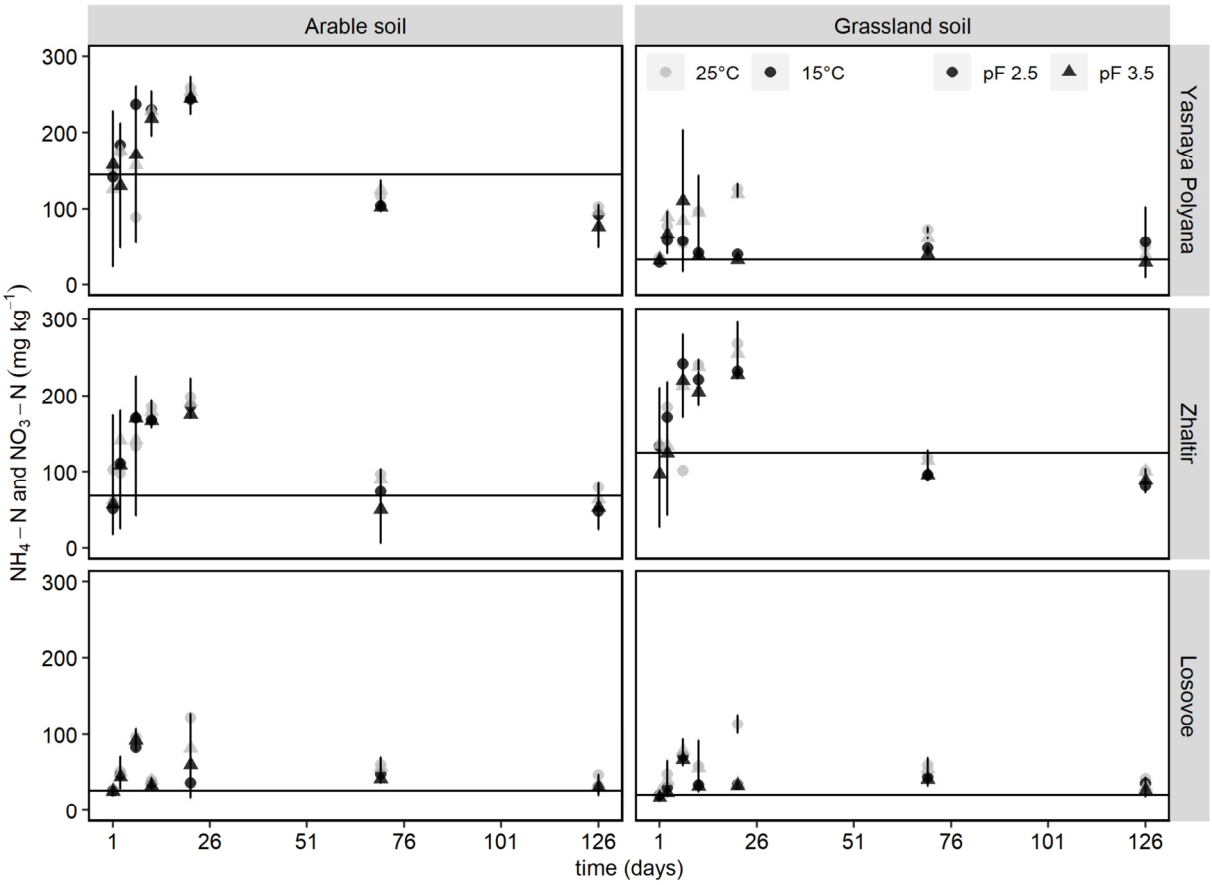

Figure S2: Mineralized N normalized to soil N during the incubation. Mineralized N was calculated as the sum of NO_3-N and NH_4-N. The contents of initial mineralized N are displayed as line in each graph. Samples are split in land use (left to right) and sites (top to bottom). Circles (●) represent matric potential of pF 2.5, rectangles (▲) matric potential of pF 3.5. Each point represents the mean of n = 3, error bars show the corresponding standard deviation. Grey colors indicate the incubation temperature of 25°C and black colors that of 15°C.

Tables

Table S1: Results of 2-way-ANOVAs with post-hoc Tukey HSD test for the influence of treatment for each site and individually for C, N, and P mineralization. Results of ANOVA and Tukey-test are ordered by sites Yasnaya Polyana (A-C), Zhaltir (D-F), Losovoe (G-I) and C (first), N (center), and P (last) mineralization. Only significant interactions are shown.

Yasnaya Polyana **A**

ANOVA mineralized C

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	4	4	0.03	0.87558	
Temperature	1	2427	2427	16.7	0.00057	***
Moisture:Temperature	1	9	9	0.06	0.80517	
Residuals	20	2906	145			

TukeyHSD - ANOVA mineralized N

Factors	comparison	diff	lwr	upr	p adj
Temperature	25-15°C	20.11	9.847	30.38	6.00E-04
Moisture:Temperature	pF3.5:25°C - pF 2.5:15°C	20.892	1.4134	40.37	0.03528
	pF 3.5:25°C - pF 3.5:15°C	21.3416	1.863	40.82	2.86E-02

Yasnaya Polyana **B**

ANOVA mineralized N

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	4.70E+01	4.70E+01	0.14	0.71	
Temperature	1	1.20E+02	1.20E+02	0.35	0.56	
Moisture:Temperature	1	2.24E+02	2.24E+02	0.66	0.43	
Residuals	20	6.82E+03	3.41E+02			

Yasnaya Polyana **C**

ANOVA mineralized P

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	3.33E+00	3.33	3.1	0.94	.
Temperature	1	5.47E+00	5.47	5.1	0.035	*
Moisture:Temperature	1	1.50E-01	0.15	0.14	0.71	
Residuals	20	2.15E+01	1.07			

TukeyHSD - ANOVA mineralized P

Factors	comparison	diff	lwr	upr	p adj
Temperature	25-15°C	0.9547	0.07269	1.837	3.53E-02
Moisture:Temperature	pF3.5:25°C - pF 2.5:15°C	20.892	1.4134	3.373	0.0458

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Zhaltir **D**

ANOVA mineralized C

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	2	2	0.03	0.87	
Temperature	1	3409	3409	38.04	5.00E-06	***
Moisture:Temperature	1	23	23	0.26	0.62	
Residuals	20	1793	90			

TukeyHSD - ANOVA mineralized N

Factors	comparison	diff	lwr	upr	p adj
Temperature	25-15°C	23.84	15.77	31.9	0.00E+00
Moisture:Temperature	pF 2.5:25°C - pF 2.5:15°C	21.868	6.57	37.17	0.036
	pF3.5:25°C - pF 2.5:15°C	23.208	7.909	38.51	0.0021
	pF 2.5:25°C - pF 3.5:15°C	24.466	9.168	39.76	1.20E-03
	pF 3.5:25°C - pF 3.5:15°C	25.806	10.508	41.1	0.0007

Zhaltir **E**

ANOVA mineralized N

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	3.12E+03	3.12E+02	0.41	0.53	
Temperature	1	1.40E+01	1.40E+01	0.02	0.89	
Moisture:Temperature	1	8.90E+01	8.90E+01	0.12	0.74	
Residuals	20	1.54E+05	7.70E+02			

Zhaltir **F**

ANOVA mineralized P

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	3.00E-02	0.03	0.21	0.65	
Temperature	1	7.36E-01	0.736	5.24	0.033	*
Moisture:Temperature	1	3.30E-02	0.033	0.23	0.635	
Residuals	20	2.81E+00	0.141			

TukeyHSD - ANOVA mineralized P

Factors	comparison	diff	lwr	upr	p adj
Temperature	25-15°C	0.3503	0.03108	0.6695	3.31E-02

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Losovoe **G**

ANOVA mineralized C

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	23	23	0.13	0.7201	
Temperature	1	2432	2432	14.23	0.0012	**
Moisture:Temperature	1	79	79	0.46	0.5055	
Residuals	20	3418	171			

TukeyHSD - ANOVA mineralized N

Factors	comparison	diff	lwr	upr	p adj
Temperature	25-15°C	20.13	9.002	31.27	1.20E-03
Moisture:Temperature	pF 2.5:25°C - pF 2.5:15°C	23.753	2.628	44.88	0.0241
	pF 2.5:25°C - pF 3.5:15°C	22.073	0.949	43.2	3.86E-02

Losovoe **G**

ANOVA mineralized N

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	90.9	90.9	8.48	0.0086	**
Temperature	1	38.5	35.5	3.59	0.0726	.
Moisture:Temperature	1	38.9	38.9	3.63	0.0712	.
Residuals	20	214.4	10.7			

TukeyHSD - ANOVA mineralized N

Factors	comparison	diff	lwr	upr	p adj
Moisture	pF 2.5 - pF 3.5	-3.892	-6.68	-1.104	8.60E-03
Moisture:Temperature	pF 2.5:25°C - pF 3.5:15°C	6.42518	1.1343	11.716	1.39E-02
	pF 3.5:25°C - pF 2.5:25°C	-6.43883	-11.7297	-1.148	0.0137

Losovoe **G**

ANOVA mineralized P

Factors	d.f.	sum squares	mean squares	F value	p	significance
Moisture	1	0.99	0.986	2.83	0.108	
Temperature	1	2.36	2.363	6.77	0.017	*
Moisture:Temperature	1	0.01	0.005	0.02	0.901	
Residuals	20	6.98	0.349			

TukeyHSD - ANOVA mineralized P

Factors	comparison	diff	lwr	upr	p adj
Temperature	25-15°C	0.6276	0.1245	1.131	1.70E-02
Moisture:Temperature	pF 2.5:25°C - pF 3.5:15°C	1.033	0.07836	1.9876	0.031

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table S2: Results of 1-way-ANOVAs with post-hoc Tukey HSD test for the influence of land use for each site and individually for C, N, and P mineralization. Results of ANOVA are ordered by sites Yasnaya Polyana (A-C), Zhaltir (D-F), Losovoe (G-I) and C (first), N (center), and P (last) mineralization.

Yasnaya Polyana **A**

ANOVA mineralized C

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	2343	2343	17.2	4.30E-04	***
Residuals	22	3003	136			

Comparison	Difference	lwr	upr	p adj
Grassland soil : arable soil	19.76	9.869	29.65	4.00E-04

Yasnaya Polyana **B**

ANOVA mineralized N

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	2.38E+03	2.38E+03	10.9	0.0033	**
Residuals	22	4.83E+03	2.19E+02			

Comparison	Difference	lwr	upr	p adj
Grassland soil : arable soil	19.93	7.388	23.47	0.0033

Yasnaya Polyana **C**

ANOVA mineralized P

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	5.00E-01	5.02E-01	0.37	0.55	
Residuals	22	2.99E+01	1.36E+00			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Zhaltir **D**

ANOVA mineralized C

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	154	154	6.70E-01	4.20E-01	
Residuals	22	5073	231			

Comparison	Difference	lwr	upr	p adj
Grassland soil : arable soil	1.014	0.641	1.387	0

Zhaltir **E**

ANOVA mineralized N

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	7.50E+02	7.50E+02	1.1	0.31	
Residuals	22	1.51E+04	6.85E+02			

Zhaltir **F**

ANOVA mineralized P

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	0.03	0.0272	0.17	0.69	
Residuals	22	3.58	0.1628			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Losovoe **G**

ANOVA mineralized C

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	2675	2675	18	0.00034	***
Residuals	22	3277	149			

Comparison	Difference	lwr	upr	p adj
Grassland soil : arable soil	21.11	10.78	31.45	3.00E-04

Losovoe **H**

ANOVA mineralized N

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	0.00E+00	3.30E-01	0.02	0.89	
Residuals	22	3.82E+02	1.74E+01			

Losovoe **I**

ANOVA mineralized P

Factors	d.f.	sum squares	mean squares	F value	p	significance
Land use	1	0.05	0.046	0.1	0.76	
Residuals	22	10.29	0.46			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table S3: Results of Kruskal-Wallis test for the influence of land use and matric potential on the Q10 value for each site individually. Results are ordered by sites Yasnaya Polyana (A), Zhaltir (B), Losovoe (C).

Yasnaya Polyana **A**

Kruskal-Wallis Q10

Factors	df	chi-square	p
Land use	1	1.3	0.3
Moisture	1	3.7	0.05

Zhaltir **B**

Kruskal-Wallis Q10

Factors	df	chi-square	p
Land use	1	0.3	0.6
Moisture	1	0.3	0.6

Losovoe **C**

Kruskal-Wallis Q10

Factors	df	chi-square	p
Land use	1	1.6	0.2
Moisture	1	4.3	0.04

Table S4: Correlation analysis matrix. Given is the correlation coefficient (upper triangle) and the significance (lower triangle). Abbreviations are: OC: organic carbon; initial N_{min}: initial mineralized N; ON: organic nitrogen; EO-P: extractable organic P; C_{min}: mineralized C; N_{min}: net mineralized N; P_{min}: net mineralized P; fPOM: free particulate organic matter; oPOM: occluded organic matter; MAOM: mineral-associated organic matter. Free and occluded POM and MAOM represented as percentage of OC and in with molar OC:ON ratio. Significant correlations are highlighted by bold numbers.

	C_{min}	N_{min}	P_{min}	OC	ON	initial N_{min}	OC:ON	Olsen-P	EO-P	Sand	Silt	Clay	fPOM	oPOM	MAOM	fPOM-CN	oPOM-CN	MAOM-CN
C_{min}		0.05	0.22	0.08	0.05	-0.02	0.02	-0.21	0.26	**0.42**	0.05	**-0.43**	**0.50**	**-0.41**	-0.34	**-0.43**	**0.47**	0.00
N_{min}	0.82		0.09	0.03	0.13	**-0.93**	**-0.47**	**-0.76**	**-0.40**	-0.26	**0.74**	-0.06	-0.31	-0.23	**0.44**	-0.33	-0.16	**-0.64**
P_{min}	0.31	0.66		-0.11	-0.09	0.04	-0.07	-0.05	0.21	-0.03	-0.04	0.05	-0.05	0.16	-0.02	0.05	-0.15	-0.07
OC	0.70	0.89	0.62		**0.99**	-0.02	**0.69**	-0.14	0.05	**-0.49**	0.05	**0.46**	-0.14	-0.30	0.29	0.36	**0.72**	**0.46**
net WEOC	0.07	0.96	0.32	0.41	-0.16	0.11	-0.10	0.08	-0.21	-0.07	0.00	0.07	-0.16	0.26	0.05	0.12	-0.29	-0.08
ON	0.81	0.53	0.69	**0.00**		-0.13	**0.60**	-0.24	-0.01	**-0.55**	0.11	**0.49**	-0.20	-0.32	0.36	0.33	**0.66**	0.35
net WETN	**0.00**	0.52	0.06	**0.02**	**0.02**	-0.08	0.29	-0.26	0.09	-0.11	0.16	0.04	0.03	-0.31	0.11	0.00	**0.46**	0.16
initial N_{min}	0.93	**0.00**	0.84	0.92	0.55		**0.51**	**0.88**	**0.49**	0.25	**-0.83**	0.11	0.29	0.32	**-0.46**	**0.42**	0.12	**0.71**
OC:ON	0.91	**0.02**	0.74	**0.00**	**0.00**	**0.01**		**0.51**	0.32	-0.15	-0.21	0.24	-0.11	0.12	0.06	**0.71**	**0.54**	**0.87**
TP	0.33	**0.00**	0.82	0.52	0.26	**0.00**	**0.01**		**0.45**	0.25	**-0.66**	0.04	0.09	0.39	-0.28	**0.53**	-0.07	**0.75**
EO-P	0.22	**0.05**	0.33	0.80	0.97	**0.02**	0.13	**0.03**		**0.52**	**-0.59**	-0.26	**0.70**	-0.16	**-0.67**	-0.16	**0.51**	**0.42**
Sand	**0.04**	0.22	0.87	**0.02**	**0.01**	0.24	0.48	0.24	**0.01**		-0.16	**-0.91**	**0.76**	-0.08	**-0.77**	**-0.54**	0.11	0.00
Silt	0.82	**0.00**	0.86	0.83	0.60	**0.00**	0.33	**0.00**	**0.00**	0.45		-0.27	**-0.49**	-0.04	**0.54**	-0.09	-0.21	-0.46
Clay	**0.03**	0.78	0.82	**0.02**	**0.01**	0.60	0.27	0.87	0.23	**0.00**	0.20		**-0.53**	0.10	**0.52**	**0.57**	-0.02	0.20
fPOM	**0.01**	0.14	0.80	0.51	0.36	0.17	0.62	0.68	**0.00**	**0.00**	**0.02**	**0.01**		-0.36	**-0.90**	**-0.67**	**0.52**	0.00
oPOM	**0.05**	0.27	0.46	0.16	0.13	0.12	0.57	0.06	0.47	0.70	0.84	0.65	0.08		-0.09	**0.56**	**-0.62**	-0.03
MAOM	0.11	**0.03**	0.94	0.17	0.08	**0.02**	0.79	0.19	**0.00**	**0.00**	**0.01**	**0.01**	**0.00**	0.68		**0.45**	-0.26	0.02
fPOM-CN	**0.04**	0.11	0.83	0.08	0.12	0.04	**0.00**	**0.01**	0.47	**0.01**	0.68	**0.00**	**0.00**	**0.00**	**0.03**		-0.15	**0.62**
oPOM-CN	**0.02**	0.47	0.47	**0.00**	**0.00**	0.59	**0.01**	0.75	**0.01**	0.61	0.33	0.94	**0.01**	**0.00**	0.22	0.48		**0.44**
MAOM-CN	0.99	**0.00**	0.73	**0.03**	0.09	**0.00**	**0.00**	**0.00**	**0.04**	0.99	0.02	0.35	0.99	0.88	0.93	**0.00**	**0.03**	

4. Study III

Competition of Plants and Microorganisms for Added Nitrogen in Different Fertilizer Forms in a Semi-Arid Climate

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Contribution: I was equally involved in the experimental design, field sampling campaigns, sample preparation, conduction of measurements, evaluation of data, data presentation and writing in the manuscript.

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Abstract

In nitrogen (N) -limited agricultural systems, a high microbial immobilization of applied fertilizer-N can limit its availability to plants. However, there is scarce information on the effect of the form of fertilizer used on the plant–microorganism competition in clay-rich soils under a severe semi-arid climate. In a field study, we investigated the wheat–microorganism competition after the direct application of $NH_4{}^{15}NO_3$ closely to seeds in arable fields in North Kazakhstan, documenting the effect of the use of liquid versus granular fertilizer and mini-tillage versus no-tillage. Our results barely showed any fertilizer-N translocation in the soil. Plants outcompete microorganisms for fertilizer-N during the vegetation period. Microbial-to-plant ^{15}N ratios revealed a predominant fertilizer-^{15}N uptake by plants. The strong competition for N was mainly related to the placement of the fertilizer close to the seeds. Moreover, the long time interval between fertilization and sampling enhanced the competition for N, meaning that previously microbially immobilized N became available to plants through the death of microorganisms and their subsequent mineralization. The fertilizer distribution between microorganisms and plants did not depend on the form of fertilizer used, owing to the good solubility of granular fertilizer. The smaller fertilizer-N uptake under the no-tilling condition was probably due to the more intense soil compaction, which caused a reduction in plant growth. The application of fertilizer close to the seeds and the small fertilizer translocation during the vegetation period ultimately resulted in a high level of plant-N being derived from the fertilizer.

Keywords: liquid fertilizer; granular fertilizer; mini-till; no-till; ammonium nitrate; ^{15}N

1. Introduction

Due to its co-variation, in semi-arid regions, besides water, nitrogen (N) is a major factor limiting the productivity and quality of wheat (Hooper and Johnson, 1999), as well as the growth and metabolism of microorganisms (Cui et al., 2018; Harder and Dijkhuizen, 1983). Consequently, plants and microorganisms may strongly compete against each other for available N (Kuzyakov and Xu, 2013). Microbial N immobilization occurs when the C:N ratio

of the decomposed substrate is higher than that of microorganisms (after taking the already microbially respired CO_2 into account) (Hodge et al., 2000a). Due to their larger surface area-to-volume ratio and rapid growth, microorganisms have been assumed to out-compete plants for N (Hodge et al., 2000a). In annual grasslands, microorganisms can assimilate nitrate (Jackson et al., 1989) and ammonium (Schimel et al., 1989) two and nine times faster than plants within the first 24 h after N application. Microorganisms may directly assimilate fertilizer N after wetting in spring (Schimel et al., 2007; Xiang et al., 2008), whereas crop plants acquire N mainly in the vegetative and reproductive growth stages (B. Chen et al., 2014). Hence, combined seeding and fertilization may be problematic for the efficient N use of plants.

The competition of plants and microorganisms for N is mostly tested by the application of ^{15}N-labeled ammonium, nitrate, or amino acids to the soil N pool and then measuring the ^{15}N in plants and organic and inorganic N forms a few hours to days (short-term) or weeks to months (long-term) later (Kaye and Hart, 1997). In ^{15}N studies on temperate humid and Mediterranean grassland soils, microorganisms were found to be strong competitors and reported to assimilate >60% of added N (Grace et al., 1993; Hodge et a., 2000a; Harrison et al., 2008) In a field experiment carried out in tallgrass prairie, up to 46% of added ^{15}N was found to be immobilized by microorganisms (Williams et al., 2001). However, after the rapid initial N capture of microorganisms, C limitation causes a halt in microbial growth and hence N acquisition (Hodge et al., 2000a). Several days to months after the N application, in situ and in vitro studies in humid and temperate grassland soils showed that most (45% to 96%) of the added ^{15}N was recovered in plants, whereas only 0% to 15% was recovered in microbial biomass (Grace et al., 2003; Kaye and Hart, 1997; Hodge et al., 2000a, b). Hence, in the long run plants out-competed microorganisms, as shown in semi-arid prairie (USA) and steppe soils (Inner Mongolia) (Hodge et al., 2000a; Wu et al., 2011; Chen et al., 2015). Due to microbial death and reassimilation, microbial assimilated N may several times contribute to the soil N pool. Consequently, formerly microbially assimilated N reenters the plant–microorganism competition, whereas plants store captured N over longer time periods

(Hodge et al., 2000a; Kaye and Hart, 1997). Hence, in the long run plant–microbe N competition is the result of numerous short-term competitions (Kaye and Hart, 1997) and mainly governed by the residence time of N in both pools (Wu et al., 2011).

Though the short- and long-term competition of plants and microorganisms has been well investigated in temperate and humid climatic conditions, there is a gap in our knowledge concerning how the application of different fertilizer forms and land use management types may affect the plant–microorganism competition in a semi-arid climate. In general, liquid N fertilizer can enhance nutrient uptake and yield as compared to its granular counterparts, as shown in Mediterranean Australia (Holloway et al., 2006, 2001). This is because of the immediate availability of nutrients and higher diffusion occurring in the soil when applied in liquid form, as shown under more humid conditions (Holloway et al., 2001; Pittawy et al., 2015). The widely adapted conservational tillage (mini-till and no-till) used in Kazakhstan improved biological and physical parameters in the soil compared to conventional tillage (Karbozova-Saljnikov et al., 2004). No-till soil management does not include any soil management until after the last harvest. At sowing time, seeds are directly seeded. Under mini-till management, in contrast, the top soil layer is shallowly managed up to a few cm by a cultivator before sowing is completed. For both conservational tillage forms, favorable conditions for microbial growth have been found (Karbozova-Saljnikov et al., 2004). However, it is unknown to what extend the presumed better N availability of liquid fertilizer impacts the plant–microorganism competition for N in different conservational tilled croplands. Our objective was thus to test the plant–microorganism competition for N supplied in liquid and granular form during seeding carried out under field conditions using the tillage forms of no-till and mini-till in semi-arid North Kazakhstan. North Kazakhstan was chosen as our study region as it represents a global bread basket (FAO, 2019; Swinnen et al., 2017). However, there have only been a few studies on the N turnover in this huge area to date. Soils in this region were formerly subjected to unsustainable land use for many years, which resulted in soil degradation (Kraemer et al., 2015; Mizina et al., 1999; Muratova and Terekhov, 2004) and low contents of mineralized N (Vasilchenko, 2014). We hypothesized

that (i) the use of liquid fertilizer can increase plant growth and N uptake in these semi-arid regions, regardless of the tillage form used, thus increasing plant competition for N in the long run. Furthermore, we assume that (ii) the plant–microorganism competition occurring in semi-arid, clay-rich soils is more severe than that reported for more humid regions.

2. Materials and methods

2.1. Study site and basic soil characteristics

In 2019, a ^{15}N labeling experiment was conducted on the site of the Scientific and Production Center for Grain Farming, which is named after A. I. Baraev, Shortandy, Akmola District, Northern Kazakhstan. The area belongs to the semi-arid steppe zone, with a mean annual temperature and mean annual precipitation of 1.8 °C and 324 mm (Baraev Institute), respectively. During the 2019 vegetation period, from the end of May to the beginning of September the mean daily temperature was 16.4 °C and the total cumulative precipitation amounted to 92 mm (Figure 1). For both fields, similar basic soil parameters were observed, without any significant differences being noted in any soil parameter for each soil increment (Table 1). Soils were shown to be fine textured (silt loam) throughout the soil profile, with high contents of clay and silt. The no-till field showed 4% higher total C and 6% higher total N contents on average than the mini-till field. However, in the no-till field the surface soil (0–20 cm) was more compacted (1.3 g cm^{-3}) than in the mini-till field (1.1 g cm^{-3}). Soils are characterized as Southern Chernozems according to the local soil classification and as Typic Haplustolls after US Taxonomy (Takata et al., 2007, 2008).

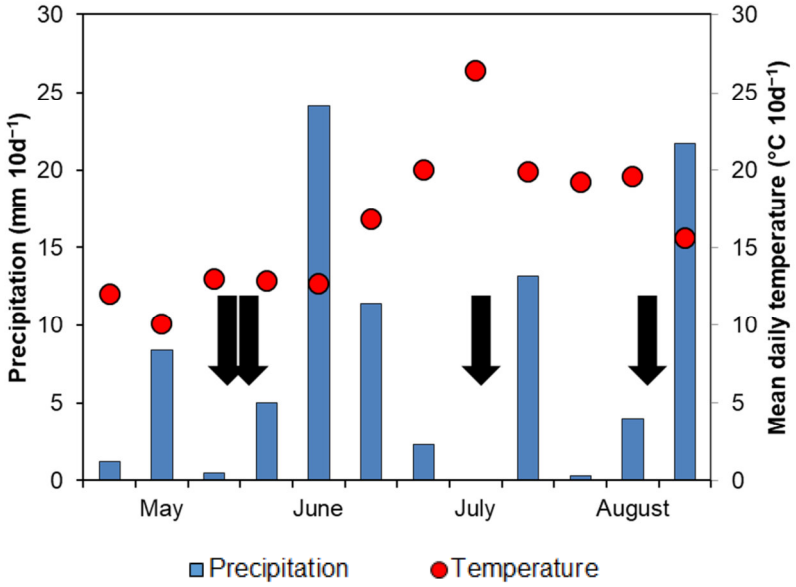

Figure 1: Climate data for the vegetation period 2019 in Shortandy, Kazakhstan. The cumulative precipitation and mean daily temperature are given in 10-day intervals. Samplings are indicated by vertical black arrows.

Table 1: Basic soil parameter for no-till and mini-till management for the soil depths of 0–20, 20–40, and 40–60 cm. Values were determined before the start of the experiment in May 2019. Texture was determined by pipette analysis (Köhn, 1928). The means of n = 2 samples ± the standard deviation are given.

Tillage	Depth	Clay	Silt	Sand	Total C	Total N	C/N	Bulk Density
	(cm)			(g kg^{-1})			(-)	(g cm^{-3})
Mini-till	0–20	369 ± 17	603 ± 11	28 ± 6	29.1 ± 0.1	2.2 ± 0.0	13.2 ± 0.1	1.1 ± 0.1
	20–40	419 ± 22	552 ± 13	29 ± 8	27.5 ± 0.3	1.9 ± 0.0	14.7 ± 0.1	1.2 ± 0.0
	40–60	420 ± 29	556 ± 27	24 ± 3	26.4 ± 0.5	1.5 ± 0.1	17.2 ± 0.7	1.3 ± 0.0
No-till	0–20	406 ± 12	570 ± 16	24 ± 4	29.9 ± 0.3	2.3 ± 0.0	12.8 ± 0.0	1.3 ± 0.1
	20–40	395 ± 19	583 ± 18	22 ± 2	29.9 ± 1.6	2.1 ± 0.1	14.5 ± 0.3	1.1 ± 0.1
	40–60	404 ± 2	567 ± 1	29 ± 3	26.8 ± 0.4	1.5 ± 0.0	17.6 ± 0.2	1.2 ± 0.0

2.2. Experimental setup and sampling

Two adjacent arable fields were chosen for the ^{15}N labeling experiment. One field was managed under mini-till (51°35.585′ N, 071°03.624′ E), while the other field was managed under no-till and direct seeding (51°35.615′ N, 071°03.707′ E) for at least 20 years. Shortly before seeding, the mini-till field was mechanically tilled to a 5–6 cm depth, while at the same time the no-till field was treated with glyphosate. In the mini-till field, 1.95 t ha^{-1} of straw remained in the field after the last harvest, while in the no-till field the amount was 2.15 t ha^{-1}. The C/N in shoots in the mini-till field was significantly higher than that in the no-till field

(Table 2). Both fields were simultaneously seeded and fertilized with granular ammonium nitrate at a rate of 20 kg N ha^{-1}. Both fields were in a wheat–wheat–fallow crop rotation, with the last fallow period occurring in 2017.

Table 2: Carbon-to-nitrogen ratio in plant compartments of the leaf and shoot, as determined at the last sampling time point in August. The means of n = 3 samples ± the standard deviation are given. Superscripted letters indicate statistically significant differences between treatments (tillage form × fertilizer form).

Tillage Form	Fertilizer Form	Compartment	C/N
Mini-till	Liquid	Leaf	46.9 ± 8.1 [a]
		Shoot	120.6 ± 13.7 [a]
Mini-till	Granular	Leaf	51.8 ± 9.5 [a]
		Shoot	116.9 ± 24.3 [a]
No-till	Liquid	Leaf	42.1 ± 2.3 [a]
		Shoot	82.5 ± 12.8 [a]
No-till	Granular	Leaf	45.3 ± 3.3 [a]
		Shoot	94.4 ± 18.5 [a]

On 30 May 2019, summer wheat (*Triticum aestivum subsp. Aestivum*) was directly seeded by a drill cultivar (Condor 1201 C, Amazone, Hasbergen, Germany) at a seeding rate of 120 kg ha^{-1} with a furrow distance of 25 cm. In total, we tested two treatment pairs (granular versus liquid fertilization, mini-till versus no-till) and their interactions. Within each of the two fields with different tillage conditions, 8 subplots with the size of 75 × 130 cm were established randomly. Of these, six subplots were used to test the effectiveness of the fertilizer form used (liquid versus granular) in triplicate, while two subplots served as controls. All subplots were trenched to the depth of 30 cm and isolated with plastic foil. This was carried out to avoid (i) fertilizer-N uptake by neighboring plants, (ii) horizontal leaching, and (iii) the surface runoff of fertilizer-N. The cut-off zones of these trenches were refilled with soil and re-seeded by hand outside of each plot.

Ammonium nitrate fertilizer ($NH_4{}^{15}NO_3$, 16.7 at% excess ^{15}N) was prepared by mixing 98 at% $NH_4{}^{15}NO_3$ (Campro Scientific, Berlin, Germany) with commercial unlabeled NH_4NO_3 (calculated after (Cabrera and Kissel, 1989)), which was applied to the soil on the day of seeding. To apply the fertilizer in liquid form, the $NH_4{}^{15}NO_3$ was dissolved in distilled water prior to fertilization. Granular fertilizer was produced by pressing the $NH_4{}^{15}NO_3$ at 100 bar for

9 min in a FTIR IR press (Beckman, 00-25, Glenrothes, UK). Labeled fertilizer was applied to each subplot by hand in the furrow (3 furrows per subplot) at a rate of 20.5 kg N ha^{-1} with a seeding depth of 3 cm directly next to the seeds. Fertilizer application should, in both cases, mimic realistic fertilizer application in Kazakhstan. For the liquid fertilization, 0.1 mL of dissolved $NH_4{}^{15}NO_3$ (5.8 M) was applied every 3 cm using a Ripette® (Ritter, Schwabmünchen, Germany). For granular fertilizer, fertilizer tablets were applied in the furrow at distances of 20 cm. For this, tablets were crushed and mixed with the surrounding soil over a length of 5 cm in each direction within the furrow (we made sure that seeds were not relocated). Hence, the actual distance between the fertilizer granules was about 10 cm, but fertilizer hotspots might have developed. Granular fertilizer was applied in this way because, over the past few years, we had usually observed the fertilization of granular fertilizer along the furrows in Kazakhstan and no continuous bands of fertilizer. Control subplots received unlabeled commercial ammonium nitrate fertilizer (GOST 2-2013) in liquid and granular form.

2.3. Soil and plant sampling

Soils from all subplots were sampled four times during the vegetation period. The first two samplings were carried out two days before and two days after seeding and fertilization. In these sampling points, the soils of each subplot were sampled at three locations directly under the furrow with cylindrical cores. Therefore, in each sampling spot the soil was dug until a 60 cm depth was reached. In each 0–20, 20–40, and 40–60 cm soil depth increment, three cylindrical cores were taken and each homogenized to a mixed sample. For the 0–20 cm soil depth increment, the first 0–4 cm of topsoil was not included in the soil sampling in order to avoid the direct sampling of fertilizer. Plant residues were removed from the soil, and samples were oven-dried at 40 °C.

The third and fourth soil samplings were conducted in July and August during the steam extension stage and the ripening stage of plant growth. These sampling time points were selected as nitrogen was highly allocated within the plants at these vegetation stages

(Beathgen and Alley, 1989; B. Chen et al., 2014). To account for the spatial heterogeneity of fertilizer distribution in the soil, subplots were sampled from three spatially distributed 20 × 25 cm microplots within each subplot. In these microplots, soil samples were taken with an N_{min} corer on two randomly chosen 5 × 5 cm squares, meaning that furrows, hills, and their interspace were sampled. Both these samples were homogenized in a mixed sample (a total of nine soil samples were generated from each subplot). Plant residues were removed and soil samples were oven-dried at 40 °C.

Aboveground wheat plant biomass growing in the furrow was collected from each microplot by cutting off the plants directly at the soil surface. Fresh plants were then separated into shoots, leaves, and grains. Each plant compartment was weighted in its fresh and 40 °C oven-dried state to determine the plant biomass.

2.4. $\delta^{15}N$ analysis

Nitrate was extracted from fresh soil samples within 30 h after sampling in a 12.5 mM $CaCl_2$ solution with a soil-to-volume ratio of 1:4 (*w:v*) (VDLUFA, 2002). Extracts were shaken for 1.5 h and filtered <0.45 µm using cellulose acetate syringe filters (Berrytech, Grünwald, Germany). All extracts were poisoned with $HgCl_2$ (350 mg L^{-1}) to prevent microbial N transformation. Extracts were stored cold in the dark until their content of nitrate and its ^{15}N abundance were determined in VCl_3 using the SPINMas technique (Stange et al., 2007). Nitrate was our main focus because of the rapid oxidation of ammonium to nitrate. Ammonia was not measured because, over our last few years of working in Kazakhstan, we always found very minor ammonium contents in these clayey and dry soils. It was also reported that the NH_4-N contents in the soils of Northern Kazakhstan are usually small and do not exceed 3 to 4 mg kg^{-1} (Vasilchenko, 2014; Черненок and Грицких, 1998).

Unfortunately, it was not possible to directly measure microbial biomass N and ^{15}N (MBN) in fresh samples; thus, these were determined in air-dried soil samples (Brookes et al., 1985b; Franzluebbers et al., 1999; Schroeder et al., 2021; Zagal, 1993). The microbial biomass N in soil samples from a 0–20 cm depth was analyzed using a modified chloroform–

fumigation–extraction (CFE) method, which includes pre-extraction with 0.05 M K_2SO_4 prior to the CFE procedure (Brookes et al., 1985b; Müller and Fragstein und Niemsdorff, 2006). Microbial biomass N was determined at a 0–20 cm depth in order to investigate the microbial fertilizer immobilization directly at the application spot. Pre-extraction should remove high amounts of mineralized N forms to enhance the determination of possibly small microbial ^{15}N. About 30 g of each dried sample was rewetted to a 60% water holding capacity and incubated for 2 weeks at room temperature. Afterwards, the samples were sieved <2 mm and the water contents were determined. Each sample was divided into two equal parts. Both aliquots were pre-extracted with 0.05 M K_2SO_4 (1:4 *w:v*) for 30 min by horizontal shaking at 200 rev. min^{-1}. Afterwards, one sample was fumigated to cause the lysis of microbial cells; the other sample was not fumigated. Fumigation was conducted under vacuum (800 mbar) for 24 h at 25 °C with ethanol-free chloroform in a desiccator. After chloroform was removed, the fumigated and unfumigated samples were extracted with 0.5 M K_2SO_4. Extracts were then filtered (Satorius hw3, Göttingen, Germany) before measurement to determine their total organic carbon (TOC) and total nitrogen (TN) contents on a multiN/C 2100S automatic analyzer (Analytik Jena AG, Jena, Germany). Afterwards, the extracts were freeze-dried and measured on an EA-IRMS (vario ISOTOPE elemental analyzer coupled with an isoprime precisION isotope ratio mass spectrometer, Elementar Analysensysteme GmbH, Langenselbold, Germany) to determine their N contents as well as $\delta^{15}N$ ratios.

To determine the total ^{15}N abundance in soil and plants, all samples were dried at 40 °C. Soil samples were sieved <2 mm and visible plant material was removed. Plant samples were shredded. All samples were milled for isotope analysis on the EA-IRMS.

2.5. Data and statistical analysis

All data analyses were carried out in R 3.6.3. (R Core Team, 2020) and Excel 2010 (Microsoft). $\delta\ ^{15}N$ ratios were transformed into at% as:

$$at\% = (100 * AR * (\delta^{15}N/1000 + 1))/(1 + (AR * (\delta^{15}N/1000 + 1))) \quad (1)$$

where at% is the atomic percentage of ^{15}N, AR is the absolute ratio of mole fractions of ^{15}N in air of 0.0036764 (Coplen, 2002), and $\delta^{15}N$ is the ratio of ^{15}N to ^{14}N in a sample to that of air as a standard.

Due to the spatially distributed soil sampling that took place in July and August, all soil-derived N-pools (based on mg kg^{-1}) and their ^{15}N values (based on at%) were interpolated by applying a linear model with a least square estimation, in which the sampling position (hill, furrow, and clearance) was used as a variable. Afterwards, the ^{15}N abundance in the samples were corrected for the background abundance and expressed as the at% excess.

Microbial biomass N was calculated from the difference between the fumigated and unfumigated TN. The ^{15}N enrichment in MBN was calculated from the mass balance (Dijkstra et al., 2006).

The plant N uptake was evaluated as the plant dry weight (g per m^2) multiplied by its N content (%). The nitrogen harvest index (NHI) was calculated as the nitrogen content in the grain related to the N content of the whole plant, which are both given in g m^2:

NHI = N yield in grains/N yield in plant (2)

The percentage of N derived from fertilizer (NdfF%) in plant samples was calculated as:

NdfF (%) = ^{15}N at% excess in plant $*$ 100/^{15}N at% excess in fertilizer (3)

The fertilizer N uptake by plants was calculated by the multiplication of the plant N yield with the NdfF. The ^{15}N recoveries in all soil and plant compartments were calculated as the % of applied ^{15}N (mg per subplot). To compare the ^{15}N recovery of the investigated N fractions at a given time, the ^{15}N recoveries in each compartment were additionally related to the total recovered ^{15}N at a given time point.

Plant–microorganism competition was calculated by two indexes. First, microbial biomass N-to-plant N ratios (Q. Liu et al., 2016) were determined to assess the competition for N. The second competition index was calculated based as the ratio of ^{15}N recoveries in MBN to plant to determine the competition for fertilizer-N (Wu et al., 2011). Hence, the first index describes the competition for N, whereas the second gives information about the

competition for fertilizer N. For both calculations, the unit mg N per m² was used for microbial biomass and plants.

We used the aov-function in R to conduct two-way analysis of variance (ANOVA) to test for significant differences between fertilizer and tillage form and their interactions on aboveground dry weight plant biomass, plant N uptake, grain yield, MBN, NdfF, NHI, as well as the ^{15}N recovery of applied ^{15}N in soil and plant, nitrate and MBN. If ANOVA assumptions were not met, data were log-transformed. Groups were compared using the Tukey's honest significant post hoc test (HSD) and statistical differences reported at a significance level of $p<0.05$. Statistic significant different groups are presented as small letters in tables. Detailed ANOVA results are given in the Supplementary Table S3.

3. Results

3.1. Soil nitrogen

Nitrate contents were small, at 0.4 to 1.9 g m^{-2} (1.5 to 12.9 mg kg^{-1}) throughout the vegetation period (Table 3). In general, the highest but most greatly deviating nitrate concentrations were observed in June after the fertilizer application, when the soil was the wettest (Supplementary Table S1). These strong variations in nitrate contents indicate that the ammonium nitrate fertilizer from the top 0–4 cm was not yet well distributed into the sampled soil > 4 cm.

Table 3: Nitrogen contents in plant and soil (0–20 cm) compartments over the vegetation period. The means ± the standard deviation of n = 3 subplots are given. Abbreviations are: MBN: microbial biomass nitrogen. For empty cells, there was no plant material available at these time points. Superscripted letters indicate statistically significant differences between treatments (tillage form × fertilizer form) at a specific sampling date.

Variant	Compartment	May	June	July	August
				($g\ m^{-2}$)	
Mini-till	NO_3-N	1.2 ± 0.5 [a]	2.5 ± 0.5 [a]	0.8 ± 0.0 [a]	0.9 ± 0.3 [a]
Liquid	MBN	8.3 ± 1.6 [a]	9.3 ± 1.7 [a]	11.7 ± 0.5 [a]	11.6 ± 1.3 [a]
	Plant			5.9 ± 0.6 [a]	10.7 ± 3.8 [a]
	Grain				8.6 ± 2.9 [a]
	Leaf			4.4 ± 0.4 [a]	1.2 ± 0.6 [a]
	Shoot			1.5 ± 0.3 [a]	0.9 ± 0.4 [a]
Mini-till	NO_3-N	1.8 ± 0.6 [a]	0.8 ± 0.4 [a]	1.1 ± 0.0 [a, c]	1.7 ± 0.1 [b]
Granular	MBN	8.0 ± 2.0 [a]	11.1 ± 1.2 [a]	12.6 ± 0.3 [a,b]	13.0 ± 1.6 [a]
	Plant			5.8 ± 0.5 [a]	9.9 ± 2.2 [a]
	Grain				7.9 ± 1.5 [a]
	Leaf			4.2 ± 0.4 [a]	1.1 ± 0.4 [a]
	Shoot			1.6 ± 0.1 [a]	0.9 ± 0.4 [a]
No-till	NO_3-N	1.8 ± 0.3 [a]	2.8 ± 1.5 [a]	2.3 ± 0.4 [b]	0.6 ± 0.1 [c,d]
Liquid	MBN	9.2 ± 1.4 [a]	11.1 ± 0.7 [a]	13.7 ± 0.1 [a,b]	11.9 ± 0.7 [a]
	Plant			7.1 ± 0.6 [a]	10.7 ± 2.3 [a]
	Grain				8.3 ± 1.7 [a]
	Leaf			5.1 ± 0.6 [a]	1.2 ± 0.2 [a]
	Shoot			2.1 ± 0.0 [a]	1.2 ± 0.4 [a]
No-till	NO_3-N	1.9 ± 0.4 [a]	1.9 ± 1.3 [a]	1.5 ± 0.6 [a,b,c]	0.4 ± 0.1 [d]
Granular	MBN	9.8 ± 1.4 [a]	10.6 ± 3.1 [a]	16.0 ± 2.0 [b]	12.1 ± 0.3 [a]
	Plant			6.3 ± 1.8 [a]	7.7 ± 1.3 [a]
	Grain				5.9 ± 1.3 [a]
	Leaf			4.4 ± 1.2 [a]	1.0 ± 0.0 [a]
	Shoot			1.9 ± 0.6 [a]	0.8 ± 0.0 [a]

Over the vegetation period, MBN ranged from 9.3 to 16.0 g m^{-2} (41.9 to 63.5 mg kg^{-1}) (Table 3). For both tillage and fertilizer forms, MBN tended to increase about 7% to 27% from May to June (Table 3) and further in July. In July, MBN was significantly higher under granular fertilized treatments, while in August the MBN was significantly higher under the mini-till condition.

3.2. Plant nitrogen uptake

In July, wheat was similarly established under all treatments. In August, however, a shift in the vegetative stage of wheat was obvious, with less developed wheat plants with milky grains and greener leaves seen under the no-till condition compared to already golden leaves and drier grains seen under the mini-till condition (about Zadoks 77 and 87, respectively) (Supplementary Figure S1a,b).

For all treatments, the above-ground plant biomass varied between 2.0 and 2.3 t ha^{-1} in July and 5.4 and 7.3 t ha^{-1} in August (Table 4), and grain yields ranged from 2.7 to 3.8 t ha^{-1} (Table 4), in both cases without any significant differences being seen between plants treated with different fertilizer or tillage forms.

Table 4: Plant biomass in July and August and grain yield in August, shortly before harvest. The means ± the standard deviation of n = 3 subplots are given. Dry weight is abbreviated to d.w. Superscripted letters indicate statistically significant differences between treatments (tillage form × fertilizer form) at a specific sampling date.

Tillage Form	Fertilizer Form	Total Plant d.w. (t ha^{-1}) July	Total Plant d.w. (t ha^{-1}) August	Grain Yield (t ha^{-1}) August
Mini-till	Liquid	2.3 ± 0.1 [a]	7.3 ± 2.3 [a]	3.8 ± 1.1 [a]
Mini-till	Granular	2.3 ± 0.4 [a]	7.3 ± 1.1 [a]	3.7 ± 0.4 [a]
No-till	Liquid	2.1 ± 0.1 [a]	6.2 ± 1.3 [a]	3.2 ± 0.7 [a]
No-till	Granular	2.0 ± 0.3 [a]	5.4 ± 1.0 [a]	2.6 ± 0.8 [a]

At the first plant sampling in July, plants were already in the beginning of the jointing stage and thus were at a major N uptake stage (B. Chen et al., 2014), taking up 5.8 to 7.1 g N m^{-2} (Table 3). Most plant N was stored in the leaves (Table 3) but was not affected by the fertilizer or tillage form used. In August, the plant biomass N ranged from 7.7 to 10.7 g m^{-2} (Table 3) and was not significantly affected by the fertilizer or tillage form used. Most plant N was stored in the grains, and N storage in leaves and shoots decreased compared to the N contents in July (Table 3). Fertilizer form and tillage form did not affect the N contents in the plant compartments. The fertilizer-N uptake (NdfF) ranged from 1.0 to 1.6 g m^{-2} (12% to 15% of the total N uptake; Table 5) and was significantly higher under the mini-till and liquid fertilization conditions than under the no-till and granular fertilization conditions. The NHI

ranged from 0.77 to 0.81 and tended to be higher under the mini-till and liquid fertilization condition. However, these differences were not significant.

Table 5: Nitrogen derived from fertilizer (NdfF) in July and August and nitrogen harvest index (NHI) in August, shortly before harvest. The mean ± the standard deviation of n = 3 subplots are given. Superscript letters denote differences between treatments (tillage form × fertilizer form) at a specific sampling date.

Tillage Form	Fertilizer Form	NdfF (g m^{-2})		NHI
		July	August	August
Mini-till	Liquid	1.3 ± 0.1^{a}	1.6 ± 0.1^{a,b}	0.81 ± 0.03^{a}
Mini-till	Granular	1.2 ± 0.2^{a}	1.3 ± 0.1^{a,b,c}	0.80 ± 0.03^{a}
No-till	Liquid	1.1 ± 0.2^{a}	1.5 ± 0.3^{a,b}	0.79 ± 0.02^{a}
No-till	Granular	1.3 ± 0.4^{a}	1.0 ± 0.1^{c}	0.77 ± 0.04^{a}

3.3. ^{15}N recovery

In the beginning of June, three days after the fertilizer application, no fertilizer granules were found in the soil any more, as they has probably already been dissolved by the soil solution. In total, 18% to 33% of the applied ^{15}N was recovered in the 4–60 cm soil (excluding the fertilization layer) (Figure 2), indicating the small translocation of fertilizer-N from the top 4 cm. However, about 8% ± 3% of the total recovered soil ^{15}N was found in MBN, showing microbial immobilization of fertilizer-N as soon as the fertilizer-N became available to microorganisms. In total, 25% ± 16% of the total recovered soil ^{15}N was found in nitrate. The high variability along with the small recovery indicates that most fertilizer remained in the top 4 cm and was only slightly translocated. The differences in recoveries were not significant.

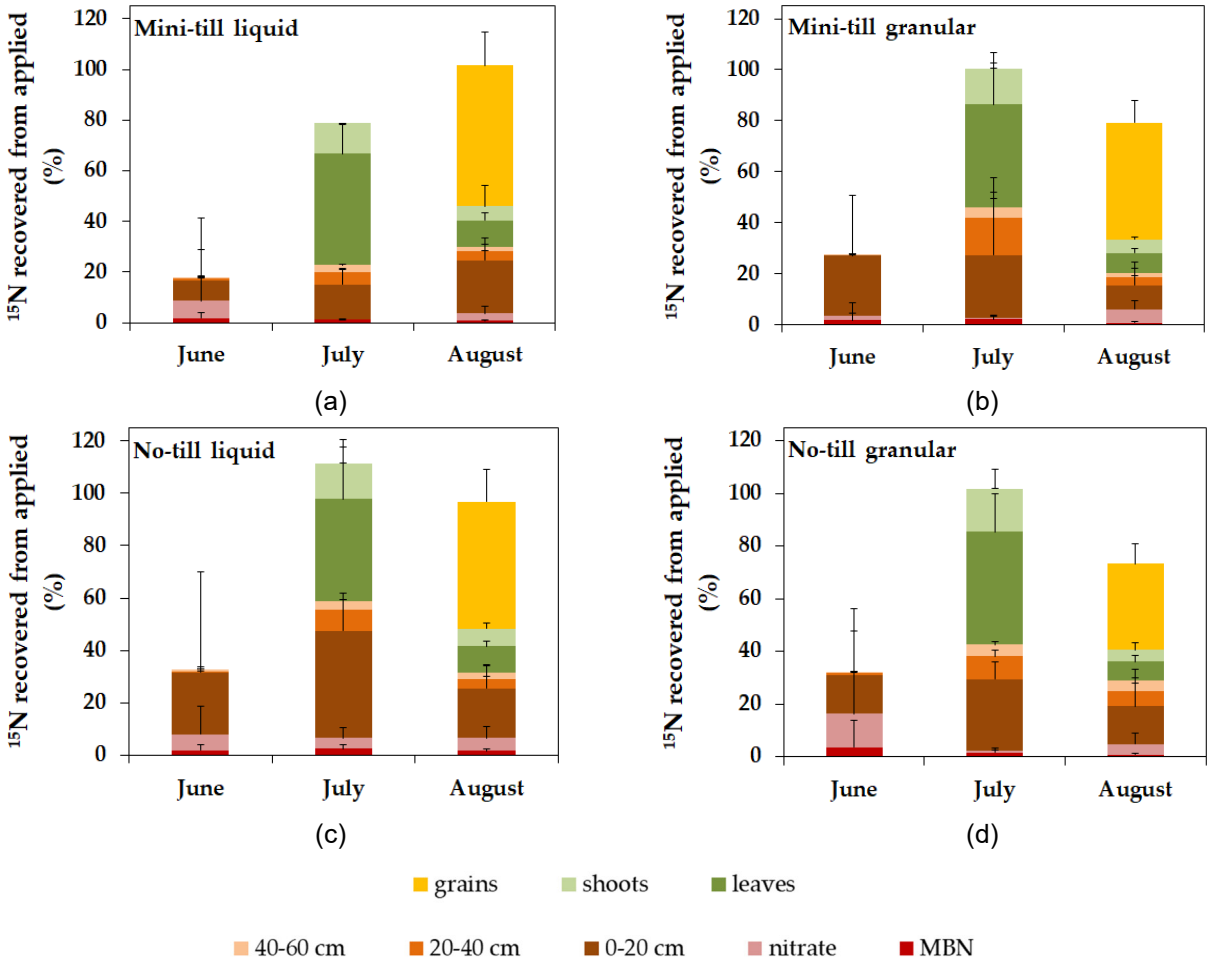

Figure 2: Recovery of applied ^{15}N in soil and plant compartments over the vegetation period for the treatment combinations: (a) mini-till liquid, (b) mini-till granular, (c) no-till liquid, and (d) no-till granular. All data values are the means of n = 3 subplots. Error bars represent the standard deviation and are displayed on top of each bar to provide a better readability.

In mid July, 79% ± 14% to 112% ± 29% of the applied ^{15}N was recovered (Figure 2). Of the 43% ± 17% of ^{15}N found in the soil depth increments, 69% ± 9% was located at 0–20 cm (Figure 2). On average, 1% ± 2% and 2% ± 1% of the total recovered ^{15}N was identified as nitrate and MBN, respectively. The fertilizer form used had no effect on the ^{15}N recovery in the different soil depth increments. In contrast, the ^{15}N recovery in 0–20 cm and nitrate was significantly higher under the no-till condition. Plants took up most of the applied fertilizer-derived ^{15}N (56% ± 3%). Most of the total recovered ^{15}N was found in the leaves (43% ± 13%). Fertilizer form had no effect on the plant ^{15}N recovery.

In August, 73% ± 16% to 102% ± 15% of the applied ^{15}N was recovered in total. Only 32% ± 8% ^{15}N was left in the soil, of which 76% ± 7% was found at 0–20 cm. The ^{15}N

recovery in soil was not affected by the fertilizer form or by the tillage method, and translocation into deeper soil depth increments was similarly small for all treatments. In total, 6% ± 5% of the total recovered ^{15}N was found as nitrate, whereas only 1% ± 1% ^{15}N was immobilized as MBN. The fertilizer and tillage form had no significant effect on the recovery in nitrate or MBN fraction. In total, 68% ± 8% of the applied ^{15}N was recovered in plants. Between July and August, a decreasing ^{15}N abundance (of the total recovered ^{15}N) in the leaves and shoots and increasing ^{15}N abundance in the grains (52% ± 8%) mainly indicates N translocation occurring within the plant (Figure 2). The ^{15}N recovery in grains and in the whole plant was significantly higher under the mini-till and liquid fertilization conditions than under the no-till and granular fertilization conditions, respectively.

3.4. Plant–microorganism competition

Microbial biomass N-to-plant N ratios were around 2.2 (mean) in July and decreased to 1.3 in August (Figure 3), indicating a superior microbial N capture. The microbial biomass ^{15}N-to-plant ^{15}N ratios were much lower, at around 0.04 in July and 0.02 in August (Figure 3). Values below one indicate that developed plants increasingly exceeded microbial ^{15}N immobilization. The lower ratios seen in August compared to July for both indexes suggest a stronger plant competitiveness. In July and August, both indexes were not affected by the fertilizer form used, but in August plant competitiveness was enhanced under the mini-till condition.

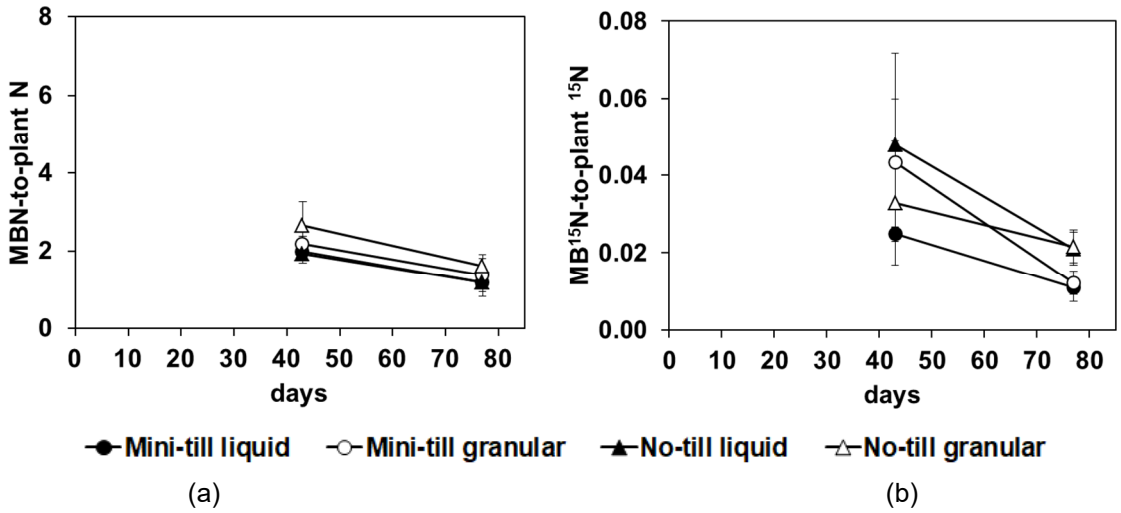

Figure 3: (a) Microbial biomass N-to-plant N ratio based on mg N m^{-2} (b) and microbial biomass ^{15}N-to-plant ^{15}N ratio based on mg ^{15}N m². The mean of n = 3 subplots is shown; error bars represent the standard deviation.

4. Discussion

4.1. Initial microbial fertilizer-N immobilization

Plant–microorganism competition for N depending on fertilizer form was investigated in clayey soils with a ^{15}N labeling study over the vegetation period of spring wheat in the semi-arid climatic zone of North Kazakhstan. In our study, fertilizer-N was effectively kept in the topsoil, as reported for arable soils in semi-arid Australia and Canada (Hancock et al., 2011; Malhi et al., 2009). The high total ^{15}N recoveries in the plant and soil of 73% ± 16% to 102% ± 15% for the applied ^{15}N in August show that the applied fertilizer-N was not lost due to (i) leaching, as precipitation with 92 mm (Figure 1) and hence translocation was small during the vegetation period (Figure 2), or (ii) volatilization, as NH_3 was applied as fertilizer in the soil, reducing NH_3 losses compared to near-surface applications (Rochette et al., 2013).

Two days after fertilization, the MBN at 0–20 cm increased by 7 to 27% (Table 3) when the soil moisture was the largest (Supplementary Table S1), suggesting the occurrence of microbial N immobilization. The assumed microbial N immobilization is supported by findings from semi-arid Texas, where the addition of water and N has been shown to increase MBN shortly after application (Zhang and Zak, 1998). Microbial N immobilization can be confirmed by an increase in ^{15}N excess in MBN after fertilization (Supplementary Table S2). Independently from fertilizer form, up to 4% of the applied ^{15}N was immobilized by

microorganisms (Figure 2), accounting for 8% of the total recovered fertilizer-N in June, excluding the top 4 cm, in which fertilizer was applied, as well as many microorganisms. Hence, the microbial fertilizer N immobilization was presumably even higher. Short-term competition studies in early vegetative plant stages report that microorganisms effectively took up most fertilizer N, as shown in temperate soils in Inner Mongolia (Q. Liu et al., 2016), in semi-arid prairie soils in the USA (Chen et al., 2015), and in semi-arid steppe soils in Inner Mongolia (Wu et al., 2011). However, previous studies in more humid climate zones have shown that microorganisms immobilize up to 60% of applied fertilizer-N within three days (Grace et al., 1993; Hodge et al., 2000a; Harrison et al., 2008). The smaller microbial fertilizer-N immobilization at this early time point in our study was probably due to the excluded sampling of the top 4 cm, in which the fertilizer was applied. Two days after fertilization, the fertilizer was not yet well distributed throughout the soil, causing comparably smaller recoveries (Figure 2). However, the relatively high recoveries in MBN for the total recovered ^{15}N in June show that if fertilizer-N became available to microorganisms, it was effectively immobilized.

In field and laboratory studies carried out under wetter conditions, it has been suggested that liquid N sources are more available to plants (Beachchamp et al., 1986; Holloway et al., 2001; Gagnon et al., 2012; Pittawy et al., 2015). Hence, we assumed that in early vegetative stages liquid fertilizer-N in particular would be taken up in high amounts by microorganisms under the drier soil conditions of our study. In contrast to this, we could observe significantly higher MBN under granular fertilization in July, but not higher MBN or $MB^{15}N$ in August (Table 3; Figure 2), when, in both cases, the soil moisture was low (Supplementary Table S1). Additionally, we did not observe significant differences in aboveground plant and grain yield (Table 4) when comparing the fertilizer forms, which disagrees with hypothesis one. This is probably because of the good water solubility of NH_4NO_3 (Carl Roth GmbH + Co KG, 2015), meaning that granular fertilizer had already been dissolved in the soil. Granular and liquid ammonium nitrate was hence similarly available to microorganisms and plants, independently of what form it was supplied in.

Interestingly, our study showed that the fertilizer form used was less important for the N uptake by microorganisms, only affecting plant and grain ^{15}N recovery and NdfF in August according to ANOVA. The good solubility of granular ammonium nitrate in water (safety data sheet; C Roth, Karlsruhe, Germany) may have resulted in the similar performance of different forms of fertilizer. Observations showing a similar efficiency of liquid and granular fertilizer forms are scarce (Tripolskaja and Verbylienė, 2014). The higher plant recoveries of fertilizer-N and NdfF seen in August under mini-till management are in line with observations that under shallow tillage conditions more ^{15}N was recovered in soil and plants than under zero tillage conditions for a urea fertilization rate of 100 kg N ha^{-1} under a high precipitation level (178 to 232 mm) in the Canadian Great Plains (Carter and Rennie, 1985). In our study, this result can be attributed to the different plant development (Supplementary Figure S1a,b) occurring under no-till and mini-till management, where plants were further developed under the mini-till condition (Supplementary Figure S1a,b). The soil cracking (Supplementary Figure S1c) of these clayey soils (Table 1) in the dry summer 2019 was seen in abundance and occurred more often under the no-till condition, suggesting the occurrence of stronger soil compaction than under the mini-till condition. The higher initial bulk density seen under no-till management (Table 1) and increased compaction by soil drying could have increased the penetration resistance of the bulk soil (excluding dry cracks) and may therefore have resulted in smaller root development (Unger and Kaspar, 1994).

Consequently, we cannot accept our first hypothesis. Our results show that in these highly competitive conditions for N between plants and microorganisms with low levels of precipitation and high clay contents in North Kazakhstan, plants compete effectively against microorganisms for fertilizer-N (Figures 2 and 3) in the long run, regardless of the form in which N was initially supplied, though this is possibly affected by the tillage form used.

4.2. Plant–microorganism competition

In July, the MBN further increased (Table 3), but at this time point plants were already far developed (jointing stage) and took up high amounts of N (Table 3) regardless of the fertilizer form used. The MBN-to-plant-N index (Figure 3a) shows that despite strong plant N uptake, microorganisms still hold 2.0 to 2.7 times more N than plants, indicating effective N immobilization by microorganisms. Four times higher values (about 10) for this index were reported in a short-term competition study in July in a temperate grassland area in Inner Mongolia under nitrate addition (Q. Liu et al., 2016). Interestingly, the MB^{15}N-to-plant-^{15}N ratio (Figure 3b) showed the opposite trend, with a stronger ^{15}N immobilization being seen in plants than in microorganisms. The MB^{15}N-to-plant-^{15}N ratios fit ratios below 1 well, which had already been found three days after N application in a non-grazed semi-arid Inner Mongolian steppe soil in which the vegetation was already established (Wu et al., 2011), indicating the effective uptake of fertilizer-N by plants. However, three days after ^{15}N application, these ratios were higher (about 0.5) (Wu et al., 2011) than those seen in our study after 43 days. The higher ^{15}N recovery in plants and therefore smaller MB^{15}N-to-plant-^{15}N ratios were due to the faster turnover times of microorganisms compared to plants (Hodge et al., 2000a; Kaye and Hart, 1997). Hence, the initially microbially immobilized ^{15}N was mineralized and released to the plant as suggested for tallgrass prairies (Williams et al., 2001). The fact that plants outcompete microorganisms for N over longer time intervals could also suggest the existence of different N pools in plants and microorganisms, with plants preferring inorganic N while microorganisms predominantly take up organic N, as shown in various ecosystems (Huygens et al., 2016; Kaye and Hart, 1997). In temperate grasslands, it has been shown that plants also compete effectively for a variety of amino acids (Bardgett et al., 2003; Harrison et al., 2007). However, inorganic N forms are still the major N source for plants in semi-arid regions (Huygens et al., 2016), and incubation experiments suggest that microbial competition is more pronounced for organic than for inorganic N forms (Dunn et al., 2006). Consequently, competition for inorganic N could be avoided, as plants and microorganisms could prefer different sources of N. Despite N-recycling and the preferred N

forms, the spatial aspect must also be considered. Fertilizer placement has been shown to strongly affect the uptake of N by plants (Hodge et al., 2000a; Petersen, 2001). The application of fertilizer into the furrow simultaneously with seeding could therefore have increased plant competition for fertilizer-N (Chen et al., 2016; Petersen, 2001) compared to spatially more distributed microorganisms (Hodge et al., 2000b), meaning that microbial ^{15}N recovery is especially small at later time points due to this "dilution effect" of soil sampling.

From July to August, plants took up even more N (Table 3) and competed more effectively against microorganisms, as indicated by the higher ^{15}N recovery (Figure 2) and decreasing microorganism-to-plant N and ^{15}N ratios seen (Figure 3). Our study showed smaller competition indexes than were found in comparable studies (Q. Liu et al., 2016; Wu et al., 2011). This is probably due to the higher precipitation occurring in these studies (mean annual precipitation of 334 and 350 mm), as a higher water availability has been shown to increase MBN in semi-arid areas (Zhang and Zak, 1998). However, the differences in time periods between ^{15}N application and sampling time also have to be kept in mind. Whereas the ^{15}N competition index rapidly decreased from about 3 (24 h) to 0.5 (72 h) after ^{15}N application (Wu et al., 2011), we sampled after 43 and 77 days. Hence, plant–microorganism competition in our case was much more a product of numerous short-term competitions, and hence the competition ratios must have been smaller due to the death and remineralization of microorganisms. Unfortunately, no information about the clay content is offered in these studies (Q. Liu et al., 2016; Wu et al., 2011), but the strong retention of mineral N seen in our study, especially abiotically of ammonium as another sink, might therefore have further increased the competition for the remaining N. In conclusion, in these clay-rich soils with very low levels of precipitation, plant–microorganism competition for N is further enhanced, meaning that our second hypothesis cannot be rejected.

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original draft preparation, M.K.; writing—review and editing, G.G., O.S., and M.K.; visualization, M.K.; supervision, O.S.; project administration, O.S.; funding acquisition, G.G. All authors have read and agreed to the published version of the manuscript.

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Supplementary Material

Figures

(**a**) (**b**) (**c**)

Figure S1: Field observations. Plant development in (**a**) July (day 43) and (**b**) August (day 77) for mini-till and no-till and (**c**) soil cracking in August (day 77).

Tables

Table S1: Gravimetric water contents in percent for each soil increment for each sampling time point. Given is the mean ± the standard deviation of n = 9 microplots.

Tillage	Depth (cm)	May	June	July	August
Mini-till	0-20	26 ± 6	16 ± 6	15 ± 7	14 ± 2
	20-40	23 ± 3	17 ± 7	17 ± 6	11 ± 2
	40-60	22 ± 2	14 ± 7	15 ± 5	11 ± 1
No-till	0-20	22 ± 5	17 ± 5	17 ± 5	13 ± 4
	20-40	23 ± 4	16 ± 5	16 ± 6	11 ± 1
	40-60	19 ± 3	14 ± 6	18 ± 6	11 ± 2

Table S2: Absolute ^{15}N amount in mg per subplot in nitrate, MBN and the 4-20 cm soil depth increment 3 shortly before (May) and after (June) fertilization. Note that in the 4-20 cm soil depth increment ^{15}N recovered in nitrate and MBN is not included.

Time	Tillage form	Fertilizer form	NO_3-^{15}N	$MB^{15}N$	0-20 cm soil
May	Mini-till	Liquid	0 ± 0	0.0 ± 0.0	0 ± 0.0
	Mini-till	Granular	0 ± 0	0.0 ± 0.0	0.1 ± 0.2
	No-till	Liquid	0 ± 0	0.0 ± 0.01	0.0 ± 0.0
	No-till	Granular	0 ± 0	0.0 ± 0.0	0.1 ± 0.2
June	Mini-till	Liquid	22.6 ± 67.7	5.8 ± 7.1	27.2 ± 80.6
	Mini-till	Granular	5.5 ± 16.6	5.5 ± 9.8	77.9 ± 177.9
	No-till	Liquid	20.4 ± 35.4	5.5 ± 6.9	78.0 ± 127.1
	No-till	granular	41.9 ± 103.6	12.4 ± 33.0	48.7 ± 82.3

Table S3: Results of 2-way-ANOVAs with post-hoc Tukey HSD test for the influence of fertilizer and tillage form and their interaction on various soil and plant parameters for the sampling times June, July, and August. Only significant interactions are shown. Input units of each parameter were: t ha^{-1} for plant yield, kg ha^{-1} for plant N uptake, mg kg^{-1} for MBN and NO_3-N, g m^{-2} for N contents in plant compartments, and % for all ^{15}N recoveries.

Aboveground dry weight August **A**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.64	0.64	0.27	0.62	
Tillage form	1	6.91	6.91	2.93	0.13	
Fertilizer:Tillage	1	0.54	0.54	0.23	0.65	
Residuals	8	18.84	2.36			

Plant N uptake August **B**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	1040	1040	1.56	0.25	
Tillage form	1	384	384	0.58	0.47	
Fertilizer:Tillage	1	376	376	0.56	0.47	
Residuals	8	5333	667			

Grain yield August **C**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.31	0.306	0.5	0.5	
Tillage form	1	1.89	1.887	3.07	0.12	
Fertilizer:Tillage	1	0.16	0.157	0.26	0.63	
Residuals	8	4.92	0.615			

MBN June **D**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	30	30	0.48	0.51	
Tillage form	1	22	22	0.36	0.57	
Fertilizer:Tillage	1	73	73	1.19	0.31	
Residuals	8	494	61.8			

MBN July **E**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	129.8	129.8	7.15	0.028	*
Tillage form	1	57.8	57.8	3.19	0.112	
Fertilizer:Tillage	1	18.1	18.1	1	0.347	
Residuals	8	145.1	18.1			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Comparison	Difference	lwr	upr	p adj
Granular - Liquid	6.577	0.9069	12.25	0.0282

MBN August **F**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	36.4	36.4	1.58	0.244	
Tillage form	1	170.1	170.1	7.42	0.026	*
Fertilizer:Tillage	1	24.1	24.1	1.05	0.336	
Residuals	8	183.5	22.9			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Comparison	Difference	lwr	upr	p adj
No-till - Mini-till	-7.531	-13.91	-1.154	0.0261

$T^{15}N$ recovery in 0-20 cm June **G**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	96	96	0.28	0.61	
Tillage form	1	378	378	1.09	0.33	
Fertilizer:Tillage	1	53	53	0.15	0.71	
Residuals	8	2764	346			

$T^{15}N$ recovery in 20-40 cm June **H**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.0358	0.0358	1.12	0.32	
Tillage form	1	0.0263	0.0263	0.82	0.39	
Fertilizer:Tillage	1	0.0678	0.0678	2.12	0.18	
Residuals	8	0.2558	0.032			

$T^{15}N$ recovery in 40-60 cm June **I**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.123	0.123	2.54	0.15	
Tillage form	1	0.047	0.047	0.97	0.35	
Fertilizer:Tillage	1	0.001	0.001	0.01	0.91	
Residuals	8	0.388	0.0485			

NO_3-^{15}N recovery June **J**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	1	1	0.01	0.92	
Tillage form	1	81	81	0.59	0.47	
Fertilizer:Tillage	1	103	103	0.75	0.41	
Residuals	8	1100	137			

MB^{15}N recovery June **K**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	2.9	2.9	0.27	0.62	
Tillage form	1	3	3	0.28	0.61	
Fertilizer:Tillage	1	3.6	3.6	0.33	0.58	
Residuals	8	85.6	10.7			

Soil ^{15}N recovery July **L**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.0051	0.0051	0.24	0.638	
Tillage form	1	0.1572	0.1572	7.33	0.027	*
Fertilizer:Tillage	1	0.1358	0.1358	6.33	0.036	*
Residuals	8	0.1715	0.0214			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
log10-transformed

Comparison	Difference	lwr	upr	p adj
NT:Liquid - MT:Liquid	0.44171	0.05887	0.8246	0.0252
NT - MT	0.2289	0.03401	0.4239	0.0267

T^{15}N recovery in 0-20 cm July **M**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	27	27	0.43	0.5314	
Tillage form	1	798	798	12.79	0.0072	**
Fertilizer:Tillage	1	570	570	9.13	0.0165	*
Residuals	8	499	62			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
log10-transformed

Comparison	Difference	lwr	upr	p adj
No-till - Mini-till	16.31	5.793	26.83	0.0072
NT:Liquid - MT:Liquid	30.09	9.435	50.752	0.007

T^{15}N recovery in 20-40 cm July **N**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.128	0.128	2.47	0.15	
Tillage form	1	0.002	0.002	0.04	0.85	
Fertilizer:Tillage	1	0.065	0.065	1.27	0.29	
Residuals	8	0.0517	0.0517			

log10-transformed

$T^{15}N$ recovery in 40-60 cm July **O**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.0718	0.0718	3.09	0.12	
Tillage form	1	0.0131	0.0131	0.56	0.47	
Fertilizer:Tillage	1	0.0008	0.0008	0.03	0.86	
Residuals	8	0.1861	0.0233			

log10-transformed

NO_3-^{15}N recovery July **P**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	10.9	10.9	4.37	0.07	.
Tillage form	1	21	21	8.44	0.02	*
Fertilizer:Tillage	1	18	18	7.23	0.028	*
Residuals	8	19.9	2.49			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

log10-transformed

Comparison	Difference	lwr	upr	p adj
No-till - Mini-till	2.647	0.5453	4.749	0.0198
NT:Liquid - MT:Liquid	5.0986	0.9709	9.2263	0.0177
NT:Liquid - MT:Granular	4.5532	0.4255	8.6809	0.0315
NT:granular - NT:Liquid	-4.3576	-8.4853	-0.2299	0.0389

$MB^{15}N$ recovery July **Q**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.03	0.03	0.07	0.8	
Tillage form	1	3.20E-01	3.20E-01	0.74	0.42	
Fertilizer:Tillage	1	1.89E+00	1.89E+00	4.35	0.07	.
Residuals	8	3.48E+00	4.35E-01			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Plant ^{15}N recovery July **R**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	18	18	0.16	0.7	
Tillage form	1	1	1	0.01	0.91	
Fertilizer:Tillage	1	47	47	0.43	0.53	
Residuals	8	873	109.1			

Leave ^{15}N recovery July **S**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0	0	0	0.99	
Tillage form	1	5	5	0.08	0.78	
Fertilizer:Tillage	1	42	42	0.74	0.41	
Residuals	8	456	57			

Shoot ^{15}N recovery July **T**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	16.5	16.5	1.6	0.24	
Tillage form	1	11.4	11.4	1.1	0.32	
Fertilizer:Tillage	1	0.1	0.1	0.01	0.91	
Residuals	8	82.7	10.34			

Competition index ^{15}N July **U**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.000009	0.000009	0.03	0.86	
Tillage form	1	0.000119	0.000119	0.43	0.53	
Fertilizer:Tillage	1	0.000833	0.000833	3	0.12	
Residuals	8	0.00222	0.000278			

Competition index N July **V**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.636	0.636	4.97	0.056	.
Tillage form	1	0.126	0.126	0.99	0.35	
Fertilizer:Tillage	1	0.217	0.217	1.7	0.229	
Residuals	8	1.023	0.128			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Aboveground dry biomass July **W**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.1	0.0095	0.12	0.74	
Tillage form	1	0.087	0.087	1.1	0.33	
Fertilizer:Tillage	1	0.007	0.0071	0.09	0.77	
Residuals	8	0.633	0.0792			

Plant N yield July **X**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	58	57.8	0.59	0.47	
Tillage form	1	224	224.1	2.28	0.17	
Fertilizer:Tillage	1	15	15.3	0.16	0.7	
Residuals	8	786	98.3			

NdfF July **Y**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	1.7	1.74	0.31	0.59	
Tillage form	1	0.1	0.14	0.03	0.88	
Fertilizer:Tillage	1	4	4.01	0.71	0.42	
Residuals	8	45.1	5.64			

Soil ^{15}N recovery August **Z**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	115.8	115.8	3.47	0.1	.
Tillage form	1	76.6	76.6	2.3	0.17	
Fertilizer:Tillage	1	37.7	37.7	1.13	0.32	
Residuals	8	267.1	33.4			

T^{15}N recovery in 0-20 cm August **AA**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	193.2	193.2	5.58	0.046	*
Tillage form	1	6.4	6.4	0.19	0.678	
Fertilizer:Tillage	1	35.6	35.6	1.03	0.34	
Residuals	8	276.8	34.6			

Comparison	Difference	lwr	upr	p adj
Granular - Liquid	-8.025	-15.86	-0.1939	0.0457

$T^{15}N$ recovery in 20-40 cm August **AB**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	2.76	2.76	1.83	0.213	
Tillage form	1	5.45	5.45	3.62	0.094	.
Fertilizer:Tillage	1	5.54	5.54	3.68	0.091	.
Residuals	8	12.04	1.51			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

$T^{15}N$ recovery in 40-60 cm August **AC**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	1.05	1.05	1.68	0.23	
Tillage form	1	5.6	5.6	9.01	0.17	*
Fertilizer:Tillage	1	1.17	1.17	1.88	0.208	
Residuals	8	4.97	0.62			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Comparison	Difference	lwr	upr	p adj
No-till - Mini-till	1.366	0.3167	2.415	0.017

NO_3-^{15}N recovery August **AD**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	1.1	1.1	0.26	0.62	
Tillage form	1	0.4	0.4	0.1	0.76	
Fertilizer:Tillage	1	7.5	7.5	1.8	0.22	
Residuals	8	33.4	4.18			

$MB^{15}N$ recovery August **AE**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	3.42E-01	3.42E-01	2.29	0.168	
Tillage form	1	7.43E-01	7.43E-01	4.98	0.056	.
Fertilizer:Tillage	1	2.68E-01	2.68E-01	1.8	0.217	
Residuals	8	1.19E+00	1.49E-01			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Plant ^{15}N recovery August **AF**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	848	848	18.97	0.0024	**
Tillage form	1	319	319	7.15	0.0282	*
Fertilizer:Tillage	1	49	49	1.1	0.324	
Residuals	8	357	45			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Comparison	Difference	lwr	upr	p adj
Granular - Liquid	-16.81	-25.71	-7.909	0.0024
No-till - Mini-till	-10.32	-19.22	-1.418	0.0282
NT:Granular - MT:Granular	-27.126	-44.6	-9.648	0.0048
NT:Granular - NT:Liquid	-20.865	-38.34	-3.386	0.0212

Grain ^{15}N recovery August **AG**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	493	493	21.74	0.0016	**
Tillage form	1	300	300	13.24	0.0066	**
Fertilizer:Tillage	1	29	29	1.29	0.2887	
Residuals	8	181	23			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Comparison	Difference	lwr	upr	p adj
Granular - Liquid	-12.81	-19.15	-6.477	0.0016
No-till - Mini-till	-10	-16.34	-3.663	0.0066

Leave ^{15}N recovery August **AH**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	19.37	19.37	5.18	0.052	.
Tillage form	1	0.62	0.62	0.17	0.694	
Fertilizer:Tillage	1	0	0	0	0.973	
Residuals	8	29.91	3.74			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Shoot ^{15}N recovery August **AI**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	6.33	6.33	3.76	0.088	.
Tillage form	1	0.06	0.06	0.03	0.857	
Fertilizer:Tillage	1	2.84	2.84	1.68	0.23	
Residuals	8	13.47	1.68E+00			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Competition index ^{15}N August **AJ**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	2.40E-06	2.40E-06	0.16	0.6971	
Tillage form	1	2.88E-04	2.88E-04	19.37	0.0023	**
Fertilizer:Tillage	1	3.00E-07	3.00E-07	0.02	0.8953	
Residuals	8	1.19E-04	1.49E-05			

Comparison	Difference	lwr	upr	p adj
No-till - Mini-till	0.009791	0.00466	0.01492	0.0023
NT:Liquid - MT:Liquid	0.0100932	1.71E-05	0.02017	0.0496

Competition index N August **AK**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.305	0.305	2.32	0.17	
Tillage form	1	0.043	0.043	0.32	0.59	
Fertilizer:Tillage	1	0.044	0.044	0.33	0.58	
Residuals	8	1.053	0.1316			

NHI August **AL**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.00052	0.000519	0.6	0.46	
Tillage form	1	0.00244	0.002444	2.84	0.13	
Fertilizer:Tillage	1	0.00013	0.000126	0.15	0.71	
Residuals	8	0.00688	0.00086			

NdfF August **AM**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	38.8	38.8	16.68	0.0035	**
Tillage form	1	13.8	13.8	5.93	0.0409	*
Fertilizer:Tillage	1	2.4	2.4	1.02	0.3426	
Residuals	8	18.6	2.3			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Comparison	Difference	lwr	upr	p adj
Granular - Liquid	-3.595	-5.625	-1.565	0.0035
No-till - Mini-till	-2.143	-4.173	-0.1131	0.0409

NO_3-N June **AN**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	9.33E+01	9.33E+01	5.17	0.053	.
Tillage form	1	7.80E+00	7.80E+00	0.43	0.529	
Fertilizer:Tillage	1	1.29E+01	1.29E+01	0.72	0.442	
Residuals	8	1.44E+02	1.80E+01			

log10-transformed

NO_3-N July **AO**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	4.30E-03	4.30E-03	0.45	0.521	
Tillage form	1	1.68E-01	1.68E-01	17.55	0.003	**
Fertilizer:Tillage	1	3.91E-02	3.91E-02	7.21	0.028	*
Residuals	8	7.67E-02	9.60E-03			

Comparison	Difference	lwr	upr	p adj
No-till - Mini-till	0.2369	0.1065	0.3673	0.003
NT:Liquid - MT:Liquid	0.3887	1.36E-01	0.64478	0.0055
NT:Liquid - MT:Granular	0.27482	1.87E-02	0.53091	0.036

NO_3-N August **AP**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	4.80E+00	4.80E+00	9	0.0171	*
Tillage form	1	4.83E+01	4.83E+01	90	1.30E-05	***
Fertilizer:Tillage	1	1.22E+01	1.22E+01	22.7	0.0014	**
Residuals	8	4.30E+00	5.00E-01			

Comparison	Difference	lwr	upr	p adj
Granular-Liquid	1.268	0.293	2.242	0.0171
No-till - Mini-till	-4.011	-4.985	-3.036	0
MT:Granular - MT:Liquid	3.2831	1.37E+00	5.1973	0.0026
NT:Liquid - MT:Liquid	-1.9952	-3.909	-0.08104	0.0413
NT:Granular - MT:Liquid	-2.743	-4.657	-0.82877	0.0077
NT:Liquid - MT:Granular	-5.2783	-7.193	-3.36416	0.0001
NT:Granular - MT:Granular	-6.0261	-7.94	-4.11189	0

C/N grain - August **AQ**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	2.52E+00	2.52E+00	0.96	0.35	
Tillage form	1	6.06E+00	6.06E+00	2.32	0.17	
Fertilizer:Tillage	1	3.10E-01	3.10E-01	0.12	0.74	
Residuals	8	2.09E+01	2.61E+00			

C/N leaf - August **AR**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	4.90E+01	4.90E+01	1.15	0.31	
Tillage form	1	9.50E+01	9.50E+01	2.22	0.17	
Fertilizer:Tillage	1	2.00E+00	2.00E+00	0.05	0.83	
Residuals	8	3.44E+02	4.30E+01			

C/N shoot - August **AS**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	5.00E+01	5.00E+01	0.14	0.713	
Tillage form	1	2.76E+03	2.76E+03	7.91	0.023	*
Fertilizer:Tillage	1	1.81E+02	1.81E+02	0.52	0.492	
Residuals	8	2.79E+03	3.48E+02			

Comparison	Difference	lwr	upr	p adj
No-till - Mini-till	-30.3	-55.15	-5.452	0.0228

Leaf N July **AT**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.69	0.69	1.3	0.29	
Tillage form	1	0.52	0.52	0.97	0.35	
Fertilizer:Tillage	1	0.16	0.16	0.3	0.6	
Residuals	8	4.23	0.529			

Shoot N July **AU**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.002	0.002	0.01	0.906	
Tillage form	1	0.539	0.539	5.25	0.051	.
Fertilizer:Tillage	1	0.058	0.058	0.57	0.473	
Residuals	8	0.822	0.103			

Leaf N August **AV**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.069	0.069	0.54	0.48	
Tillage form	1	0.019	0.019	0.15	0.71	
Fertilizer:Tillage	1	0.001	0.001	0.01	0.92	
Residuals	8	1.022	0.1278			

Shoot N August **AW**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.086	0.086	0.77	0.41	
Tillage form	1	0.009	0.009	0.08	0.79	
Fertilizer:Tillage	1	0.129	0.129	1.15	0.32	
Residuals	8	0.899	0.1123			

Grain N August **AX**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	7.12	7.12	1.85	0.21	
Tillage form	1	3.66	3.66	0.95	0.36	
Fertilizer:Tillage	1	2.38	2.38	0.62	0.45	
Residuals	8	30.78	3.85			

MBN May **AY**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	1	1	0.02	0.9	
Tillage form	1	5	5	0.1	0.76	
Fertilizer:Tillage	1	10	10	0.21	0.66	
Residuals	8	392	48.9			

NO_3-N May **AZ**

	D.f.	Sum Sq.	Mean Sq.	F value	p value	significance
Fertilizer form	1	0.032	0.032	1.92	0.2	
Tillage form	1	0.0157	0.0157	0.94	0.36	
Fertilizer:Tillage	1	0.0236	0.0236	1.41	0.27	
Residuals	8	0.1334	0.0167			

log10-transformed

5. Synthesis

5.1. Processes affecting the nitrogen availability under the current agricultural practice

5.1.1. Nitrogen availability and retention in the soil

To meet their N demand, plants in semi-arid ecosystems prefer inorganic over organic N forms (Huygens et al., 2016). The mineralization of ON into inorganic N forms is a key process in soil fertility. However, NH_4-N contents were mostly not detectable neither during our field campaigns (data not shown; Reck, 2019), nor in incubation experiments (study II), which agrees with observations of low NH_4-N contents in Northern Kazakh soils (Vasilchenko, 2014; Черненок and Грицких, 1998). Also NO_3-N contents are often limited in Northern Kazakh soils (Figure 5.1.; Reck, 2019). Low contents of mineral N fit well to our findings of low overall net N mineralization (study II Figure 2) and similar high gross N mineralization and consumption rates (study I Figure 2), despite experimental conditions (warm temperature, moderate soil moisture) were potentially favorable for microbial decomposition of SOM. Radiocarbon dating revealed that fPOM was the major source of N (study II Figure 4). However, released element ratios did not correlate with the initial SOM element ratios of fPOM which disagrees with H2.3. Calculated theoretical mineralized N amounts (under the assumption of a fixed OC:ON ratio of fPOM and based on the amount of mineralized C) are much higher than the observed values (study II Figure 2). Also, after an initial flush of mineralized N forms (study II supplementary Figure S2), their contents rapidly decreased. Both observations indicate strong removal of inorganic N forms from the soil solution.

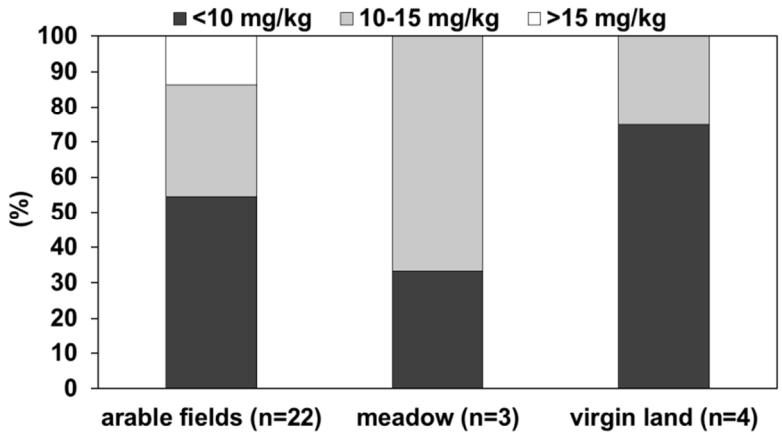

Figure 1: Proportion of content-classes of NO_3-N in all investigated fields in North Kazakhstan in 2017 and 2018. <10 mg NO_3-N kg^{-1} indicate low, 10-15 mg NO_3-N kg^{-1} adequate and > 15 mg NO_3-N kg^{-1} oversupply of NO_3-N in the soil as commonly recommended for arable soils in Kazakhstan (Гамзиков, 2018). In this figure n gives the amount of sampled fields. Data were adapted from a Master of Science thesis (Reck, 2019).

Low overall net N mineralization and inorganic N contents, and similar gross N mineralization and NH_4^+ consumption rates (study I Table 4, study II Figure 2 and supplementary Figure S2) were attributed to strong biotic (microorganisms) (Geisseler et al., 2009) and abiotic (SOM, pedogenic minerals) (Knicker, 2011; Sollins et al., 2006) N retention processes. These concurrent soil processes strongly limited the N availability for plants (study I and II), also after fertilization in the field, and resulted in a strong competition between plants and microorganisms for N (study III Figure 3). Though many studies reported high microbial N immobilization (e.g. Grace et al., 1993) in soils, biotic NH_4^+ consumption was low under no N addition (study I). Similarly in a global meta study on gross N transformation low NH_4^+ consumption rates are observed at low N addition (Song et al., 2021). This result suggests that mineral N is most likely predominantly abiotically fixed (study I) as also suggested by Davidson et al. (1991) and agrees with H1.3. In sterilized soils, without biotic competition for N, high abiotic fixation of applied N was shown (study I Table 3). High SOM contents (study II Table 2) and reactive mineral phases (e.g. clay: study I Table 1; study II Table 2) provide sorption sites for mineralized N forms and thus may buffer released mineralized N in natural soils (Nieder et al., 2011; Nommik and Vahtras, 1982). Hereby, N retention by clay minerals should exceed N retention by SOM (Braun et al., 2018). A strong indicator for high clay fixation is the abundant presence of expandable clay minerals

(montmorillonite, vermiculite) in the studied soils (study II; Prays, 2018, unpublished; Bräunig, 2020), which can partially incorporate NH_4^+ as the primary product of N mineralization into their interlattice (Allison and Roller, 1955; Scherer et al., 2014). An additional experiment on the NH_4^+ fixation on dried soils showed that 29 to 52 % of the of the applied 20 kg $(NH_4)_2SO_4$-N ha^{-1} fertilizer could be retained within 24 h after N addition (Bräunig, 2020), confirming the assumption of rapid clay NH_4^+ retention within hours (Nieder et al., 2011). Overall, losses of N are considered insignificant as mineralized and fertilized N was effectively kept in the soil system. Gaseous N losses by denitrification processes were small (<3 µg kg^{-1} during 126 days; study II data not shown), and losses of fertilizer N via leaching were small on field scale (study III Figure 2).

In conclusion, net N mineralization in the studied clayey steppe soils of North Kazakhstan is low. The N availability of released mineralized N is rapidly diminished by strong retention processes, especially by sorption to reactive mineral phases. Consequently, the natural supply of available inorganic N forms in these soils is limited which ultimately demands fertilization necessary in the near future.

5.1.2. Effects of land use and fertilization on the nitrogen availability

Land use strongly changes nutrient cycles (chapter 1.). Higher N mineralization under grassland soils (Lang et al., 2016) could be explained by the higher contents of SOM compared to arable soils (study II, Booth et al., 2005) and hence more available substrate for microorganisms. Predominant fPOM decomposition (study II Figure 4) supports the assumption that mineralization may be higher under grassland soils than arable soils. However, clear evidence for a higher mineralization under grassland soil was only found for C mineralization (study II Figure 2). In the studied soils net N mineralization and gross N mineralization rates, in general, did not significantly vary between land uses (study I, II) which disagrees with H1.1. This result was attributed to strong retention processes of mineralized N forms as discussed in section 5.1.1., which withdraw mineral N forms from the soil solution. Nitrogen retention was similar for arable soils and grassland soils (study I).

These retained N forms are hence not completely extractable as mineral N (Motavalli et al., 1995; Russow et al., 2008) and, thus, strongly impact the calculation of net N mineralization. Consequently, strong retention of N in these SOM- and clay-rich soils might overshadow land use effects on net N mineralization.

Fertilization strongly impacts available N forms in soils (Carpenter-Boggs et al., 2000; Lu et al., 2021; Song et al., 2021). Nevertheless, at no and at 60 kg N ha^{-1} fertilization gross N mineralization rates were similar (study II Figure 5), contradicting H1.2. A reduced microbial N uptake with higher N fertilization (Hao et al., 2020; Li et al., 2010; Treseder, 2008; Zhang et al., 2008) is a result of a reduced microbial activity because of fertilization-induced soil acidification (Kemmitt et al., 2006; Rousk et al., 2009) and might explain similar gross N mineralization rates for no and moderate N fertilization. Likewise, abiotic N retention increased (absolute values) because biotic N immobilization remained constant at the high ion pressure at N fertilization (study I).

The field study showed that fertilizer N was not lost due to leaching, but for both fertilizer forms effectively kept in the topsoil (>70 % in 0-20 cm) and only slightly translocated into deeper soil compartments (study III Figure 2). Similarly, high fertilizer N recoveries in the top 10 to 15 cm have been reported in other semi-arid regions (Hancock et al., 2011; Malhi et al., 2009). Most of the fertilizer N, in contrast to H3.1., regardless of its form (granular *versus* liquid), was taken up by plants (about 60 %; study III Figure 1). The competition index as ratio of fertilizer N recovery in microorganism and plants further showed that plants are superior in fertilizer N uptake and that this competition is much more pronounced than observed in wetter semi-arid steppe soils in Inner Mongolia (Wu et al., 2011), which is in accordance with H3.2. Both results suggest that the fertilizer placement close to the seed with seeding is useful to obtain high fertilizer N use by plants (study III; Hodge et al., 2000; Petersen, 2001).

As a result of high N retention (section 5.1.1.) and N output with harvest, N fertilization seems necessary in the studied soils. Wheat as the primary crop in North Kazakhstan mainly needs N in certain vegetative stages (Beathgen and Alley, 1989; B. Chen

et al., 2014) and not directly in the beginning of its vegetative stage. However, after the current common agricultural practice, fertilizer is applied with seeding. An initial retention of fertilizer N (section 5.1.1.) might therefore even be advantageous, if biotic and abiotic retained fertilizer N could be remobilized (Knicker, 2011; Nieder et al., 2011; Sollins et al., 2006) in the later vegetation period. Desorption experiments on these soils are needed to examine how fast and how much of the retained N could be remobilized. Alternatively, additional N fertilization in later vegetative stages can be a valid option to increase fertilizer N use by plants (Tran and Tremblay, 2000) and therefore also reduce abiotic N retention in the soil. However, this will be at the cost of an additional driving over the field that is likely economically not feasible.

5.2. Towards a more sustainable, climate-adapted agricultural practice

5.2.1. Effect of adapted fertilizer and tillage form on the nitrogen availability

In contrast to the hypothesized higher plant N uptake under liquid NH_4NO_3 fertilization (H3.1.), no difference in neither fertilizer N recovery nor plant N uptake was observed (study III Table 2). This result is in contrast to reports showing a higher availability of N applied in liquid form than granular fertilizer from, tested with manure, urea, and mono-ammonium phosphate or zinc sulfate combined with ammonium nitrate (Beauchamp, 1986; Gagnon et al., 2012; Holloway et al., 2001; Pittawy et al., 2015). Increased volatilization of liquid NH_4^+ fertilizers (e.g. urea) might cause similar N efficiencies for liquid and granular fertilizer forms (Watson et al., 1992). However, the high ^{15}N recovery and application of fertilizer in 4-5 cm soil depth, hence a reduced volatilization (Rochette et al., 2013), contradict this assumption. The similar performance of liquid and granular NH_4NO_3 fertilizer is probably because of the good water solubility of NH_4NO_3 (Carl Roth GmbH + Co KG, 2015). At the time of fertilization in the end of May the soil was moist (study III supplementary Table S1) so that granular NH_4NO_3 has been already dissolved in the soil shortly after fertilization (study III), and consequently yielded in similar N uptake by plants compared to liquid fertilization.

In study III the effect of tillage form on the N uptake was tested. In July there were no differences between tillage form for fertilizer N and total N uptake by wheat. In August, at the end of the vegetation period, the fertilizer N uptake was higher in plants grown on mini-till than on no-till (study III Tables 3 and 4). Similarly, higher fertilizer N recovery in plants under shallow-till compared to zero-till were found for urea fertilized fields in the Canadian Great Plains (Carter and Rennie, 1985). We further found a trend to higher total plant N uptake, biomass production and grain yield under mini-till (study III Table 3). A study on NH_4NO_3 fertilized fields in Switzerland demonstrated higher grain yields for mini-till than no-till and showed that the N availability under no-till was not a limiting factor in plant productivity (Rieger et al., 2008). In fact, a shift in vegetative stage was observed between the samplings in July and August, where the wheat plants were 2 to 3 weeks further developed under mini-till (study III supplementary Figure S1). Probably, the dry summer in 2019 in Shortandy (study III Figure 1) affected the soil and hence plant growth. The water content of both tillage forms was, however, similar (study III supplementary Table S1). But the topsoil of no-till fields has already been compacted before the experiment (study III Table 1). The abundant soil cracking suggests that drying of the topsoil caused by high temperatures and low precipitation may have further increased compaction of these clayey soils (study III supplementary Figure S1). This enhanced compaction probably further increased penetration strength and decreased penetration depth of wheat roots (Unger and Kaspar, 1994) under no-till and, hence, may have decreased plant N uptake and growth at the time of sampling. It should be noted that tillage form was not replicated, so that these results must be regarded carefully.

In conclusion, the adaption of liquid instead of granular NH_4NO_3 fertilizer did neither increase N availability nor plant productivity, likely due to the high solubility of this fertilizer. Mini-till tended to positively affect N uptake and growth of wheat compared to no-till soil management, and was probably related to physical soil properties in the clayey soil and the dry summer. Overall, the attempt to adapt agricultural practice appeared to be less important

than regional soil properties, which, however, demonstrates that the current agricultural practice is already well adapted to current climatic conditions.

5.2.2. Effects of climatic changes on the nitrogen availability

Moderate increasing temperatures generally tend to enhance the microbial activity (Franzluebbers et al., 2000; Gao and Yan, 2019; Wallenstein et al., 2011). Moreover, higher temperatures further lower the temperature sensitivity resulting in a higher decomposition rate of more stable SOM (Davidson and Janssens, 2006). Thus it was expected that, depending on land use, raising temperatures is accompanied by an increase in net N mineralization (H2.1.). However, temperature and land use were in general not found to be an influencing factor on the net N mineralization after the 125-day incubation period (study II). Radiocarbon dating suggests fPOM to be the major source of released N, even more so at the end of the incubation, and no evidence was found for an enhanced decomposition of MAOM under elevated temperatures (study II Figure 4). Therefore, H2.1. must be rejected. Low effects of increased temperature on net N mineralization have been reported before, but for smaller temperature ranges (5 °C) (Wang et al., 2007). The lacking temperature effect at 15 and 25 °C in study II was mainly attributed to the strong retention of released mineralized N as discussed in section 5.1.1. and 5.1.2. These retention processes probably strongly contributed to the low net N mineralization so that possible effects of an increased temperature over 125-day incubation period were not observed. In this context, a following study on the temperature-dependency of gross N mineralization and consumption rates in these clay-rich soils would be interesting.

At low soil moisture (e.g. during droughts) the supply of soluble substrates limits the microbial respiration (Skopp et al. 1990) while the osmotic equilibrium of microbial cells has to be maintained (Schimel et al., 2007). A moderate lowered matric potential of these dry areas was expected to increase net N mineralization (H2.2.) as reviewed by Moyano et al. (2013). In contrast to H2.2., matrix potential did not affect net N mineralization (study II Figure 2), though soil moisture has been reported to be a controlling factor in the net N

mineralization in semi-arid and temperate grasslands (Hu et al., 2019; Wang et al., 2006). While the difference in the investigated matric potential was huge, the difference in absolute water contents was low due to the abundant fine pores of these clay-rich soils (study II). The small difference in absolute water contents may have been too low to influence the microbial activity. Consequently, matric potential had little effect on the net N mineralization and H2.2. was not confirmed.

In conclusion, changes net N mineralization due to climatic changes were not observable (study II). Despite the expected absence of a higher natural N supply, it is assumed that Kazakhstan might even be a winner of global climate change due to longer vegetation periods (Lioubimtseva and Henebry, 2009) and higher precipitation (Hu et al., 2017; Huang et al., 2014). However, the assumption is too shortly sighted. Especially C mineralization and hence SOM losses were enhanced at increased temperatures (Study II Figure 2). Differences in net N mineralization caused by climatic changes were not visible, but regarding the effect of temperature on C mineralization, climate-adapted soil management practices might be necessary to mitigate decline in soil quality.

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6. Conclusion

6.1. Critical evaluation

Though the N cycle has been studied intensively in various environments, there is a lack of empirical data in clay-rich soils in severe semi-arid regions. This study aimed at investigating N availability and its fate in North Kazakh steppe soils. Hereby, the current status and sustainable and climate adapted soil managements were evaluated. To address these aims, three individual experiments were conducted. However, it must be noted that these are results of separate experiments a direct comparison or transfer of results from one to another experiment or to the field has to be done with caution. First of all, both laboratory incubation experiments were conducted under similar (0-10 cm topsoil, temperature, land use) but slightly different experimental conditions (e.g. fertilization, soil moisture) but also soil parameters varied, most importantly the clay content (e.g. 275 g kg^{-1} (study I) versus 338 to 505 g kg^{-1} (study II)). Secondly, the transfer from laboratory results (study I, II) to the field (study III) has been shown to be strongly skewed, with lower mineralization (Honeycutt, 1999; Sistani et al., 2008) and immobilization (Booth et al., 2005b; Davidson et al., 1991) under field conditions. This was related to the varying climatic conditions in the field and external factors compared to optimal or controlled varying climatic conditions in laboratory studies (Sistani et al., 2008). Therefore, the obtained results are not directly comparable, but give strong indications and drew special attention to the N retention in these soils.

Our first goal was to determine processes and the fate of N in these clay-rich, semi-arid steppe soils of North Kazakhstan under the current agricultural practice. Overall, our results show that mineral N forms were mostly in deficiency and there was a strong competition for available inorganic N. However, also abiotic N retention in the soil was high, further reducing the potentially available N released by mineralization and/or fertilization for plants and microorganisms. Secondly, we tested if the transition of the current agricultural practice to a more sustainable and climate adapted form might increase the N availability. However, we found that a changed fertilizer form did not improve plant N uptake or growth. In contrast, a change in tillage form could improve plant growth in a dry summer. Simulated

wetter and warmer climate conditions did apparently not affect N mineralization. In conclusion, site specific soil properties, before the soil management, appeared to be the dominating influencing factors on the N availability. The soil management in these intensively agricultural used steppe soils is already well adapted to the current and future specific needs in this semi-arid region.

6.2. Outlook

This study shows the importance of N retention processes (study I, II, III). Naturally, this can partly be accounted to different soil parameters such as the clay content mineralogy, or the SOM content (study I, II). But in general the observed high N retention is a very important result because especially in steppe soils under arable use, the N availability is highly important for the quality and productivity of crop plants (Barker and Pilbeam, 2015; Hooper and Johnson, 1999). If N is applied with seeding (current agricultural practice) when the plants do not need N yet, N may get retained in the soil as NH_4^+ immobilized in the interlayer of lattice clay minerals and by microbial immobilization. This may be advantageous as leaching processes (study III) or gaseous losses (study II) of N around the plant are reduced. However, in this case the reversibility of this retention must be assured. The release of microbial N immobilization depends on their live cycle and by death and remineralization, microbial immobilized N may several times contribute to the soil N pool during a vegetation period (Kaye and Hart, 1997). In contrast the reversibility of NH_4^+ incorporated into clay mineral interlayers depends on NH_4^+ and K^+ concentrations in the soil and is therefore retained over a longer time period (Nieder et al., 2011). If appropriate reversibility of retained N is not the case or only to a minor degree, then N availability may be too low to meet the plant demands. Then, N could be applied in higher doses, in form of different fertilizer types, or at later plant vegetative stages at which the plant requires N most. Higher N fertilization rates are, however, economically difficult to implement in this region (personal communications with farmers). Moreover, in this case, also more N would be retained (study II). Hence due to the long term N depletion, the abundant free sorption sites

must first be occupied before the N availability in the soil increases. The application of poor water soluble N fertilizer types is also not practical as during dry vegetation periods there would barely any N be available to plants and hence the whole harvest is at risk. Studies on N application as urea and ammonium nitrate during the vegetation phase of wheat in semi-arid Australia (Wallace et al., 2020) and continental Canada (Tran and Tremblay, 2000) showed higher N efficiencies compared to early fertilization at sowing. But this would result in additional expenses (machine employment, time) and therefore costs. Hence, it should be calculated/tested if these additional costs justify the possibly higher plant productivity of crop plants. This thesis demonstrated strong N retention processes and hence low availability of mineral N forms in this region. This study helps to understand the N dynamics and to adjust N fertilization in semi-arid and clay-rich steppe soils of North Kazakhstan to assure successful agriculture under changing environmental conditions in the future.

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Further I thank my colleagues at my second employment at the University in Halle, namely the soil science and geography groups. First of all, I thank G. Schmidt and D. Kley for giving me the opportunity to finish my PhD work. M. Koza for many talks and exchanges. Special thanks are going to A. Prays, for many work and non-work related talks, good collaboration and spending a lot of time together. K. Kaiser and R. Mikutta I am grateful for co-supervising, improving my writing style, and also just having a good time. And lastly, lots of thanks to C. Krenkewitz and A. Kroner from the lab team Halle, who helped a lot in stressful times.

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Curriculum vitae

MARKUS KOCH

WORK EXPERIENCE

Work

10.2020/12.2021 · Scientific assistant and PhD-Student at the institute for geoscience and geography, Martin Luther University Halle-Wittenberg, Halle, Germany
Reference: Georg Schmidt · +49 345 552-6063 · gerd.schmidt@geo.uni-halle.de
Duty: Working on the same sub-project of ReKKS (see below) and other duties of an additional project

08.2017/09.2020 · Scientific assistant and PhD-Student at the institute for soil science, Leibniz University Hannover, Hannover, Germany
Reference: Georg Guggenberger · +49 511 762-2623 · guggenberger@ifbk.uni-hannover.de
Duty: Working on a sub-project of ReKKS (Innovations for sustainable agricultural soil management in the dry-steppe of Kazakhstan and Southwest Siberia): Nutrient availability and use efficiency

Internships

11.2015/02.2016 · Student assistant at the institute for soil science (LUH)
Reference: Georg Guggenberger · +49 511 762-2623 · guggenberger@ifbk.uni-hannover.de
Duty: Supervision of a soil science tutorial

09.2015/10.2015 · Student assistant at the institute for soil science (LUH)
Reference: Georg Guggenberger · +49 511 762-2623 · guggenberger@ifbk.uni-hannover.de
Duty: Supervision of a soil science tutorial

03.2015/05.2015 · Student assistant at the institute for mineralogy (LUH) in cooperation with the federal institute of geoscience and resources (BGR)
Reference: Secretary · +49 511 762-2222 · k.kortlang@mineralogie.uni-hannover.de
Duty: Assistance with preparations and conduction of a conference

11.2014/02.2015 · Student assistant at the institute for soil science (LUH)
Reference: Georg Guggenberger · +49 511 762-2623 ·
guggenberger@ifbk.uni-hannover.de
Duty: Standard soil sample preparations and analysis

11.2013/12.2013 · Student assistant at the institute for soil science (LUH)
Reference: Robert Mikutta · +49 345 552-2530 ·
robert.mikutta@landw.uni-halle.de
Duty: Production of pedogenic iron(hydr-)oxides, sorption experiments with pedogenic iron(hydr-)oxides, sample preparation, production of microbial nutrient solutions, extractions of mineralized nitrogen, extraction of cations

08.2013/10.2013 · Student assistant at the institute for soil science (LUH)
Reference: Robert Mikutta · +49 345 552-2530 ·
robert.mikutta@landw.uni-halle.de
Duty: Production of pedogenic iron(hydr-)oxides, sorption experiments with pedogenic-iron(hydr-)oxides, sample preparation, production of microbial nutrient solutions, extractions of mineralized nitrogen, extraction of cations

04.2013/06.2013 · Student assistant at the institute for soil science (LUH)
Reference: Robert Mikutta · +49 345 552-2530 ·
robert.mikutta@landw.uni-halle.de
Duty: Production of pedogenic iron(hydr-)oxides, sorption experiments with pedogenic iron(hydr-)oxides, sample preparation, production of microbial nutrient solutions, extractions of mineralized nitrogen, extraction of cations

12.2012/02.2013 · Student assistant at the institute for mineralogy (LUH)
Reference: Harald Behrenz · +49 511 762 8054 ·
h.behrens@mineralogie.uni-hannover.de
Duty: Digestion of rocks with hydrofluoric acid

EDUCATION

PhD student 08.2017/12.2021 · PhD-Student in the BMBF supported ReKKS sub-project (Innovations for sustainable agricultural soil management in the dry-steppe of Kazakhstan and Southwest Siberia): Nutrient availability and use efficiency.

M.Sc. 10.2014/05.2017 · Leibniz University Hannover (LUH), Hannover, Germany
Master of geoscience, grade: 1.1
Theme*:* Methodical analysis of the influence of soil moisture on the spectral signature of soils considering its physical and chemical properties (in cooperation with the federal institute of geoscience and resources (BGR))
Supervision: Prof. Dr. Georg Guggenberger (LUH) & Dr. habil. Susanne Stadler (BGR)

Erasmus 02.2016/07.2016 · L'Universitá della Calabria, Cosenza, Italy
ERASMUS exchange program

B. Sc. 10.2011/08.2014 · Leibniz University Hannover (LUH), Hannover, Germany
Bachelor of Geoscience, grade: 1.8
Theme: Influence of soil organic matter on the siderophore-induced dissolution of ferrihydrite-organic associations
Supervision: Prof. Dr. Georg Guggenberger (LUH) & Prof. Dr. Robert Mikutta (LUH)

ABILITIES

Computer R, ArcGIS, LATEX, MS Office (Excel, Word, PowerPoint)

Laboratory EA, EA-IRMS, LiquiTOC, UV-VIS, ICP-OES, AAS, BET, Skalar, MS, SPINMas, ASD, SisuROCK

FURTHER INFORMATION

Publications *2021* · **Koch**, Akshalov, Carstens, Shibistova, Stange, Thiedau, Kassymova, Sauheitl, Meinel, Schaarschmidt, Guggenberger: Competition of plants and microorganisms for added Nnitrogen in different fertilizer forms in a semi-arid climate. Agronomy, 11, 2472.

2021 · **Koch**, Schodlok, Guggenberger, Stadler: Effects of water tension and surface roughness on soil hyperspectral reflectance. Geoderma, Vol. 385, 114888.

2021 · Koza, Schmidt, Bondarovich, Akshalov, Conrad, Pöhlitz: Consequences of chemical pretreatments in particle size analysis for modelling wind erosion. Geoderma, Vol. 396, 115073. **acknowledged contributions**.

2018 · Poggenburg, Mikutta, Liebmann, **Koch**, Guggenberger: Siderophore-promoted dissolution of ferrihydrite associated with adsorbed and coprecipitated natural organic matter. Organic Geochemistry, Vol. 125, 177-188.

Communication

2020 · IALE-Russia International Conference 2020, Moscow. Landscape Science and landscape ecology: considering responses to global challenges. Session: Land use and land management (LULM) V.4.

2020 · Talk on the Young Scientists Conference 2020. Barayev Kazakh Research and Production Center of Grain Farming

2018 · Participation in statistical intensive course I-IV for R of the German Soil Science society (DBG): basics, experimental designs, modelling, regression, analysis of variance, multivariate statistics (PCA; PCR, PLS)

2016 · Participation in EUFAR (European Facility for Airborne Research) Workshop, Hyperspectral Remote Sensing Expert Working Group: Implementation and Soil Spectroscopy for digital mapping and monitoring of soils: Toward space applications and transfer technology office activities

2016/17 · Voluntary participation in the Study Buddy Program: Supervision of international students

Awards

2016/17 · Scholarship of Lower Saxony
2015/16 · Scholarship of Lower Saxony
2014/15 · Scholarship of Lower Saxony

Languages

German · Mother tongue

English · Business fluent

Italian · Fluent

French · Good knowledge

Publications

Working paper · **Koch**, Stange, Carstens, Shibistova, Guggenberger: Gross mineralization versus biotic and abiotic retention in steppe soils of North Kazakhstan.

Working paper · **Koch**, Prays, Kaiser, Mikutta, Schrumpf, Gentsch, Carstens, Shibistova, Guggenberger: Sensitivity of carbon, nitrogen, and phosphorus mineralization in semi-arid steppe soils to temperature and moisture. *To be submitted to Biology and Fertility of Soils.*

2021 · **Koch**, Akshalov, Carstens, Shibistova, Stange, Thiedau, Kassymova, Sauheitl, Meinel, Schaarschmidt, Guggenberger: Competition of plants and microorganisms for added Nnitrogen in different fertilizer forms in a semi-arid climate. Agronomy, 11, 2472.

2021 · **Koch**, Schodlok, Guggenberger, Stadler: Effects of water tension and surface roughness on soil hyperspectral reflectance. Geoderma, Vol. 385, 114888.

2021 · Koza, Schmidt, Bondarovich, Akshalov, Conrad, Pöhlitz: Consequences of chemical pretreatments in particle size analysis for modelling wind erosion. Geoderma, Vol. 396, 115073. **acknowledged contributions**.

2018 · Poggenburg, Mikutta, Liebmann, **Koch**, Guggenberger: Siderophore-promoted dissolution of ferrihydrite associated with adsorbed and coprecipitated natural organic matter. Organic Geochemistry, Vol. 125, 177-188.