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Nitrogen turnover in steppe soils of Inner Mongolia as affected by sheep grazing

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SUMMARY

Annual sum and seasonal patterns of gross nitrogen (N) turnover in soils of terrestrial ecosystems in general and steppe soils in particular are poorly understood due to limited temporal resolution in previous studies. Based on whole-year round sampling with monthly to submonthly temporal resolution at replicated ungrazed (UG) and wintergrazed (WG) steppe plots in Inner Mongolia, we show that annual gross ammonification was 240 and 215 kg N ha^{-1} $year^{-1}$, while annual net ammonification was -9 and -6 kg N ha^{-1} $year^{-1}$ for UG and WG respectively. Annual gross nitrification was 417 and 362 kg N ha^{-1} $year^{-1}$, while annual net nitrification was 31 and 19 kg N ha^{-1} $year^{-1}$ at UG and WG, respectively. Furthermore, no relationship was found between gross and net N turnover. Net rates of N turnover did not provide insight into dynamics and magnitude of actual (gross) rates of N turnover.

In the whole-year round experiment, four different seasons with characteristic patterns of N turnover were identified: (1) Growing season characterized by drying/rewetting cycles and counter-rotating cycles of microbial growth and gross rates of ammonification, contributed 46% to annual cumulative gross ammonification and 30.5% to nitrification, and almost all net N turnover . (2) Transition to winter with first freeze events characterized by a sharp decline in microbial biomass in conjunction with a peak of gross nitrification, contributed 6.5% to gross ammonification while 27.5% to nitrification. (3) Winter with constantly frozen soil characterized by low rates of N turnover, while there was slow buildup of microbial biomass; contributed 8.5% to gross ammonification and 8.5% to nitrification (4) Spring freeze-thaw period characterized by peaks of gross N turnover and soil nitrate concentrations at highest soil moisture values, contributed 39% to gross ammonification and 33.5% to nitrification. Winter grazing significantly decreased gross N turnover and microbial biomass only in freeze-thaw period by decreasing soil moisture and temperature. This study shows that freeze thaw periods are key periods for understanding patterns and magnitudes of gross N turnover and that low temporal resolution studies on gross N turnover as well as net N turnover studies in general

may not allow for a functional insight into ecosystem N turnover.

During the last decades, scientists are considerably interested in the competition for nitrogen between plant and microbe which are crucial regulators of belowground nitrogen cycling in terrestrial ecosystems. However, such interactions have mostly been excluded from experimental setups for the investigation of gross inorganic N fluxes and N partitioning to plants and microorganisms. Ungulate grazing is likely to feed back on soil N fluxes, and hence it is of special importance to simultaneously investigate grazing effects on both plant and microbial N fluxes in intact plant-soil systems, where plant-microbe interactions persist during the experimental incubation. Based on the homogenous $^{15}NH_4^+$ labelling of intact plant-soil monoliths we investigated how various stocking rates (0, 1.5, 4.5, and 7.5 sheep ha^{-1} grazing $season^{-1}$) in steppe of Inner Mongolia feedback on gross rates of N mineralization and short-term inorganic N partitioning between plant, microbial and soil N pools. Our results showed that the effect of grazing on gross N mineralization was non-uniform. At low stocking rate gross N mineralization tended to decrease but increased with higher grazing pressure. Hence, there was no significant correlation between stocking rate and gross N mineralization across the investigated grazing intensities. Grazing decreased ^{15}N recovery both in plant and microbial N pools but strongly promoted NO_3^- accumulation in the soil and thus egatively affected potential ecosystem N retention. This appeared to be closely related to the grazinginduced decline in easily degradable soil C availability at increasing stocking rate.

Grazing affects not only the N cycling in the steppe grsaaland, but also the soil labile organic carbon (LOC) oxidation, which drives the flux of CO_2 between soils and the atmosphere. However, the impact of grazing management and the contribution soil aggregate size classes (ASC) to LOC from grassland soils is unclear. We evaluated the effects of grazing intensity and soil ASC on the soil LOC, including CO_2 production, microbial biomass carbon (MBC) and dissolved organic carbon (DOC) and nitrogen mineralization (N_{min}) in topsoils (0-10 cm) in Inner Mongolia, Northern China. Soil samples were separated into aggregate size classes of 0-630 um (fASC), 630-2000 um (mASC) and >2000um (cASC). The results showed that heavy grazing (HG) and continuous grazing (CG) increased LOC significantly compared to

an ungrazed site since 1999 (UG99) and an ungrazed site since 1979 (UG79). For winter grazing site (WG), no significant differences were found. CO_2 production was highest in cASC, while lowest in fASC. MBC and DOC showed the highest values in mASC and were significantly lower in fASC. Grazing increased N_{min} in bulk soils while exhibited complex effects in the three ASCs. The results suggest that the rate of carbon mineralization was related with the rate of nitrogen accumulation. To reduce CO_2 emission, nutrient loss, and improve soil quality and productivity, moderate grazing is suggested.

To summarize, this study shows the freeze-thaw process is the key period to understand the whole-year round N cycling which produces approx. 50% inorganic N. Grazing effects were mostly pronounced in the spring freeze thaw period by decreasing the temperature and soil moisture in winter grazed site, but not in other periods of the year. Net rates of N turnover did not provide insight into dynamics and magnitude of actual (gross) rates of N turnover. Gross nitrification exceeded gross ammonfication both during freeze-thaw periods and at the annual scale. Grazing reduced both plant and microbial N acquisition but increased nitrification and nitrate accumulation in the soil and thus negatively affected potential ecosystem N retention. The grazing stock of approximately 1.5 sheep ha^{-1} y^{-1} is recommended to establish sustainable summer grazing in semi-arid steppe of Inner Mongolia. Heavy grazing (i.e. HG and CG) increased CO_2 production significantly as well as N_{min} in bulk soils; however, moderate grazing (i.e. WG) exhibited no significant effects, which is consistent with the finding that moderate grazing increases C and N sequestration.

ZUSAMMENFASSUNG

Sowohl die Gesamtjahresleistung wie auch saisonale Schwankungen von Brutto-N-Umsetzungen in Böden terrestrischer Ökosysteme sind aufgrund von stark eingeschränkter zeitlicher Auflösung früherer Studien bisher kaum verstanden. Dies gilt insbesondere für semi-aride kontinentale Steppenböden. Auf Basis von Ganzjahresmessungen mit mindestens monatlicher zeitlicher Auflösung zeigt diese Dissertation, dass die jährliche Brutto-Ammonifikation in unbeweideten (UG) und im Winter beweideten (WG) Steppenböden der Inneren Mongolei 240 und 215 kg N ha^{-1} $Jahr^{-1}$ betrug, während eine Netto-Ammonifikation von -9 and -6 kg N ha^{-1} $Jahr^{-1}$ für UG und WG bestimmt wurde. Die jährliche Brutto-Nitrifikationsleistung betrug 417 (UG) und 362 (WG) kg N ha^{-1} $Jahr^{-1}$, während die Netto-Nitrifikation nur 31 und 19 kg N ha^{-1} Jahr betrug. Es konnte kein Zusammenhang zwischen Netto- und Brutto-Raten der Ammonifikation und Nitrifikation gefunden werden, so daß die Netto-Raten keinen Einblick in die Dynamik und Größenordnung der tatsächlichen N-Umsetzungen erlaubten.

Im Rahmen von Ganzjahresmessungen konnten vier Jahreszeiten mit charakteristischen N-Umsetzungsmustern identifiziert werden: (1) Wachstumsperiode mit Austrocknungs/Wiederbefeuchtungszyklen und gegenläufigen Zyklen von mikrobiellem Wachstum und Brutto-Ammonifikation. Diese Periode trug 46 bzw. 30.5 % zur jährlichen Brutto- Ammonifikation bzw. –Nitrifikation bei, während gleichzeitig fast die gesamte Netto-N-Mineralisierung beobachtet wurde. (2) Übergangsphase zum Winter mit ersten Frostereignissen im Oberboden. Dieser Zeitraum war gekennzeichnet von einem scharfen Einbruch der mikrobiellen Biomasse im Boden und gleichzeitig einem starken Anstieg der Brutto-Nitrifikation. Insgesamt wurden hier 6.5 % bzw. 27.5 % der jährlichen Brutto-Ammonifikation bzw. –Nitrifikation beobachtet. (3) Winter mit permanent geforenem Boden. Dieser Zeitraum war durch niedrigste N-Umsetzungsraten (jeweils 8.5% der jährlichen Brutto-Ammonifikation wie auch der Brutto-Nitrifikation) gekennzeichnet, während ein Netto-Aufbau der mikrobiellen Biomasse beobachtet werden konnte. (4) Die Frühlings-Frost-Tau-Periode war gekennzeichnet durch hohe Brutto-N-

Umsetzungsraten (39% bzw. 33.5% der jährlichen Brutto-Ammonifikation bzw. Brutto-Nitrifikation) und Boden-Nitrat-Konzentrationen.

Winterbeweidung führte nur in der Frost-Tau-Periode des Frühlings zu reduzierten Brutto-N-Umsetzungen und verringerter mikrobieller Biomasse, was auf reduzierte Temperaturminima und Bodenfeuchte auf den beweideten Untersuchungsflächen zurückgeführt werden konnte. Die vorliegende Arbeit zeigt somit, daß Frost-Tau-Perioden der Schlüssel zum Verständnis der Größenordnung von Brutto-N-Umsetzungen in kontinentalen Steppenböden sind und daß Studien, die auf geringerer zeitlicher Auflösung und auf Netto-N-Umsetzungen basieren, kein funktionales Verständnis des N-Kreislaufes dieser Ökosysteme zulassen.

Die Konkurrenz um mineralischen N zwischen Pflanzen und Mikroorganismen ist ein wesentlicher Steuerfaktor der Boden-N-Umsetzungen. Jedoch wurden Pflanze-Mikroorganismen-Boden-Interaktionen in bisherigen experimentellen Ansätzen zur Untersuchung mikrobieller N-Umsetzungen und zur Allokation von mineralischem N im Pflanze-Boden-Mikroorganismen-System in der Regel ausgeblendet. Beweidung durch Paarhufer dürfte sehr sich sehr wahrscheinlich auf solche Interaktionen auswirken. Daher ist es in Beweidungsexperimenten von besonderer Wichtigkeit, simultan sowohl mikrobielle wie auch pflanzliche N-Umsetzungen in intakten Pflanzen-Boden-Systemen, in denen Pflanze-Mikroben-Interkationen fortdauern, zu untersuchen. So wurden im Rahmen dieser Arbeit intakte Pflanzen-Boden-Monolithe homogen mit $^{15}NH_4^+$ gelabelt, um zu untersuchen, wie sich verschiedene Beweidungsintensitäten (0, 1.5, 4.5, und 7.5 Schafe pro Hektar und Beweidungsperiode) auf Brutto-N-Mineralisierung und kurzfristige Allokation von mineralischem N in pflanzlichen, mikrobiellen und abiotischen Boden-N-Pools auswirken. Es zeigte sich, dass der Beweidungseffekt auf Brutto-N-Mineralisierung nicht-linear war. So tendierte die Brutto-N-Mineralisierung im Vergleich zur unbeweideten Kontrolle bei niedriger Beweidungsintensität abzunehmen, während eine Zunahme bei größeren Beweidungsintensitäten beobachtet wurde. Die Beweidung reduzierte die ^{15}N-Wiederfindung sowohl in der Pflanze wie auch in den Bodenmikroorganismen, während die Nitrat-Akkumulation im Boden mit der Beweidung zunahm, so daß mit zunehmender Beweidung eine Abnahme der

potenziellen N-Retention im Ökosystem beobachtet wurde. Dies schien in engem Zusammenhang mit einer beweidungsbedingten Abnahme der Verfügbarkeit an labilem C im Boden zu stehen.

Beweidung beeinflusst nicht nur den N-Kreislauf in semi-ariden Grasländern, sondern wirkt sich auch auf die Oxidation von labilem organischem Kohlenstoff (LOC) aus, was den CO_2-Fluß zwischen Boden und Atmosphäre steuert. Über die genauen Auswirkungen des Beweidungsmanagements und den Beitrag von Aggregatklassen (ASC) auf LOC gibt es allerdings keine Erkenntnisse. Für eine Abschätzung der Auswirkungen der Beweidungsintensität und von Bodenaggregatklassen auf LOC wurden für Oberböden (0-10 cm) der Inneren Mongolei in Norchina die CO_2-Produktion, Kohlenstoff der mikrobiellen Biomasse (MBC), gelöster organischer Kohlenstoff (DOC) und Stickstoffmineralisierung (Nmin) gemessen. Die Bodenproben wurden in Aggregatklassen von 0-630 um (fASC), 630-2000 um (mASC) und >2000um (cASC) aufgeteilt. Die Ergebnisse zeigen, dass intensive Beweidung (HG) and kontinuierliche Beweidung (CG) zu signifikant höheren Anteilen an LOC führten verglichen mit Flächen, die seit 1999 (UG99) und 1979 (UG79) unbeweidet sind. Für Flächen mit Winterbeweidung (WG) konnten keine signifikanten Unterschiede festgestellt werden. Für cASC wurde die höchste und für fASC die niedrigste CO_2-Produktion gemessen. Bezüglich MBC und DOC wurden die höchsten Werte für mASC und significant niedrigere Werte für fASC festgestellt. Beweidung führte zu generell höheren Nmin-Werten in Gesamtböden, wohingegen sich unterschiedliche Reaktionen für die drei ASCs zeigten. Die Ergebnisse legen nahe, dass die Rate der Kohlenstoffmineralisierung mit der Rate der Stickstoffakkumulation zusammenhängt. Für eine Reduzierung der CO_2-Emissionen und des Nährstoffverlust sowie für eine Verbesserung der Bodenqualität und –produktivität wird ein extensiveres Beweidungsmanagement empfohlen.

Zusammenfassend ist festzuhalten, daß Frost-Tau-Perioden mit einem Beitrag von etwa der Hälfte der jährlichen Brutto-Produktion von mineralischem Stickstoff sich als Schlüssel für das Verständnis des N-Kreislaufes der untersuchten kontinentalen Steppen erwiesen haben. Auch die Beweidungseffekte auf Brutto-N-Umsetzungen waren im wesentlichen auf diese Zeiträume beschränkt und wurden über die

Auswirkung beweidungsbedingt reduzierter Vegetationshöhe auf Schneemengehöhe und damit Bodentemperatur und Bodenfeuchte gesteuert. Nicht nur in der Frost-Tau-Periode, sondern auch auf jährlicher Skala war die Brutto-Nitrifikation größer als die Brutto-Ammonifikation. Zunehmende Beweidungsintensität führte zu einer Reihe ökologisch unerwünschter Konsequenzen wie reduzierte N-Aufnahme durch Pflanzen und Mikroorganismen wie auch Nitrat-Akkumulation im Boden und somit zu reduzierter N-Retention im Ökosystem. Diese Folgen blieben unterhalb einer Beweidungsintensität von 1.5 Schafen pro Hektar und (Sommer-) Beweidungssaison aus, so daß dieser Schwellenwert für eine nachhaltige Beweidung in den untersuchten semi-ariden Steppen empfohlen wird. Intensive Beweidung (z.B. HG und CG) führte zu einer signifikant erhöhten CO_2-Produktion sowie zu signifikant erhöhten N_{min}-Werten in Gesamtböden; im Gegensatz dazu zeigten sich auf moderat beweideten Flächen (z.B. WG) keine signifikanten Auswirkungen, was sich mit der Feststellung deckt, dass moderate Beweidung zu einer erhöhten Sequestrierung von C und N führt.

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GLOSSARY

C: Carbon

CERN: the Chinese Ecological Research Network

CG: Continuously grazed

CO_2: Carbon dioxide

Ctot: Total carbon

DIN: Dissolved inorganic nitrogen

DOC: Dissolved organic carbon

DON: Dissolved organic nitrogen

HG: Heavy grazed

HG: Heavy grazed

IBCAS: Institute of Botany, the Chinese Academy of Sciences

IMGERS: Inner Mongolia Grassland Ecosystem Research Station

MBC: Microbial biomass carbon

MBN: Microbial biomass nitrogen

N: Nitrogen

Nmin: N Mineralization

OC: Organic carbon

OM: Organic matter

SOC: Soil organic carbon

SOC: Soil organic carbon

SOM: Soil organic matter

TN: Total nitrogen

UG: Ungrazed

UG79: Ungrazed since 1979

ASC: aggregate size classes

mASC: middle aggregate size classes

fASC: fine aggregate size classes

cASC: coarse aggregate size classes

LOC: labile organic carbon

AGBM: aboveground plant biomass

BGBM: belowground plant biomass

N. S.: not significant

SAZ: soil aggregate size

GI: grazing intensity

1. INTRODUCTION AND AIMS

1.1 Introduction

Inner Mongolia grassland is a part of Eurasian Steppe, the largest contiguous grassland area in the world (Bai et al., 2004), which is more than 8% of the Earth‘s land surface. Eurasian continental, semi-arid steppe areas had undergone a considerable change in the last decades which was associated with the sedentarization of nomads, the collectivization of their livestock and, after reprivatisation, the increase of livestock. Subsequently, population increase in conjunction with poor management caused over-grazing and over-cropping that reduced vascular plant cover, accelerated soil loss, and decreased soil nutrient level (Graetz 1994, Kang et al. 2007), which lead to detoriation and desertification in approx. 60-70% of the grasslands of China, Mongolia and the Asian parts of the former Soviet Union (Li et al. 2000; Graetz, 1994), following the rapid expansion of the livestock industry after 1980 (Tong et al., 2003). The Inner Mongolian grasslands are of denotative ecological and economical importance (He et al., 2011).

The ecological significance of the N cycle, in terms of regulating plant productivity, ecosystem N retention and N loss, which can affect soil acidification, streamwater quality, eutrophication, as well as atmospheric chemistry and radiative forcing, is well acknowledged (Galloway et al. 2008, Butterbach-Bahl et al. 2011). However, due to its complexity and methodological difficulties in the quantification of actual soil N turnover processes, our understanding is still fragmentary. The current state of knowledge on N cycling may correspond to the state of knowledge on the Carbon (C) cycle several decades ago (Schlesinger 2009). Nitrogen cycling in steppe in general and the effect of grazing in particular have been studied predominantly in temperate grasslands of North America (Chen and Stark 2000; Corre et al. 2002; Verchot et al. 2002), but rarely in semi arid grass land of Asia.

Belowground nitrogen (N) cycling in terrestrial ecosystems is characterized by a variety of N transformation processes and fluxes involving organic as well as inorganic N species mediated by both plants and microorganisms. Grazing in general is thought to increase N mineralization by the dropping of readily decomposable faeces (Tracy and Frank 1998) and by incorporation of plant litter into the soil via

ungulate trampling (Zacheis et al. 2002). Further grazing impacts, which may feedback on soil N conversion, involve reduction of aboveground plant biomass (van Wijnen et al. 1999) or altered plant species composition (Olofsson et al. 2001). However, the effect of grazing on N mineralization reported in previous studies remains contradictory – ungulate grazing has been reported to both increase (Groffman et al. 1993, Le Roux et al. 2003) and decrease (Biondini et al. 1998, Bardgett and Wardle 2003) N mineralization. These contradictory results may be explained by variable responses of N mineralization at different levels of grazing intensity (Xu et al. 2007).

Most previous studies focused on the measurements of net rates of ammonification and nitrification across a wide variety of terrestrial ecosystems. However, it is well acknowledged that net rates comprise both production and consumption of inorganic N, and that such studies do not necessarily provide insight into actual gross rates of N turnover. Gross N mineralization, i. e. the microbial production of ammonium (NH_4^+) from organic N compounds is a key processes of soil N cycling, since free NH_4^+ in soil is subject to a variety of competing processes and fates, e. g. microbial nitrification to nitrate (NO_3^-), microbial immobilization (i. e. incorporation in microbial cell walls), and plant uptake (plant N nutrition). After nitrification, NO_3^--N may also either be taken up by plants or microorganisms, or undergo denitrification i. e. a stepwise reduction to N gases with nitrous oxide (N_2O) as an intermediate and molecular dinitrogen (N_2) as the end-product, and thus get lost from the ecosystem. Therefore, net mineralizaiton is a poor approximation to the real N status of ecosystems (Davidson et al. 1991, 1992, Schimel and Bennett 2004). Few studies on gross N turnover are available for such ecosystems (Holst et al. 2007).

Grazing is also one of the most important factors that could change the soil C stock in grassland ecosystems (Cui et al., 2005), which influences organic matter input and associated soil properties (Steffens et al., 2009b, Wiesmeier et al. 2009). Moreover, the rate of changes in soil C over time in the processes of biotic community development is tightly coupled with soil nitrogen mechanisms (Knops and Tilman, 2000). Previous studies have shown that heavy grazing could reduce SOC(soil organic carbon) contents and stocks associated with higher bulk densities in topsoil in semiarid steppes in Inner Mongolia (Cui et al., 2005; He et al., 2011; Steffens et al., 2008, Wiesmeier et al. 2011). However, light grazing pressure for 20 years caused no

significant decrease of SOC contents (Cui et al., 2005). The influence of grazing on soil C turnover in grasslands is complex and difficult to predict. Until now, only few studies have been conducted on the impact of grazing on LOC.

1.2 Objectives

The aim of this work is to elucidate the seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia. As more and more studies indicate that plants can efficiently compete for inorganic N with microbese, we then evaluate the grazing intensity effects on gross rates of N mineralization and short-term inorganic N partitioning in intact plant-soil systems of semi-arid steppe of Inner Mongolia. Finally, an incubation experiment of CO_2 production is developed to assess the labile organic C and N mineralization of soil aggregate size classes in semi-arid grasslands as affected by grazing management.

For the estimation of the seasonality of soil microbial nitrogen turnover, the whole year-round ^{15}N dilution experiment was developed which comprise 20 - 21 times at 8 - 38 days intervals in total of 14 months. In every plot, 5 - 10 spots were sampled and mixed to account for spatial variability. All soil samples were immediately processed after sampling within 24 hours in order to avoid storage artifacts. For the evaluation of the grazing effects on gross mineralization and short-term inorganic N competiton in intact plant-soil systems, in situ ^{15}N application experiment was conducted in the controlled field with the grazing intensity of 0, 1.5, 4.5, 7 sheeps ha^{-1} y^{-1}. On each plot, 18 stainless steel soil cores with the inner diameter of 15 cm and height of 20 cm were driven into the soil within an area of 160 m^2. To reveal the impact of grazing on the labile organic C and N mineralization of soil aggregate size classes, an incubation experiment of CO_2 production was carried out for one month with the soil sampled from five different grazing intensities sites that is CG, HG, WG, UG99, UG79.

In this work three main objectives were examined in detail:

Objective Ⅰ: To investigate the seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia (The whole year-round ^{15}N dilution experiment, Chapter 3, partly taken from Wu et al. 2012)

Biogeochemical nitrogen (N) cycling in terrestrial ecosystems is complex, since it comprises many players ranging from microorganisms to higher plants performing a wide range of processes with very different magnitudes of N turnover (Schimel and Bennett 2004; Booth et al. 2005; Kreutzer et al. 2009; Rennenberg et al. 2009, Butterbach-Bahl et al. 2011). The terrestrial N cycle is dominated by the soil microbial N turnover processes of ammonification, i. e. the conversion of organic N compounds to ammonium, nitrification (conversion of organic N or ammonium to nitrate), and the subsequent allocation of bioavailable N such as ammonium and nitrate to plants, microorganisms as well as to N loss pathways.

Since the determination of gross rates of ammonification and nitrification is based on time- and resource-intensive techniques mostly involving the use of stable ^{15}N isotopes, actual gross rates of N turnover have been determined less often than net rates. However, a few points in time measurements are available for a range of terrestrial ecosystems (Booth et al. 2005), though the temporal resolution of such studies is strongly limited. The few available studies examining the temporal variability of gross N turnover in soils (e. g. Dannenmann et al., 2006, Rosenkranz et al., 2010) showed that its rates may markedly fluctuate across seasons. Hence, the available studies on gross N turnover in general do neither allow to understand seasonal dynamics nor reliable estimates of annual rates of gross N turnover. However, actual gross rates of N turnover in soil subjected to winter conditions have only been determined few times in laboratory studies (Müller et al. 2002, Ludwig et al. 2004, Freppaz et al. 2007), but not in situ.

The latter studies determined significant rates of gross ammonification and/or nitrification in soil subjected to freeze thaw events, however also were restricted to single point in time measurements. So far, no study has investigated gross N turnover in permanently frozen soil. However, based on more indirect parameters of N turnover, several studies showed that significant biogeochemical C and N turnover can occur in frozen soils and during freeze/thaw periods (Vogt et al. 1986; Clein & Schimel 1995; Brooks et al. 1999).

Soil Microbial N turnover may persist in winter under snowpack in the soil, as indicated e. g. by measurements of significant net N turnover rates in continental steppe of China (Zhou et al. 2009, Zhao et al. 2010), boreal forests (Kielland et al. 2006), arctic tundra (Schimel et al. 2004), and temperate hardwood forests (Groffman

et al. 2001b). Microbial biomass and several soil enzyme activities were even found to peak in late winter in alpine soils (Lipson et al. 1999, 2002). Furthermore, significant plant litter decomposition was found in winter in seasonally snow-covered ecosystems (Taylor and Jones 1990, Hobbie and Chapin 1996, Schmidt and Lipson 2004).

Soil freeze thaw cycles have been reported to kill a portion of soil microbial biomass (DeLuca et al. 2002, Clein and Schimel 1995, Schimel and Clein 1996). The resulting increase of easy degradable N and C substrates in soil during spring snowmelt has been shown to prime fine root turnover, increase net N mineralization (Groffman et al. 2001a, Schmidt et al. 2007, Matzner and Borken 2008), nutrient leaching (Brooks et al. 1999), or N_2O emissions from soil (Wolf et al. 2010).

The lack of insight into actual soil N turnover in soils subjected to harsh winter conditions still hampers a functional and quantitative understanding of soil N biogeochemistry in frozen soil, under freeze-thaw conditions and at the annual scale. In particular, it remains uncertain whether our understanding of the contribution of cold seasons to annual N flux, as estimated from dynamics and magnitude of measurements of net rates of N turnover, enzyme activities and microbial community parameters, is valid.

However, also these studies were based on single or few measurements in the growing seasons and thus could neither provide detailed insight in the temporal dynamics and environmental controls of N turnover at the annual scale. Hence, also for semi-arid, winter-cold continental steppe ecosystems of Asia, understanding of both the magnitude of annual gross N and net N turnover as well as the contribution of the cold seasons to the annual budget of N turnover and thus a functional understanding of the importance of the winter period for annual soil N cycling has not yet been achieved.

Therefore, we measured gross and net N turnover as well as dynamics of soil inorganic N and microbial biomass concentrations in monthly or bi-weekly temporal resolution over an entire year at replicated plots of two contrasting steppe systems, i.e. grazed and ungrazed. The aim of this study was to characterize and quantify both the temporal dynamics and annual sum of microbial N turnover over a full year (Chapter 3; partly taken from Wu et al., 2012).

In particular, we tested the following hypotheses:

(1) There is significant gross N turnover in permanently frozen steppe soils
(2) Both N turnover in the growing season as well as during freeze thaw periods are of major importance for the annual budget of gross N turnover
(3) To elucidate the significance of potential environmental controls (temperature, soil moisture, microbial biomass) on gross N turnover
(4) To investigate whether net rates of N turnover provide insight into magnitude and dynamics of gross N turnover over the annual course

Objective Ⅱ: To evaluate the grazing intensity effects on gross rates of N mineralization and short-term inorganic N partitioning in intact plant-soil systems of semi-arid steppe of Inner Mongolia (In situ ^{15}N application experiment, Chapter 4, mostly taken from Wu et al. 2011a)

While the general ecological significance of the N cycle in terms of regulating ecosystem N retention, N loss (which can affect atmospheric chemistry, climate change and water quality), and plant nutrition has remained unchallenged, our perception of the functioning of this complex network of closely interlinked processes has changed considerably during the last decades (Schimel and Bennett 2004, Chapman et al. 2006, van der Heijden et al. 2008, Rennenberg et al. 2009). Among others, central paradigm shifts were: (1) plants actively compete for nitrogen (organic and inorganic species) with microbes, and they are not a priori inferior in this competition (Schimel and Bennett 2004, Harrison et al. 2007, 2008, Xu et al. 2008) and (2) not only microorganisms control plant growth but plants may actively control microbial nitrogen conversion in soils (Chapman et al. 2006). Microorganisms regulate plant productivity, e.g. by acquisition of plant nutrients by mycorrhizal fungal plant symbionts, or by mineralization of, and competition for, nutrients by free-living microbes (van der Heijden et al. 2008). Vice versa, plants influence microbial N turnover in soil by the determination of organic matter composition via plant residues and root exudates, thus influencing substrate quality for N mineralization (Chapman et al. 2006), and by direct carbon (C) allocation to microorganisms via root exudation (Kuzyakov 2000, Bais et al. 2006).

Surprisingly, the view of close reciprocal interconnections and interactions between plant and microbial belowground N fluxes has not been reflected in

experimental setups for process-oriented investigations: soil ecologists/microbiologists still exclude the plant part for the determination of soil N turnover processes while plant physiologists frequently determine N uptake rates after removing soil (Gessler et al. 2005, Högberg and Read 2006, Rennenberg et al. 2009). Thus, competitive and other plant-microbe interactions are excluded in these experiments of ecosystem N turnover / immobilization. However, comparing N turnover in root-free soil with N turnover in root enclosures revealed different patterns and rates of N fluxes, especially with respect to internal cycles of N turnover (Jones et al. 1994; Burger and Jackson 2004). In order to reduce the current major deficits in knowledge on N cycling, it is essential to link plant physiology and microbial N metabolism in experimental designs where plant-microbe competition and further interactions persist (Rennenberg et al. 2009).

The available studies investigating grazing effects on soil N conversion conducted in Asia in general confined themselves to the determination of net rates of N turnover only (Xu et al. 2007), which comprise both production and consumption of inorganic N, and thus do not allow an insight into actual N turnover in soil. An exception is the study of Holst et al. (2007), who investigated gross rates of N turnover in Inner Mongolia, China, and found that grazing tended to decrease gross rates of N turnover. However, experiments on gross rates of N turnover in the latter study was based – like in nearly all other comparable studies on gross rates of N turnover – on experiments with disturbed soil where living plants and roots were excluded. Hence, such experiments do not provide insight into simultaneously occurring N fluxes in the plant-soil system. As outlined above, the exclusion of plant-microbe interaction is likely to significantly alter soil N cycling. It may be of special importance to investigate soil N cycling in intact plant-soil systems when the plant compartment is affected by a treatment, as is the case in the example of grazing. Hence, we developed a method for the determination of gross rates of N mineralization and subsequent partitioning of inorganic N to plants and microorganisms in large soil cores where plant-microbe interactions persisted throughout the experimental incubation (Chapter 4; mostly taken from Wu et al., 2011a). We applied this method to plots subjected to different stocking rate in Inner Mongolia.

The hypotheses are:

(1) Grazing increases gross N mineralization

(2) Grazing alters short-term plant-microbe competition for inorganic N in favour of microorganisms and at the expense of plants

Objective Ⅲ: To assess the labile organic C and N mineralization of soil aggregate size classes in semi-arid grasslands as affected by grazing management (incubation experiment of CO_2 production, Chapter 5, mostly taken from Wu et al. 2011b)

Soil labile organic carbon (LOC) is a soil fraction with turnover time of less than a few years (even less than weeks) as compared to recalcitrant carbon with a turnover time of several thousand years (Parton et al., 1987; Schimel et al., 1985). LOC as the most active fraction of soil organic carbon (SOC) can be readily influenced by disturbance and management (Harison et al., 1993). Therefore, LOC oxidation drives the flux of CO_2 between soils and the atmosphere (Zou et al., 2005) and makes a greater contribution to nutrient cycling than stable SOC (Whalen et al., 2000). As most studies are focusing on total organic carbon storage and sequestration, mineralization of LOC is not well understood, particularly for typical grassland soils.

SOC protection mechanisms are intimately tied to the processes of aggregate turnover and stabilization at multiple scales (Steffens et al., 2009b). The deposition and transformation of organic matter plays a major role in aggregate stabilization and there are strong feedbacks between aggregate turnover and SOC dynamics (Jastrow et al., 2007; Lützow et al., 2006). Differences in turnover rates of SOM fractions may be due to physical protection of organic matter within soil aggregates as well as chemical protection from humification (Cambardella and Elliott, 1993). The fractions of SOM that turn over rapidly are believed to make a greater contribution to nutrient cycling than fractions that turn over slowly because they provide a more readily accessible source of energy for the saprotrophic soil organisms responsible for nutrient cycling (Janzen et al., 1992). It is becoming increasingly important to determine not only how land management practices affect the retention or loss of these fractions of SOM, but also how they affect nutrient cycling from SOM fractions. Improved understanding of these processes will provide valuable information for maintaining or implementing

environmentally-sustainable land management practices in agricultural and forest soils (Whalen et al., 2000).

The effects of grazing on soil N mineralization have been intensively studied. However, variable grazing effects have been reported with both increasing (Groffman et al., 1993; Le Roux et al., 2003) and decreasing (Bardgett and Wardle, 2003; Biondini et al., 1998) N mineralization. Furthermore, N mineralization of soil aggregate size classes (ASC) is not well understood, especially under the influence of grazing (He et al., 2011). Estimating N mineralization would be helpful to understand C mineralization of ASCs in semi-arid grasslands affected by grazing management (Chapter 5; mostly taken from Wu et al., 2011b).

The objectives of this research are:

(1) To evaluate the influence of different grazing intensities on soil LOC and N mineralization
(2) To estimate the effect of different ASCs on soil LOC and N mineralization
(3) To elucidate the interactions of LOC and N mineralization

I note that major parts of the thesis have been published previously with me as the leading author. I have meationed this in the upper text and have cited them in this thesis. The text passages from these papers have been used in this thesis.

2. MATERIAL AND METHODS

2.1 Plot description

Our study was carried on in the Inner Mongolia grassland, a part of the largest contiguous grassland area in the world (Bai et al., 2004). The sites located in the Xilin River Basin, Inner Mongolia Autonomous Region, China (43°38′ N, 116°42′ E). These sites are managed by the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), which belongs to the Chinese Ecological Research Network (CERN).

The climate in this area belongs to the continental middle temperate semi-arid zone (Chen 1988). Winter is cold and dry, while summer is warm and wet.The mean annual temperature in the Xilin River Basin is about 0.3 ℃ with mean monthly temperatures ranging from - 21.6 ℃ in January to 19.0 ℃ in July. The mean annual precipitation is 346 mm with 60–80% falling during the growing season from May to September with mean monthly temperature ≥5°C (figure 1) and approximately 10% of which falls as snow. The soil is classified as alkalescent loamy sand.

The steppe grassland covers 72% of the Xilin River Basin with a large number of typical plant species. The mainly vegetation types are Leymus chinensis and Stipa grandis in this region. Leymus chinensis in general dominated steppe communities at areas that is lightly or moderately grazed with relatively wet soil conditions, Stipa grandis mostly dominated communities in drier area that is usually heavily grazed(Tong et al., 2004; Chen et al., 2005b). The other species are Agropyron michnoi, Carex korshinskii, Puccinellia tenuiflora, Salsola collina, Melissitus ruthenica, Axyris amaranthoides and Caragana microphylla. Cleistogenes squarrosa and Artemisia frigida appears when the steppe grassland degraded (Schroeder, 2010).

The Inner Mongolia grassland is the most important area of livestock grazing in China, which covers 78.8×10^7 ha and accounts for 39% Chinese grassland (alata，2006). Over the past half century, the herders change their nomadic life style and move to the permanent settlements with an intensive livestock production system. The sedentarization of the nomads and improved livestock (Figure 2) resulted in a massive degradation of the grasslands. The grasslands are nearly fully grazed with high

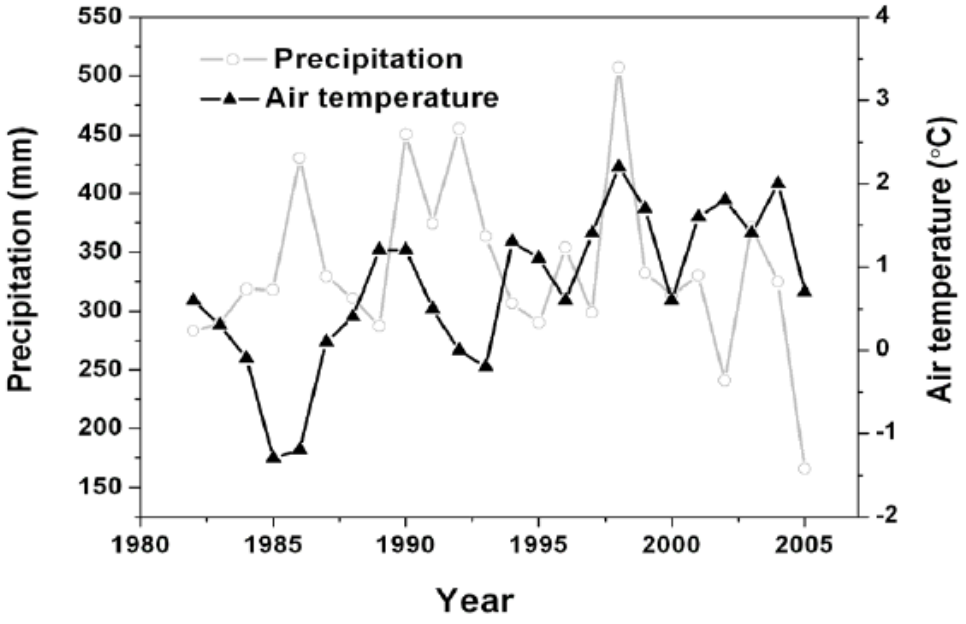

Figure 1： The mean annual temperature and precipitation（1982-2005,data given by IMGERS)

stocking rates around villages, farms and streets. According to the remote sensing, until 1995 the degradation area is 38.7×10^7 ha, which accounts for 60.1% available grassland of Inner Mongolia (Wei Z. J. and Shuang Q.，2001). The aboveground fresh biomss decreased 45%, from 1912 kg ha^{-1} in 1950' to 1050 kg ha^{-1} in 1980'.

2.1.1 Fenced experiment

Five experimental sites with different grazing intensities (Fig. 3) were chosen in this study. The whole area is grazed by herds that are composed of 70–90% sheep and 10–30% goats. The whole experimental area was grazed before with low intensity. In 1979, one plot (24 ha) was fenced and excluded from grazing (UG79). After 20 years of moderate grazing, two plots were fenced again: one was completely excluded from grazing (25 ha; UG99), the other is still grazed during winter (34 ha; WG), equivalent to a grazing intensity of 0.5 sheep unit ha-1 year-1. The ungrazed site was a Leymus chinensis grassland.

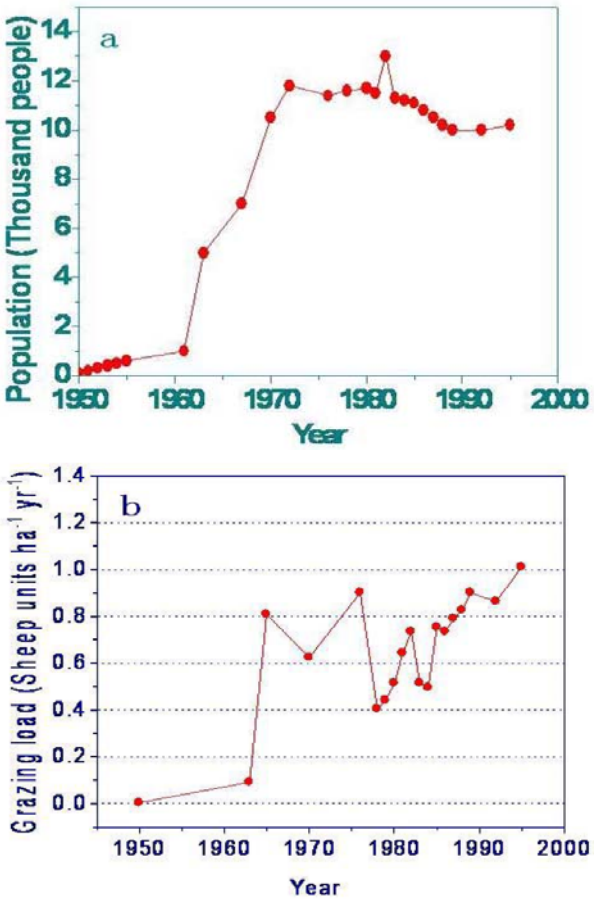

Figure 2: population(a) and grazing stock, (b) change during the past half century in xilinhot grassland, Inner Mongolia（Xu et al.，2003）

Another site (24 ha) was grazed during the whole year (continuously grazed; CG), equivalent to a grazing intensity of 3 sheep units ha^{-1} $year^{-1}$. An unfenced site was grazed with approximately 4 sheep units ha^{-1} (HG) during the vegetation period that is located approximately 2 km away from the other sites (table 1). The vegetation composition in this site has significantly changed, that is the abundances of Potentilla acaulis L., Artemisia frigida Willd. and the abundances of C_4 grasses [Cleistogenes squarrosa (Trin.) Keng] increased dramaticaly, at the same time, the C_3 grasses [L. chinensis (Trin.) Tzvel., Stipa grandis P. Smirn.] reduced. These vegetation changes indicated typically the effect of overgrazing (Wang and Ripley 1997; Wang 2002; Tong and others 2004).

Table 1: Main charactersistics of the different experimental sites

	UG99	WG	UG79	CG	HG
Geographic coordinates	43°33.0′N	43°33.0′N	43°33.1′N	43°33.1′N	43°34.7′N
	116°40.1′E	116°40.1′E	116°40.5′E	116°40.0′E	116°40.6′E
Height above sea level(m)	1,268	1,267	1,252	1260	1,218
Maximum vegetation height 2005 (cm)	n.d.	19.6 ± 3.0	24.2 ± 2.8	n.d.	6.9 ± 1.9
Slope(°)	2.2–2.5	2.5–2.7	1.9–2.9	Flat	Flat
pH, 0–4 cm, ± s.d	6.8 ± 0.3	6.7 ± 0.3	6.6 ± 0.2	6.6 ± 0.35	6.6 ± 0.3
Bulk density, 0–4 cm, ± s.d. (g cm^3)	1.09± 0.12	1.09± 0.08	0.94± 0.10	1.17±0.07	1.28 ± 0.08
C to N ratio, 0–4 cm, ± s.d.	.9.7 ± 0.7	9.5 ± 0.4	9.8 ± 0.3	9.6 ± 0.4	9.7 ± 0.4
Organic C content, 0–4 cm, ± s.d. (%)	2.55± 0.63	2.59± 0.45	3.10± 0.55	2.30± 0.41	1.70 ± 0.42
Soil texture, 0–10 cm: sand (%)	48.3	54.9	64.2	54.8	66.2
Silt (%)	25.8	18.2	13.5	21.1	15.8
Clay (%)	25.9	27.0	22.3	24.2	18.0
Grazing intensity (sheep units ha^{-1} y^{-1})	0	1.2	0	3	~4

n.d. = not determined

For further details on soil data see Steffens and others (2008)

2.1.2 Meteorological data

At the site UG99 and WG, soil temperature at 0.05 m depth was recorded continuously with PT100 thermometers (Th2-h, UMS GmbH, Munich, Germany) at one minute intervals. Soil moisture was recorded with the same frequency using three FD probes (ECH2O-5, Decagon Devices, Pullman, WA, USA) per site. The FD sensors were installed in a way that they integrated soil moisture over a depth of 0 - 0.05 m. During wintertime when soil temperatures dropped below 0°C, soil samples from 0 - 0.05 m soil depth were taken by means of 100 ml core cutters at least twice a week. The samples were dried in the oven at a temperature of 105°C for 24 hours in order to determine volumetric water content. Precipitation data was provided by the IMGERS station in daily resolution (from Wu et al. 2012).

2.1.3 Controlled grazing experiment

The controlled grazing experiment was established in 2005 (Schönbach et al. 2009) on a grazed typical steppe next to a farm. Until 2003, the area was moderately grazed

by local famers. Thereafter, it was recovered from grazing for 2 years before the experiment started in June 2005. Approximately 160 ha were fenced and divided into plots of 2 ha with different stocking rates and land use management. We choose four different stocking rates: ungrazed, low, moderate and heavy (0, 1.5, 4.5 and 7.5 sheep ha^{-1} grazing $season^{-1}$) for this experiment (figure 4). Typically single plots (n=4) of 2 ha size were grazed continuously by sheep from June to September.

2.2 Experimental design and sample analysis

2.2.1 Whole-year round field N cycling experiment (Chapter 3)

2.2.1.1 Sampling description

Net rates of N turnover, microbial biomass C and N, gross N turnover were measured 20 times at 8-38 days interval from August 16, 2007 to October 13, 2008 in our study. The intervals changed according to seasons (short interval in summer while long interval in winter). Soil sampling was conducted on three replicated plots both in the ungrazed and winter grazed site. Sampling in each plot was conducted at 6-10 spots to reduce the spatial variability (see details for each measurement). All the measurements were carried on immediately after sampling and situ incubations were used both for gross and net rate assays of N turnover to avoid any storage of soil and thus released factitious effect on N turnover. The soil samples for determination of net rates of N turnover, soil mineral N concentrations and microbial biomass C and N were sampled after the sampling for gross N turnover. On average, the delay between sampling for gross rates of N turnover and microbial biomass, mineral N and net N turnover determination was 5 days (from Wu et al. 2012).

2.2.1.2 Gross rates of microbial N turnover

Gross rates of ammonification and nitrification were determined using an in situ 15N pool dilution technique described previously by Dannenmann et al. (2006) and Wolf et al. (2010). The experimental procedure was adapted to the logistic and climatic conditions at the remote experimental site. Sampling was conducted at ten

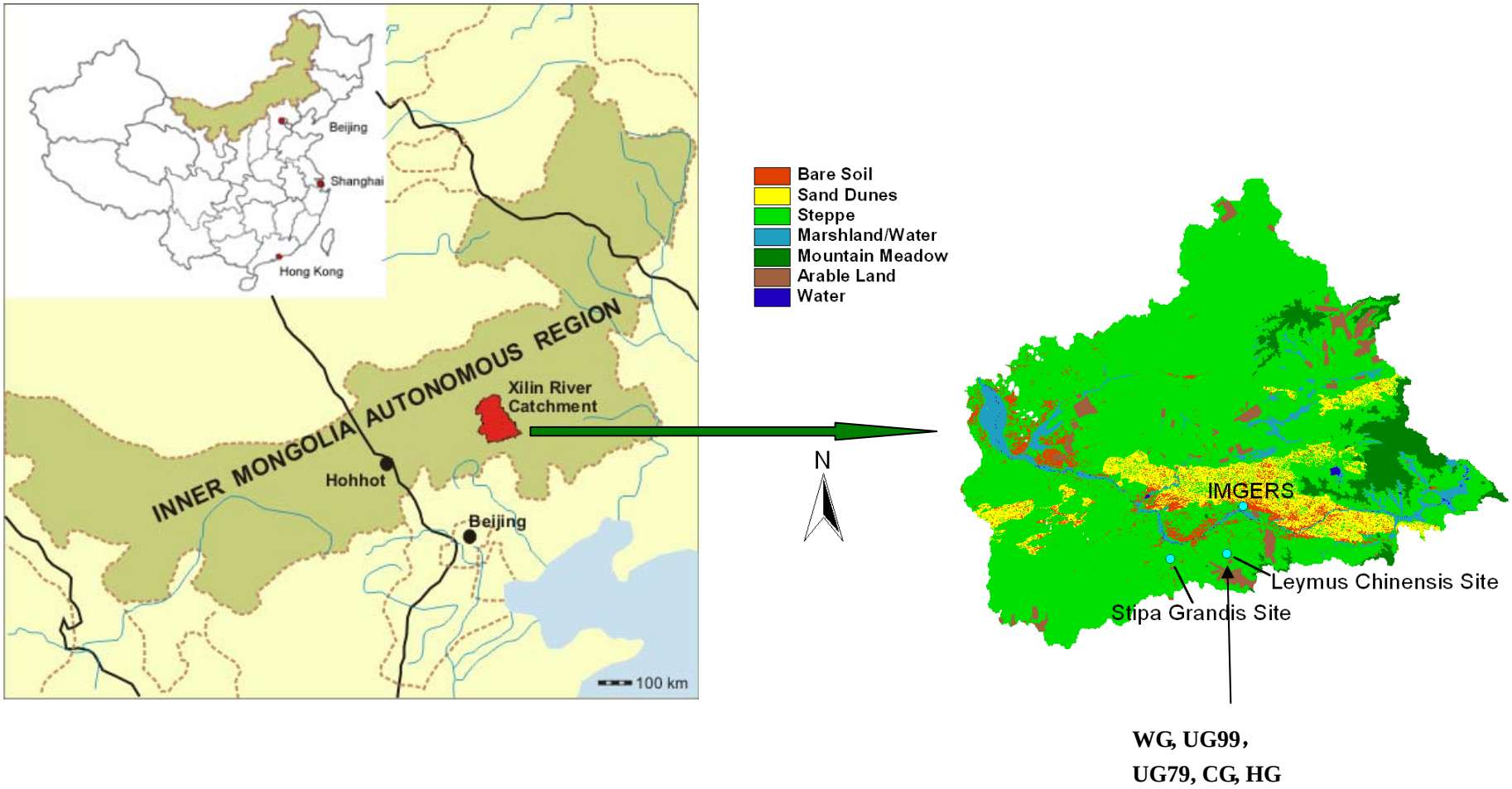

Figure 3: The details of sites (provided by IBCAS)

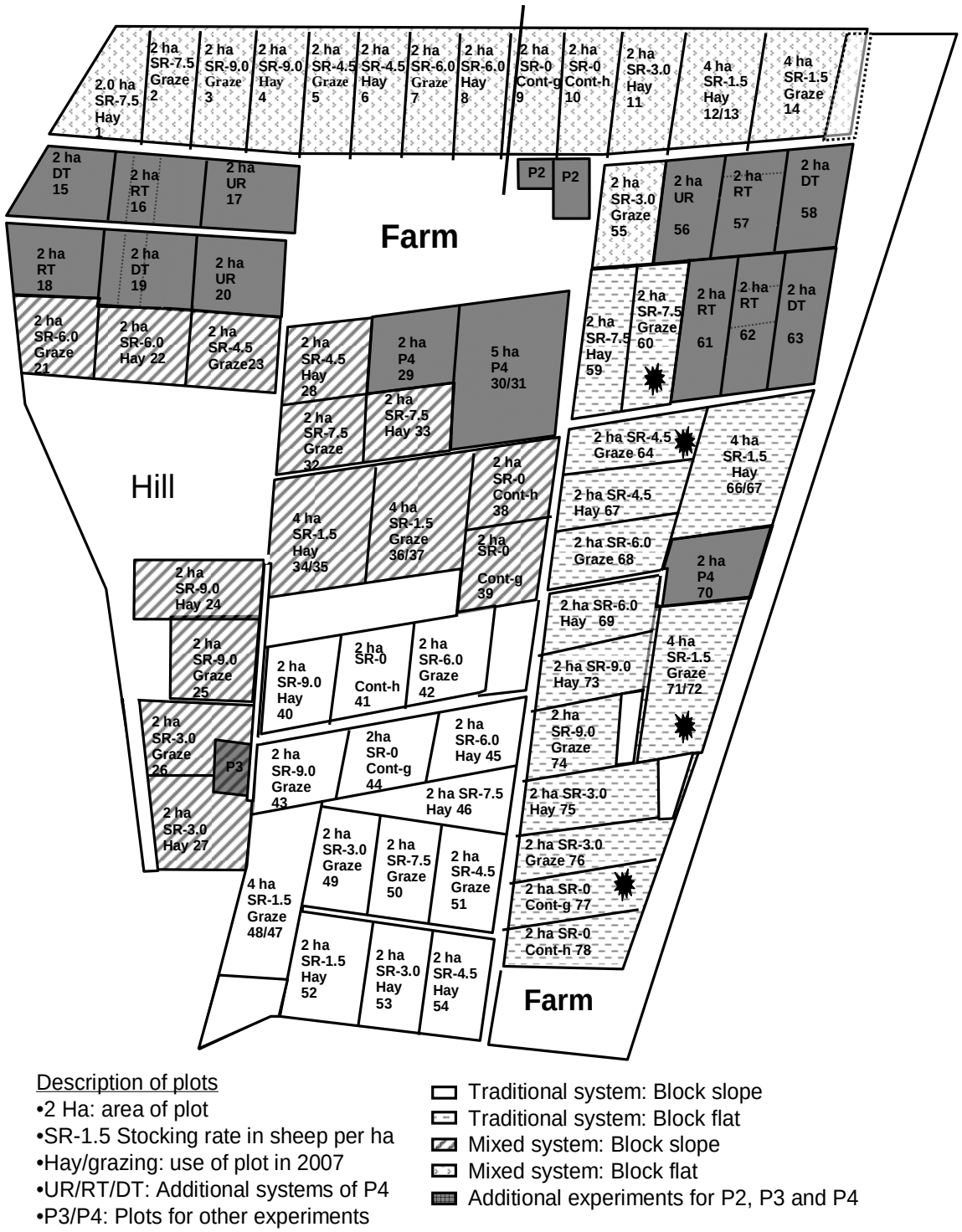

Figure 4: The grazing map (the asterisk is experimental plot, provided by IMGERS).

sampling spots at every plot. For these experiments, sample were pooled for single plots (n=6 plots, 3 plots in WG, 3 plots in UG), sieved (5mm mesh width) and homogeneously sprayed with $(NH_4)_2SO_4$ or KNO_3 solution at 30 atom% ^{15}N enrichment (Dannenmann et al. 2009) at the same day of sampling. Label application increased the soil water content by 3% and the total NH_4^+-N or NO_3^--N content by approximately 1 mg N kg^{-1} sdw, which is well below the mean pool sizes in this soil (compare Fig. 6 F, G). Six subsamples of 30 g each for every labelling treatment and plot were placed in parafilm-sealed 250 ml plastic bottles and buried close to the sampling location after labelling. Eighteen hours (time 1) and 42 hours after labelling (time 2), half of the bottles, i. e. 3 bottles per labelling treatment and plot were excavated and immediately extracted with 60 ml 1M KCl as described by Dannenmann et al. (2006). Diffusion steps for trapping NH_4^+ and NO_3^- on acid filter disks (Dannenmann et al. 2006) and subsequent GC-IRMS analyses for 15N-enrichment (Dannenmann et al. 2009) were performed as described earlier. A heater blower ensured temperatures well above 25 °C during the diffusion steps also in winter. Total ammonium and nitrate concentrations in the extracts were determined as described above. In 2008, gross nitrification is available with reduced temporal resolution only (see Fig. 6 C) (from Wu et al. 2012).

Gross ammonification (gross N mineralization) and nitrification rates were calculated using the equations given by Kirkham and Bartholomew (1954). For frozen soil conditions, a different labelling technique was applied (Wolf et al. 2010). Here, the labelling solution was amended with triple hot washed and autoclaved quartz sand at a ratio of 1:2.5, frozen and then crushed to a fine powder. This powder was homegenuously mixed with the frozen soil for labelling. All experimental steps until the amendment of the KCl solution for soil extraction were performed outdoors at air temperatures below -10°C in order to ensure that the investigated soil and the labelling solution remained constantly frozen. Incubations took place in situ at the plots by burying the incubation bottles also in winter (from Wu et al. 2012). The

process about gross rates of mineralization and nitrification and NH_4^+ and NO_3^- immobilization (Shaw, M. R. & Harte J., 2001) was given in Figure 5.

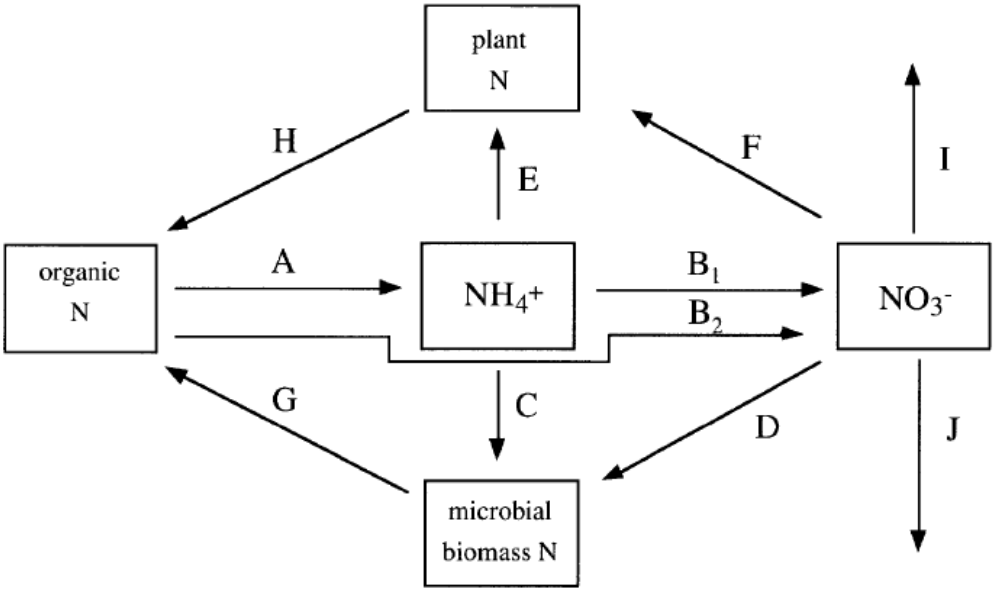

Figure 5: Diagram of N cycle including relevant transformations (Shaw, M. R. & Harte J., 2001)

A=gross ammonification
B1=gross nitrification (autotrophic)
B2=gross nitrification with organic N (heterotrophic)
C= gross immobilization (microbial assimilation) of NH_4^+
D=gross immobilization (microbial assimilation) of NO_3^-
E=plant uptake of NH_4^+
F=plant uptake of NO_3^-
G= organic N inputs from microorganisms
H= organic N inputs from plants
I=denitrification
J = NO_3^- leaching
Net ammonification=A-(B1+C),
Net nitrification= (B1+B2)-D
Net mineralization= (A+B2)-(C+D)

2.2.1.3 ^{15}N dilution calculation

Gross ammonification (gross N mineralization) and nitrification rates were carried

out according to Kirkham and Bartholomew (1954):

$$m = \frac{M_0 - M_1}{t} \frac{\log(H_0 M_1 / H_1 M_0)}{\log(M_0 / M_1)} \quad (1)$$

$$c = \frac{M_0 - M_1}{t} \frac{\log(H_0 / H_1)}{\log(M_0 / M_1)} \quad (2)$$

NH_4^+ and NO_3^- consumption rates were calculated directly using the equations given by Kirkham and Bartholomew (1954). It is assumed that gaseous N losses and other possible fates of inorganic N were negligible (Davidson et al. 1991). Microbial NH_4^+ immobilization was determined by subtracting nitrification rates from NH_4^+ consumption rates while NO_3^- immobilization was equal to microbial NO_3^- consumption.

where

M0: initial (after 18 h incubation) $^{14+15}NH_4^+$ pool (mg N kg^{-1} soil)

M1: post-incubation (after 42 h incubation) $^{14+15}NH_4^+$ pool (mg N kg^{-1} soil)

H0: initial (after 18h incubation) $^{15}NH_4^+$ pool (mg N kg^{-1} soil) above natural abundance

H1: post-incubation (after 42 h incubation) $^{15}NH_4^+$ pool (mg N kg^{-1} soil) above natural abundance

m: gross mineralization rate (mg N kg^{-1} soil)

c: gross NH_4^+ consumption rate(mg N kg^{-1} soil)

t: time (24h for the present study) between initial and post-incubation harvest (days)

It should be kept in mind, the microbial NH_4^+ immobilization is likely to be overestimated since immobilization is determined as the difference between NH_4^+

consumption (Immobilization and nitrification may have been stimulated by addition of NH_4^+) and nitrification (Nitrification should not have been stimulated as it is the outcome that was added).

2.2.1.4 Net rates of N turnover and mineral N concentrations

Net rates of N turnover were determined according to the method of Wang *et al.* (2006). For the first 4 sampling dates paired soil cores from 10 sampling spots (5 cm diameter, 10 cm depth) were taken as spatial replicates on each plot. Based on analysis of variance, the spatial replication was reduced to eight sampling spots starting from the fifth sampling date both for net rates of N turnover and microbial biomass C and N determination. One of the paired soil cores was immediately harvested (time 1; in total 60 or 48 soil cores), soil was removed out of the core and coarse material was separated from the soil. Subsamples of single cores were analysed for inorganic N concentrations, microbial biomass C and N (see below). Soil extraction for mineral N concentrations was conducted with 30 g of soil by use of 1M KCl (soil to solution ratio 1:2) as described in detail by Dannenmann et al. (2006). Concentrations of inorganic N (NH_4^+-N and NO_3^--N) in the filtered extracts were conducted at IMGERS using a flow injection autoanalyzer (Zhou *et al.* 2009, FIAstar 5000 Analyzer, Foss Tecator, Denmark). Soil water content was determined with subsamples being dried at 105℃ for 24 h. The expression of soil inorganic N concentrations was based on dry soil weight. After two weeks, the remaining 60 or 48 soil cores, were harvested, extracted and analyzed for mineral N (time 2). The ammonium and nitrate concentrations from the first harvesting date (time 1) are referred to soil ammonium and nitrate concentrations (Fig. 6 F, G). Net rates of ammonification and nitrification (Fig. 6 H, I) were calculated from the changes in the concentrations of extractable ammonium and nitrate between the 2 soil core harvesting dates (Wang *et al.* 2006) (from Wu et al. 2012).

Under frozen soil conditions, the use of the PVC soil cores was not possible.

Here, an in situ incubation buried bag technique (Dannenmann *et al.* 2006) was used for the determination of net N turnover. For this purpose, paired intact portions of soil and vegetation cover (10*5 cm are, 10 cm depth) were gained by use of a spate. One half was immediately extracted and analyzed as described above (time 1). The second half was packed into polyethylene bags with pinholes (Dannenmann *et al.* 2006) and reburied for the time 2 extraction. If applicable, the incubated soil was covered with snow of a representative height. Soil samples were kept frozen during transport from the field sites to the lab until extraction (from Wu et al. 2012).

2.2.1.5 Net mineralization calculation

Net ammonificaiton and nitrification were calculated according to the difference of inorganic N (NH_4^+, NO_3^-) ammount between initial and post incubation.

The calculation of net mineralization（Wang *et al.* 2006）:

$A_a=A_{t2}-A_{t1}$

$N_a=N_{t2}-N_{t1}$

$N_{Amm}= A_a /（t2-t1）$

$N_{Nit}= N_a /（t2-t1）$

A_{t1}：initial (time1) NH_4^+ pool (mg N kg^{-1} soil)

A_{t2}：post-incubation (time2) NH_4^+ pool (mg N kg^{-1} soil)

A_a: the amount of NH_4^+ produced between t2 and t1(mg N kg^{-1} soil)

N_a: the amount of NO_3^- produced between t2 and t1(mg N kg^{-1} soil)

N_{Amm}：Net ammonificaiton(mg N kg^{-1} d^{-1})

N_{Nit}：Net nitrification(mg N kg^{-1} d^{-1})

2.2.1.6 Microbial biomass C and N

Microbial biomass C and N was estimated using the chloroform fumigation-extraction (FE) method (Brookes *et al.*, 1985; Vance *et al.*, 1987; Tate *et*

al., 1988) as described in detail by Dannenmann *et al.* (2006). Time 1 subsamples of the soil sampled for the determination of net N turnover (see above) was used for this purpose. For the first 3 sampling dates, ten samples per plot were taken at randomly selected spots, then the spatial replication was decreased to six sampling spots per plot. After removal of coarse organic materials and stones, samples were divided in paired subsamples of 30 g each. One subsample was immediately extracted with 60 ml 0.5 M K_2SO_4 while the second subsample was fumigated under chloroform vapour for 24 h in a desiccator. Subsequently, ten vacuum/release purge cycles ensured the complete removal of chloroform and fumigated subsamples were extracted as described above. Extracts were filtered using a 0.45 µm syringe filter (Schleicher and Schuell, Dassel, Germany) and immediately frozen until analysis for total organic carbon (TOC) and total chemically bound nitrogen (TNb) using a TOC analyzer with a coupled TNb module (Dimatec Analysentechnik GmbH, Essen, Germany). Total carbon (TC) and total inorganic carbon (TIC) were determined based on non-dispersive infrared photometrical detection of evolving CO_2 after thermic and chemical oxidation of the samples. TOC was calculated as TC – TIC. TNb was analyzed by use of a chemoluminescence detector. Correction factors (0.54 for microbial biomass N and 0.379 for microbial biomass C, Brookes *et al.*, 1985; Vance *et al.*, 1987) were applied to the difference in total extractable N and TOC between paired untreated and fumigated subsamples to estimate microbial biomass C and N. Soil samples were kept frozen until extraction (from Wu et al. 2012).

2.2.2 In situ ^{15}N application experiment (Chapter 4)

The in situ ^{15}N application experiment was conducted in the controlled grazing plots. we choose four different stocking rates: ungrazed, low, moderate and heavy (0, 1.5, 4.8 and 7.5 sheep ha^{-1} grazing season^{-1}). The plots were grazed continuously by sheep from June to September every year. For the low stocking rate of 1.5 sheep ha^{-1} grazing season^{-1} 4 ha plots were used. On August 15 and August 16, 2007, 18 stainless steel soil cores were driven into the soil on each of the four plots within an

area of 160 m^2. The inner diameter of the soil cores was 15 cm and the height was 20 cm. The minimum distance between the individual cores was 2m. On August 17 and August 30, 2007, all soil cores were irrigated with 18 mm of water in order to prevent drying or death of enclosed grass plants due to inevitable cutting of roots. The simulated rainfall equals typical midsummer convective rainfall events in the investigation area. Subsequently the soil cores were left undisturbed in situ for nearly a complete year in order to facilitate regeneration of the enclosed plant-soil system from the coring and trenching impacts (from Wu et al. 2011a).

From May to June 2008, labelling tests by use of Brilliant Blue FCF colour dye solutions were performed with additional soil cores in order to find an optimum labelling method for the intact soil monoliths. Various injection patterns, numbers of injections per soil core and injection volumes per single injection were tested in order to comply with the opposing requirements 1) 3-dimensional homogeneous distribution of the label solution in the soil monolith; 2) minimization of water addition in order to minimize stimulation of N turnover and leaching of labelling solution out of the cores. Harvesting of the soil cores was performed 24 hours after colour dye application. For harvesting, soil was removed stepwise from bottom to top in 2 cm layers and the distribution of the colour dye in the soil was monitored visually. Outflow at the bottom of the labelled plant-soil-system was monitored by storing the soil cores on white paper sheets. Finally 38 single injections into the soil cores of 3 ml solution each turned out to be the best compromise. The injections were applied to four different depths of the soil cores by use of custom-made stainless steel side-port cannulas. This labelling treatment increased the water content of the soil by approx. 2.2 % soil dry weight and lead to a homogeneous distribution of the colour dye over the soil profile, while only occasionally some solution was leached out along root channels. Custom-made PVC calibres were built to fit into the soil cores in order to ensure reproducible labelling (from Wu et al. 2011a).

Labelling of all soil cores was done between 03:00 and 06:30 pm on June 14, 2008 by means of a solution containing NO_3^- at natural abundance and NH_4^+ at 60 atom % ^{15}N enrichment. The amount of added N was approximately 0.9 mg NH_4^+-N

g^{-1} sdw and 0.9 mg NO_3^--N g^{-1} sdw. This corresponded to approximately 30-50% of the ambient NH_4^+-pool and approximately 5-40 % of the ambient NO_3^- pool (from Wu et al. 2011a).

2.2.2.1 Sampling and sample preparation

Half of the 18 soil cores on each plot were harvested on June 15, 2008, (time 1) and the other half on June 17, 2008 (time 2), starting at 7:00 am. Soil cores were dug out carefully with shovels and an even ending was maintained at the bottom of the soil cores to ensure that the identical volume was enclosed in every soil core. Soil cores were placed in plastic bags and transferred to IMGERS for immediate processing. Aboveground biomass (AGB) was cut, dried at 70°C for 48 h and subsequently weighed (from Wu et al. 2011a).

Soil was completely removed from the soil cores, sieved (3.15 mm mesh) to separate belowground biomass (BGB). Belowground biomass was washed and separated by hand to obtain the living part which was then dried and weighed. Fresh weight of the whole soil contained in the soil core and its gravimetric water content were determined. Total sieved soil gained from each core was mixed by hand for at least ten minutes in order to assure full mixing to a homogenous sample. Out of the total soil sample, subsamples were taken from 10 spots and composited (200-300g in total). This representative soil sample was used for further processing of the soil, i. e. analyses of NH_4^+, NO_3^-, dissolved organic N (DON) and microbial biomass C (MBC) and N (MBN) concentrations, and, furthermore the ^{15}N enrichment in the respective pools. Plant samples were used for analyses of total N and ^{15}N enrichment. Extraction of the soil samples took place 24 hours (time 1) and 72 hours (time 2) after ^{15}N-labelling (from Wu et al. 2011a).

2.2.2.2 Analysis of total N and ^{15}N concentrations in plant biomass.

Aboveground and belowground biomass samples were milled in a ball mill (MM200, Retsch, Germany). ^{15}N:^{14}N ratios and total N of all milled plant samples were analysed by EA-IRMS (Thermo Finnigan, Bremen, Germany) as measured

against a certified plant isotope reference (from Wu et al. 2011a).

2.2.2.3 Analysis of total N, C and ^{15}N concentrations in soil pools.

Out of the representative soil sample of every soil core, a subsample of 30 g each was extracted with 0.5 M K_2SO_4 (soil: solution ratio 1:2). Subsequently, sequential diffusion steps for trapping NH_4^+-N, NO_3^--N and DON-N on acid filter disks were performed, based on the transformation of all N compounds to NH_4^+, conversion to NH_3 by pH increase, and subsequent capturing on acid traps in order to determine the ^{15}N enrichment in the respective pools. Ammonium and nitrate diffusion was performed by use of MgO and Devarda's alloy as described earlier (Dannenmann et al. 2006). After NH_4^+ and NO_3^- had been completely exhausted from the solution by additional open shaking, extracted organic nitrogen was quantitatively converted to NO_3^- by adding 30 ml of a persulfate reagent (50 g $K_2S_2O_8$, 30 g H_3BO_3, 100 ml of 3.75 mol l^{-1} NaOH in 1 l of solution) (Cabrera and Beare 1993, Corre et al. 2007) and heating to 121 °C for 4 hours. Subsequently, the NO_3^--N originating from DON-N was transformed to NH_4^+-N by addition of 0.5 g of Devarda's alloy and the pH was increased to a value of 13 by adding NaOH pellets in order to facilitate a third diffusion step for capturing DON-N on an acid filter traps analoguously as was performed for NH_4^+ and NO_3^- (Dannenmann et al. 2006) (from Wu et al. 2011a).

Further soil subsamples of 30 g each for control and fumigated treatment were used to determine microbial biomass C, N and ^{15}N enrichment by use of the chloroform-fumigation extraction technique as described in detail by Dannenmann et al. 2009. One subsample of the extract was used for auto-analysis of total organic carbon and total chemically bound nitrogen (see below). Further extract subsamples of 30 ml were used for analysis of total ^{15}N content in the sample. For this purpose, both control and fumigated soil extraction solutions (30 ml each) were amended with 30 ml of persulfate reagent (see above) immediately after extraction and the gastight and pressure resistant bottles containing the solution were heated at 121°C for 4 hours. Hence, dissolved organic N and NH_4^+-N were converted to NO_3^--N. Subsequently, Devarda's alloy and NaOH were added and the diffusion on acidified filter disks was performed as described above (from Wu et al. 2011a).

For GC-IRMS analyses of ^{15}N-enrichment (Dannenmann et al. 2009), filter traps were dried in desiccators over silica gel, transferred into tin capsules and transported to the Centre of Stable Isotopes of IMK-IFU, Germany. Total C and N pool sizes in the extracts were analyzed directly with different (in comparison with diffusion on acid traps), and more precise methods by use of subsamples of the extract solution. Ammonium and NO_3^- concentrations were determined colorimetrically by a commercial laboratory (see Dannenmann et al. 2006), after frozen samples had been transported to Germany. Total carbon (TC) and total inorganic carbon (TIC) were determined based on non-dispersive infrared photometrical detection of evolving CO_2 after thermic and chemical oxidation of the samples (Dannenmann et al. 2007). Extractable dissolved organic carbon (DOC) was calculated as TC – TIC. Total chemically bound nitrogen (TNb) was analyzed by use of a chemoluminescence detector coupled to the TOC analyzer (Dannenmann et al. 2007). Correction factors (0.54 for microbial biomass N and 0.379 for microbial biomass C, (Brookes et al. 1985, Vance et al. 1987) were applied to the difference in TNb and TOC between paired untreated and fumigated subsamples to estimate microbial biomass C and N. DON-N concentrations were calculated as TNb (NH_4^+-N + NO_3^-N concentrations). The ^{15}N enrichment in microbial biomass was calculated from the ^{15}N enrichment in total N in control and fumigated samples as determined by mass spectrometry and the respective TNb pool sizes in the control and fumigated treatment extracts gained from the chemoluminescence detection (from Wu et al. 2011a).

Gross N mineralization was calculated by use of the $^{15}NH_4^+$ pool dilution formula given by Kirkham and Bartholomew (1954). In view of the patchiness of vegetation cover and hence, large small-scale spatial variability of soil organic matter distribution (Wiesmeier et al. 2009), we did not use paired soil cores in this study. Hence, only one calculation was performed for every plot based on the mean values of ^{15}N excess in the NH_4^+-pool and the total NH_4^+ pool size determined at time 1 and time 2. Uncertainty analysis was performed based on the standard error of the mean as a quality criteria of the input parameters derived from the nine replications. Hence, it was not possible to conduct statistical tests for significant differences in gross N mineralization across the plots based on analysis of variance (from Wu et al. 2011a).

^{15}N recovery rates in the investigated N pools were calculated for every single soil core under consideration of added excess ^{15}N and the specific amount of soil and aboveground/belowground plant biomass contained in the individual plant-soil microcosms (from Wu et al. 2011a).

2.2.3 Incubation experiment of CO_2 production (Chapter 5)

The soil was sampled from UG79, UG99, WG, HG, CG. At all experimental sites, three randomly located soil pits were sampled at the upper 10 cm of the topsoil using a steel cylinder with a volume of 100 cm^3 to determine soil properties (from Wu et al. 2011b).

2.2.3.1 Physical fractionation

The upper 10 cm of one pit in each of the five plots were used for physical fractionation (4 kg for each sample). The air-dried soil was dry sieved gently by hand to three ASCs: 2000–6300 mm, 630–2000 mm and < 630 mm (referred to as coarse ASC=cASC, medium ASC=mASC and fine ASC=fASC; Steffens et al., 2009b) (from Wu et al. 2011b).

2.2.3.2 Soil incubations

Carbon and N mineralization of the ASCs were determined after soil incubation at 25 ± 0.5°C for 30 days. 50g of dry soil material were adjusted to 60% field moisture capacity in100 ml glass bottle with an open mouth, which was put in 1-L glass jars with a septum to keep it gas tight. ASCs were inoculated with 1ml dilute suspensions (soil to solution ratio 1:10) of the corresponding fresh whole soil. A thin film of water was put into the bottom of the jars to prevent the soil drying (from Wu et al. 2011b).

2.2.3.3 Determination of soil properties

In order to characterize LOC mineralization, CO_2 production after incubation, microbial biomass carbon (MBC) and dissolved organic carbon (DOC) were

determined which were related to SOC contents of the samples. Additionally, ammonium (NH_4^+) and nitrate (NO_3^-) contents were analyzed which were related to total nitrogen contents (TN) of the samples. All the analyses were performed in triplicate (from Wu et al. 2011b).

SOC and TN were determined in duplicate by dry combustion on a Vario Max CNS elemental analyser (Elementar, Hanau, Germany). The measured C concentrations of the samples that were free of carbonate represent the SOC concentration. Samples that contained $CaCO_3$ were heated to 500 °C for 4 hours to remove organic carbon and the concentration of inorganic C of the residual material was determined by dry combustion. The content of inorganic C was subtracted from the C concentration of the untreated material and represents the SOC content (from Wu et al. 2011b).

CO_2 production after incubation was determined by incubating the soil fractions in 1-L airtight jars with a vial of 20 ml 0.1 M NaOH. The NaOH solution was removed and replaced with fresh solution during sampling. The NaOH solution was sampled on 2^{th},5^{th},8^{th},11^{th},14^{th},17^{th},23^{th},30^{th} day after the incubation started. At the sampling dates, the captured CO_2 was determined by titration with 0.1 M HCl (Zibilske 1994) after precipitation of the carbonate with excess $BaCI_2$. The CO_2 produced after the incubation was used to calculate the C mineralization rate (from Wu et al. 2011b).

MBC was determined using the fumigation-extraction (FE) method (Vance, 1987, Dannenmann et al., 2006). 10g sample was immediately extracted with 30 ml 0.5 M K_2SO_4 for 60 min on a rotary shaker at 150 rpm. The second sample was fumigated under chloroform vapor for 24 h in a desiccator and then extracted as described above. Extracts were frozen under -20℃ and analyzed within one month for DOC(Dimatec Analysentechnik GmbH, Essen, Germany). Correction factors (0.379 for microbial biomass C, Vance et al. 1987) were applied to the difference in total DOC between untreated and fumigated subsamples to estimate MBC (from Wu et al. 2011b).

After rewetting the soil fractions, 10g subsample was immediately (t_1) extracted as described above in order to analyze NH_4^+ and NO_3^- concentrations. The other subsample were incubated in the glass jar for one month, and thereafter extracted with

0.5 M K_2SO (t_2). The extract solution was immediately frozen until the colorimetrical determination of NH_4^+ and NO_3^- concentrations (FIAstar 5000 Analyzer, Foss Tecator, Denmark). The difference of NH_4^+, NO_3^- and inorganic N between t_2 and t_1 were the net ammonification, nitrification and mineralization respectively (from Wu et al. 2011b).

2.3 Statistics and calculations

In general, the plot mean values were used as statistical unit in this study (n = 3 for grazing and n = 3 for ungrazed). The Wilcoxon test was applied to test for significant differences of the determined parameters at a given sampling date.

For the calculation of cumulative annual N turnover, gross and net N turnover was calculated on a daily basis by linear interpolation considering the mean bulk density of the uppermost 10 cm of the soil. Thus, the given cumulative curves of N turnover are representative for the uppermost 10 cm of the soil. Furthermore, Pearson`s correlation coefficients were calculated to illustrate dependency between soil moisture and microbial biomass (n = 618).

Changes of the analyzed parameters between time 1 and time 2 for the controlled grazing intensities were investigated by use of the Wilcoxon test (N=9). Linear regression analysis was used to investigate the effect of grazing intensity on the investigated plant and soil parameters. Pearson coefficients were calculated.

To test the significance of grazing and soil aggregate size effects on the examined parameters, a two-way analysis of variance (ANOVA) was applied.

The threshold value for significant correlations or differences was set at $P < 0.05$. All statistical analyses were performed with SPSS 10.0 (SPSS Inc., Chicago, USA).

3. Seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia*

3.1 Results

3.1.1 Meteorological data

The meteorological dataset of this study begins in August 2007 with a drying event, i. e. a decrease in soil moisture from approx. 25 vol % to values well below 10% (Fig. 6 A). After a few weeks of such low soil moisture values, precipitation events during three consecutive days in mid of September increased soil moisture again to nearly 30 vol %, followed again by a rapid drying out of soil in the last September week. Throughout this period, soil temperature was higher at the winter grazed plots (from Wu et al. 2012).

Following these two drying/rewetting cycles, the mean daily soil temperature in 5 cm depth declined slowly towards the 0°C mark indicating the first freeze events in topsoil which actually took place in the first week of October as also indicated by the several slight de-/increases in soil moisture at low moisture level of approx. 5-10 vol %. By mid of October 2007, the soil was constantly frozen until the beginning of March. Throughout this whole winter period with severe soil frost, both soil temperature in 5 cm depth and soil moisture were continuously lower at the winter grazed plots.

The subsequent period characterized by frequent and strong freeze/thaw-period lasted from beginning of March until beginning of May. The beginning of this period is clearly marked by a sharp increase in soil moisture both at WG and UG. While soil

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* Wu H.H., Dannenmann M., Wolf B., Han X.G., Butterbach-Bah K. (2012). Seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia – insights from a full year dataset of gross and net nitrogen turnover. Ecosphere, in press.

I note that this chapter is partly from Wu et al. 2012.

moisture at UG clearly reached the highest values of the whole dataset, it was constantly lower at WG than at UG within the 2 months-freeze-thaw-period. Following the spring freeze-thaw period, there were series of pronounced drying-rewetting cycles caused by episodic strong convective rainfall events throughout the whole growing season (Fig. 6 A) until the onset of the transition to winter in 2008.

3.1.2 Growing season with drying/rewetting cycles: opposite cycles of microbial growth and gross rates of N mineralization

The drying-rewetting cycles in the growing seasons, i. e. strong to extreme precipitation events followed by dry and hot weather conditions (Fig. 6A) coincided with oscillations in soil microbial biomass N (range approx. 20 to 100 mg N kg^{-1} sdw, Fig. 6D) and simultaneous counterrotating cycles of gross rates of ammonification (range from close to zero to 3 mg N kg^{-1} sdw d^{-1}, Fig. 6B). Microbial biomass was not only positively correlated to soil moisture during the growing season, but significantly correlated throughout the whole year (Fig. 9). The two major events of microbial decline at the beginning of July and at the end of summer 2008 were accompanied by a more than doubling of the ratio between microbial biomass C and microbial biomass N (Fig. 6E, partly from Wu et al. 2012).

Oscillation patterns of microbial biomass N in soil and gross ammonification were inversely pronounced in summer 2008 (Fig. 6 B, D). Consequently, gross ammonification was negatively correlated with soil water content in summer 2008 ($R=-0.579$, $p=0.012$, $N=18$). These interrelationships indicate that microbial residues from dieback events served as a substrate for gross ammonification.

Gross nitrification is available for five points in time in summer 2008 and was in the range between approx. 0 and 1.5 mg N kg^{-1} sdw d^{-1} (Fig. 6C). At least data based on this – compared to gross ammonification - lower temporal resolution do not give evidence for a strong oscillation of gross nitrification during the summer drying-rewetting events as was observed for gross ammonification (Fig. 6B).

Net ammonification was close to zero over the whole year, but became significantly negative in summer 2008 (Fig. 6 H). This did not affect extractable soil

ammonium concentrations which were low and little variable both in summer 2008 and throughout the whole investigated time span (Fig. 6 F). In contrast, net nitrification was characterized by two peaks at the beginning and end of the 2008 vegetation period and a final decline in September 2008 (Fig. 6 I). However, this dynamics rarely affected extractable soil NO_3^- concentrations (Fig. 6 G).

Grazing did not affect the pattern of temporal dynamics of the determined parameters in summer 2008 (Fig. 6B) However, occasionally grazing significantly decreased soil microbial biomass N (Fig. 1 D), the microbial C:N ratio (Fig. 6E) and soil ammonium concentrations (Fig. 6 F).

3.1.3 Transition to winter with first freeze events: Sharp decline in microbial biomass in conjunction with a peak of gross nitrification

The first freeze/thaw events in the uppermost cm of the topsoil in autumn 2007 occurred by mid of October and lasted approx. 3 weeks until the soil was constantly frozen (Fig. 6A). Compared to the previous period without soil frost, microbial biomass declined dramatically by more than 80% (Fig. 6 D) while at the same time the microbial C:N ratio was more than doubled (Fig. 6 E). Simultaneously, a moderate increase of gross ammonification at low level occurred (Fig. 6 B) while gross nitrification was increased dramatically and within in this period of first topsoil freeze thaw events and was up to an order of magnitude larger than gross ammonification (Fig. 6 C). These dynamic changes in gross rates of N turnover and microbial biomass N were not reflected by net rates of N turnover (Fig. 6 H, I) and inorganic N concentrations in soil (Fig. 6 F, G). Grazing did not affect the determined parameters of N turnover in this period (Fig. 6, partly from wu et al. 2012).

3.1.4 Winter with constantly frozen soil: Low rates of N turnover, recovery/buildup of microbial biomass

From the beginning of November 2007 until beginning of March 2008, the soil was constantly frozen (Fig. 6A). Both soil temperature and soil moisture were significantly and continuously smaller at WG than at UG (Fig. 6A). This coincided

with lower snow height at WG compared to UG due to reduced surface roughness length at the grazed plots (Wolf et al. 2010).

Both gross ammonification and gross nitrification were either not significantly different from zero or slightly but significantly positive throughout the soil frost period, and characterized by the absence of temporal dynamics throughout the whole freeze period. Low but significant $^{15}NH_4^+$ and $^{15}NO_3^-$ consumption occurred within these experiments indicating microbial immobilization of inorganic N (data not shown), which is in accordance with an increase of microbial biomass N over time at the beginning and end of winter (Fig. 6D). Similarly, net rates of ammonfication were not significantly different from zero within this period of constantly frozen soil, while net nitrification was slightly larger than zero at the end of the winter period (Fig. 6 H, I). Both ammonium and nitrate concentrations were constantly at low level (Fig. 6 F, G). After the initial increase, microbial biomass N declined in the beginning of January 2008 in conjunction with an intensification of soil frost, i. e. a drop in soil temperatures to approx. -10°C at the ungrazed plots and to approx. -15°C at the wintergrazed plots (Fig. 6 A, D). The first winter-measurements of microbial biomass N revealed significantly smaller microbial biomass N at the wintergrazed site than at the control site (Fig. 6 D). Other parameters were not significantly affected by grazing in the freeze period (Fig. 6, partly from wu et al. 2012).

3.1.5 Spring freeze thaw period: peaks of gross N turnover and soil nitrate concentrations at highest soil moisture values

The first thaw events beginning of March 2008 lead to a sharp increase of soil moisture due to snow melt (Fig. 6 A) to the highest soil moisture values of the whole investigated period at UG and to values which equal the summer values following extreme precipitation events at WG (Fig. 6 A). Considerably larger snow accumulation at UG than at WG (Wolf et al. 2010) resulted in a more than doubling of volumetric soil moisture content at UG compared to WG in the spring freeze thaw period (Fig. 6 A). The sudden increase in soil moisture due to spring thaw events coincided with an increase in soil microbial biomass N, gross rates of ammonfication and nitrification as well as soil NO_3^- concentrations. In contrast, net rates of

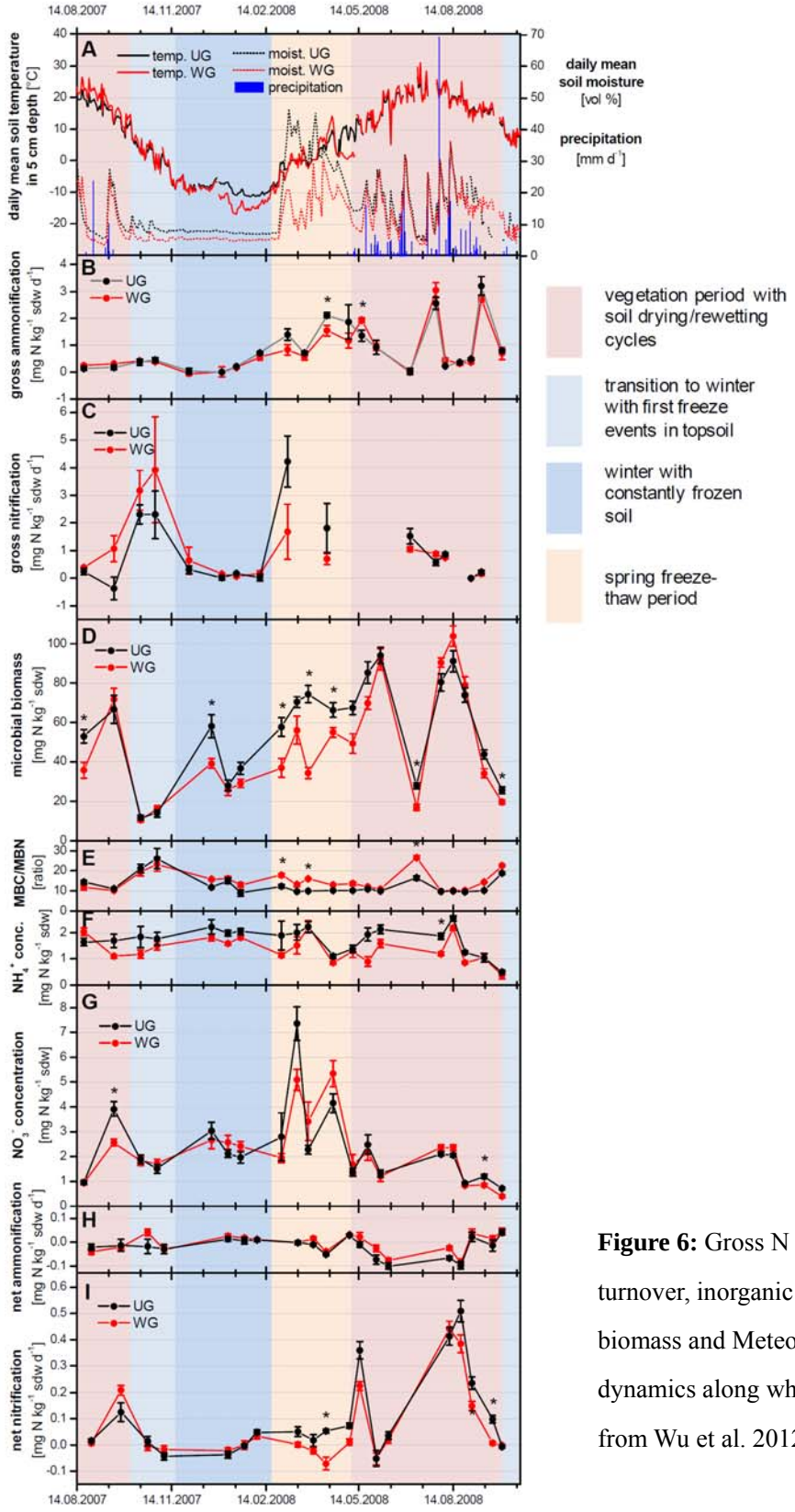

Figure 6: Gross N turnover, net N turnover, inorganic N, microbial biomass and Meteorological data dynamics along whole year (Taken from Wu et al. 2012.).

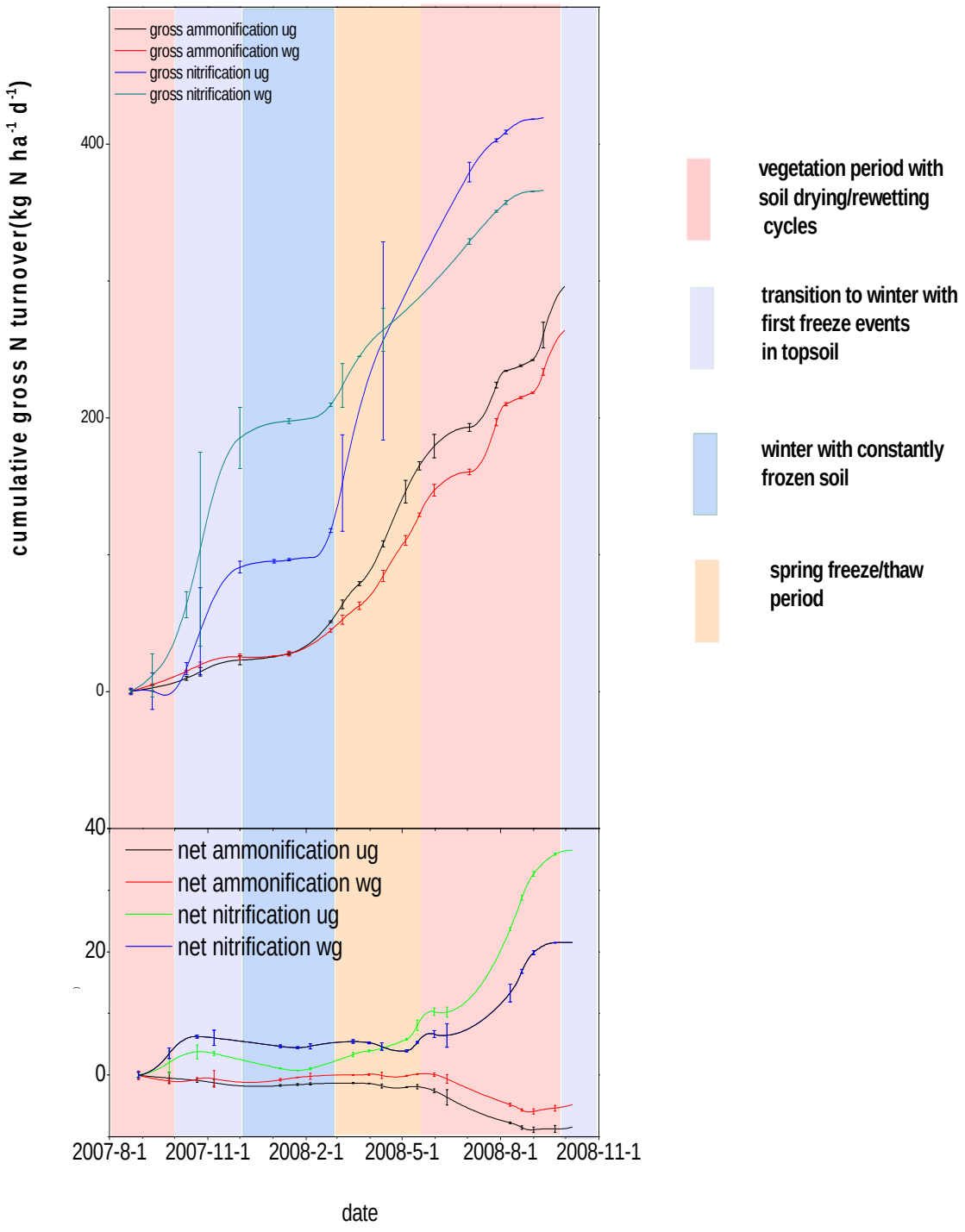

Figure 7: Cumulative gross N turnover and net N turnover along whole year (Taken from Wu et al. 2012.).

nitrification and ammonification as well as soil NH4+ concentrations did not strongly increase after winter (Fig. 6 F, H, I).

3.1.6 Cumulative annual N turnover and contribution of seasons

Cumulative N turnover as gained by linear interpolation between the measured values of N expressed on areal basis further illustrated the importance of both autumn and spring freeze-thaw periods for annual gross N turnover (Fig. 7). Annual gross ammonification for the uppermost 10 cm of soil was 240 and 215 kg N ha^{-1} $year^{-1}$, while annual gross nitrification was 417 and 362 kg N ha^{-1} $year^{-1}$ for UG and WG, respectively. In contrast, annual net ammonification was -9 and -6 kg N ha^{-1} $year^{-1}$, while annual net nitrification was 31 and 19 kg N ha^{-1} $year^{-1}$ at UG and WG, respectively. Neither magnitude nor dynamics of net rates of N turnover were related to gross rates of N turnover.

Freeze-thaw period are particularly important for gross N turnover, which contributed 50 % and 41 % to annual cumulative gross ammonification (Table 2), 65 % and 57 % to annual cumulative gross nitrification in UG and WG respectively. In contrast, growing season contributed 40 % and 52 % to annual cumulative gross ammonification, 29 % and 32 % to annual cumulative gross nitrification in UG and WG respectively, while the winter period was of minor importance for both annual gross ammonification and annual gross nitrification (Table 2). However, totally different patterns occurred for net N turnover. Significant net N turnover only happened in growing season for WG with nearly zero values in freeze-thaw period and winter. In contrast, freeze-thaw period contributed 16% in UG both for net ammonification and nitrification.

3.1.7 Effects of soil temperature, moisture and microbial biomass N on N turnover

Soil temperature positively related with net nitrification ($r^2 = 0.14$, $P = 0.0047$, Fig. 8), net mineralization ($r^2 = 0.20$, $P = 0.0006$) and microbial biomass N ($r^2 = 0.07$, $P = 0.0359$) while negatively related with net ammonification ($r^2 = 0.17$, $P = 0.0022$),

nitrate N (r^2 = 0.10, P = 0.0225) and inorganic N (r^2 = 0.12, P = 0.0118). The relationship between soil temperature and gross ammonification (r^2 = 0.31, P < 0.0001), gross nitrification (r^2 = 0.22, P = 0.0046) was a hump-shaped form, which means that gross ammonification and gross nitrification increased at low levels of soil temperature and decreased at high levels of temperature. The threshold temperature was 6℃ for both gross ammonification and gross nitrification.

Gross ammonification was positively related with soil moisture (r^2 = 0.14, P = 0.0036, Fig. 9). Net ammonification (r^2 = 0.14, P = 0.02) and soil ammonia (r^2 = 0.14, P = 0.0035) decreased with soil moisture at low levels of soil moisture and increased at high levels of soil moisture. Net nitrification (r^2 = 0.35, P < 0.0001), net mineralization (r^2 = 0.24, P = 0.0006), and microbial biomass N (r^2 = 0.73, P < 0.0001) showed an opposite relationship, which increased with soil moisture at low levels of soil moisture while decreased at high levels. The threshold soil moisture was about 0.22 for all net N turnover factors (net ammonification, net nitrification and net mineralization). However, it was 0.12 for soil ammonia and 0.25 for microbial biomass N.

Microbial biomass had significant effects on both net N turnover and gross N turnover. Net ammonification was negatively related with microbial biomass (r^2 = 0.19, P = 0.0007), while positive relationship occurred for net nitrification (r^2 = 0.33, P < 0.0001), net mineralization (r^2 = 0.15, P = 0.0035) and gross ammonification (r^2 = 0.13, P = 0.0046).

3.2 Disscussion

3.2.1 Net rates of N turnover did not provide insight into dynamics and magnitude of actual (gross) rates of N turnover.

Annual gross ammonification was 240 and 215 kg N ha^{-1} $year^{-1}$, while annual net ammonification was -9 and -6 kg N ha^{-1} $year^{-1}$ for UG and WG respectively. Annual gross nitrification was 417 and 362 kg N ha^{-1} $year^{-1}$, while annual net nitrification was 31 and 19 kg N ha^{-1} $year^{-1}$ at UG and WG, respectively (partly from wu et al. 2012). So, net rates of N turnover were less than 8% of gross rates of N turnover, which

correspondence well with the report that gross N mineralization rates were 23 times higher than net mineralization rates in a forest soil (Stottlemyer and Toczydlowski 1999). Furthermore, no relationships were found between net N turnover and gross N

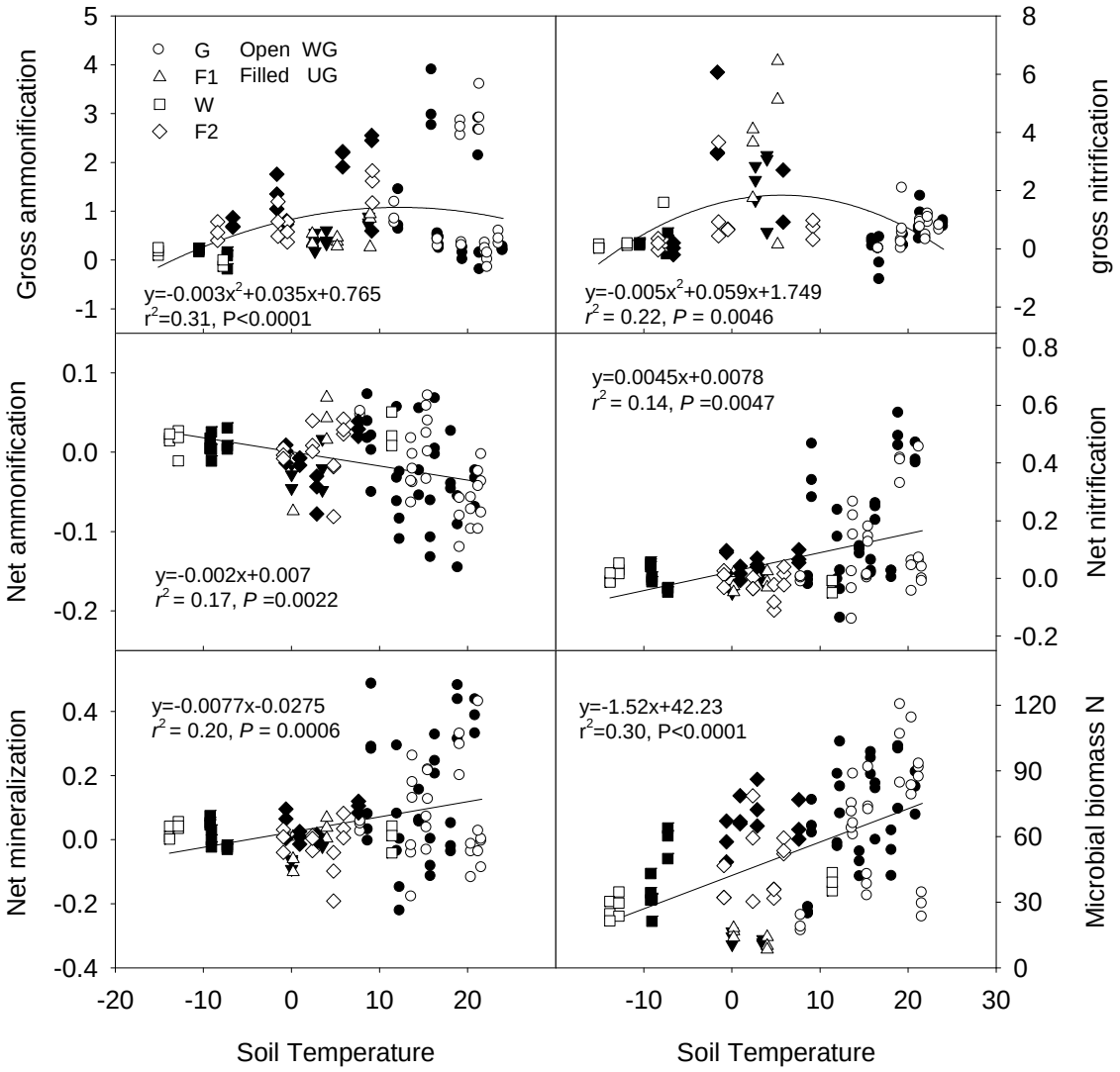

Figure 8: Effects of daily mean soil temperature in 5 cm depth soil temperature on gross N turnover, net N turnover, inorganic N and microbial biomass.

turnover in our study. It is not consistent with the findings in agroforestry systems that strong positive correlations were obtained between gross and net rates of N

mineralization (Zaman and Chang 2004), but for most studies no relationship were found (Booth 2005). Thus, net N mineralization rates do not give a clear picture of the total rate of microbial activities, because they do not provide information on the rate of the microbial immobilization which is concurrent with the N mineralization process (Zaman and Chang 2004). Net rates of N turnover therefore are a poor approximation to actual N turnover in semi-arid steppe ecosystems. However, net nitrification may be a stronger predictor for trace gas emission than gross nitrification (Stark et al. 2002). Additionally, inorganic N that is available to plants lies somewhere between net rates and gross rates, and microbes take up more N than roots (Schmidt et al. 2002).

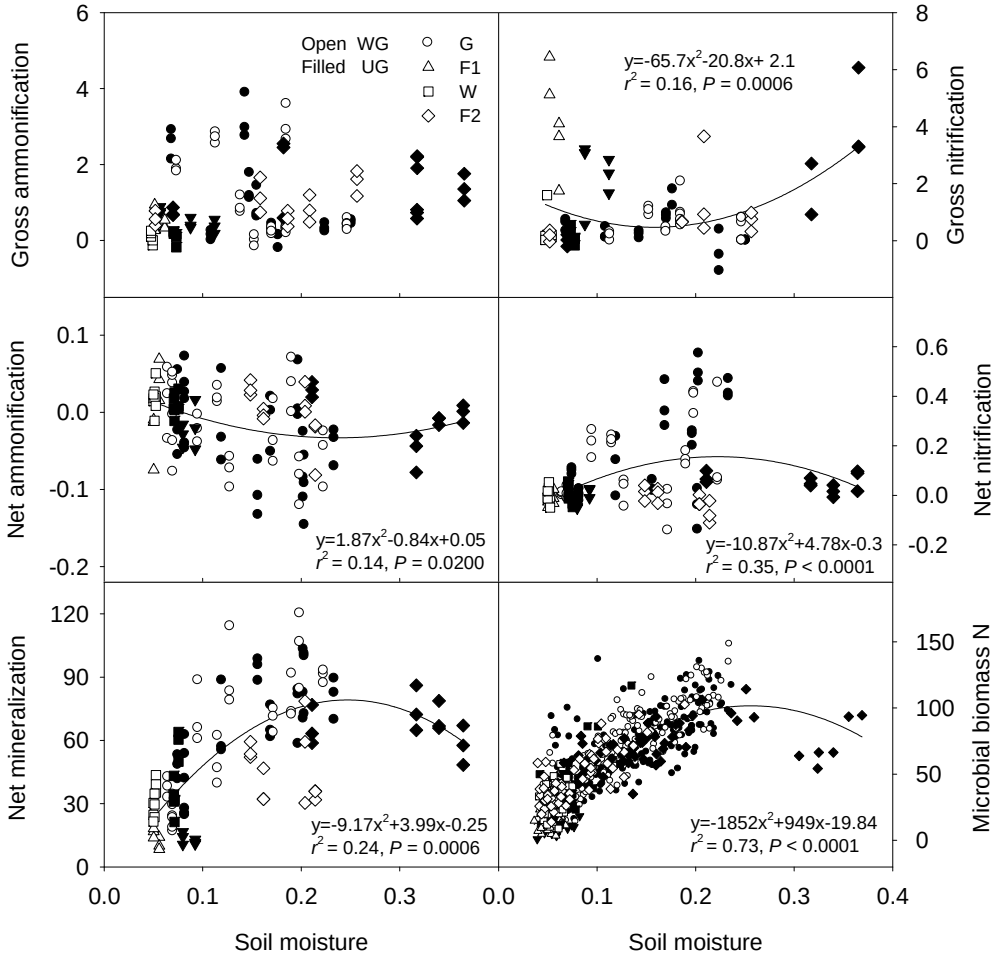

Figure 9: Effects of soil moisture on gross N turnover, net N turnover and microbial biomass.

Furthermore, N mineralization (both gross and net) is typically assayed in bulk soils, but experimental evidence suggests that N cycling rates are higher in rhizosphere soils than bulk soil (Norton and Firestone 1996), which may lead to higher rates of both N mineralization and microbial assimilation, but spatial or temporal segregation of these two processes may allow roots to acquire mineralized N as it "goes by" that might otherwise be taken up by microbes if the roots were not present (Booth et al. 2005).

3.2.2 Gross nitrification exceeded gross ammonfication both during freeze-thaw periods and at the annual scale

Gross nitrification is lower than gross ammonfication usually because gross nitrification is the process that translate the NH_4^+ (the production of ammonfication) to NO_3^- in the classical paradigm of soil N cycle (Schimel and Bennett 2004, Booth et al. 2005). However, gross nitrification exceeded gross ammonfication both during freeze-thaw periods and at the annual scale in our study, which also occasionally occur in the study adjacent to our experiment site (Holst et al. 2007). Nitrogen dynamics study in an Australian semiarid grassland soil showed heterotrophic nitrification rate explained >50% of total nitrification (Cookson et al. 2006). We also found that nitrate concentrations were higher than ammonium concentrations, MBN was positively correlated with NO_3^- but negatively correlated with NH_4^+ concentrations. Therefore, we predicted that N turnover may be nitrification-prone in the investigated ecosystem. Heterotrophic nitrification (i. e. direct oxidation of organic substrate to NO_3^- without a free NH_4^+ pool, Barraclough and Puri 1995; Corre et al. 2003) is hypothesized to have facilitated gross nitrification exceeding gross ammonification.

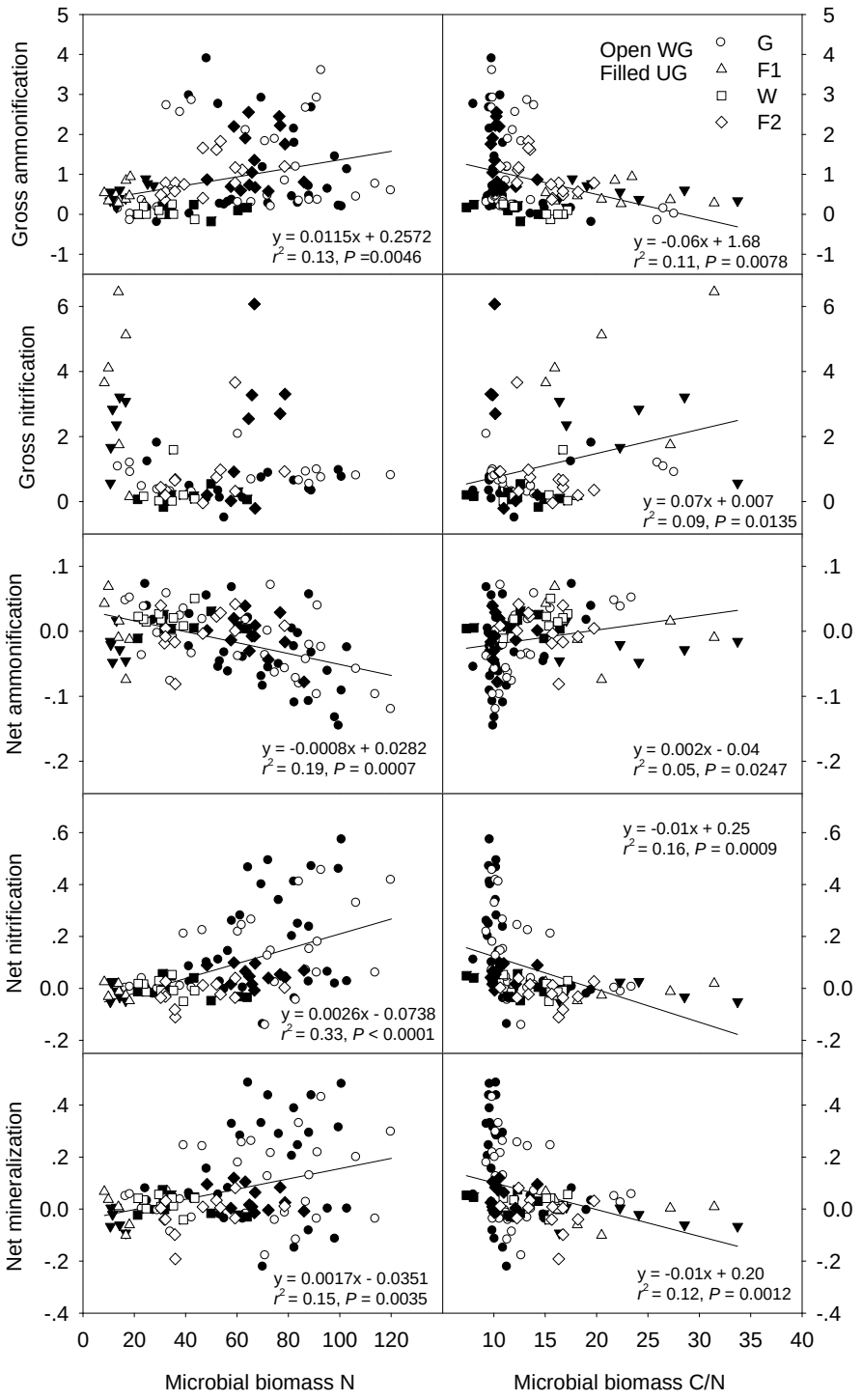

Figure 10: Effects of microbial biomass and microbial biomass C/N on gross N turnover and net N turnover.

Table 2: Contributions of Seasons to Gross N turnover.

Sites	Seasons	Gross Ammonification			Gross Nitrification		
		Amount (kg N $ha^{-1}y^{-1}$)	Contri-bution	Average (kg N ha^{-1} d^{-1})	Amount (kg N $ha^{-1}y^{-1}$)	Contri-bution	Average (kg N ha^{-1} d^{-1})
WG	Freeze-thaw 1	14.12±4.02	0.07	0.31±0.05	129.55±11.45	0.36	2.82±0.15
	Freeze-thaw 2	73.52±6.98	0.34	0.92±0.09	76.80±19.92	0.21	0.96±0.27
	Growing season	111.98±17.42	0.52	0.77±0.11	116.98±17.58	0.32	0.80±0.11
	Winter	15.57±6.45	0.07	0.17±0.05	38.63±109.42	0.11	0.41±0.83
	Whole year	215.19±34.87		0.59±0.1	361.97±158.36		0.99±0.44
UG	Freeze-thaw 1	14.95±2.72	0.06	0.32±0.04	79.36±36.03	0.19	1.73±0.48
	Freeze-thaw 2	106.54±4.72	0.44	1.33±0.06	190.60±62.66	0.46	2.38±0.84
	Growing season	95.02±28.58	0.40	0.65±0.19	122.24±31.41	0.29	0.84±0.21
	Winter	23.15±9.29	0.10	0.25±0.07	24.71±47.09	0.06	0.26±0.36
	Whole year	239.66±45.31		0.65±0.13	416.91±177.19		1.14±0.49

Days of seasons: first freeze-thaw period, 46 days, second freeze-thaw period, 80 days; growing season, 146 days; winter, 94 days. The values are expressed by mean ± se (Taken from Wu et al. 2012.).

3.2.3 Freeze-thaw periods are key periods for understanding annual N turnover in soils of semi-arid steppe

Averagely, freeze-thaw period contributed 53% to annual cumulative gross N turnover (Table 2 and Fig.7), growing season contributed 38%, winter contributed 9 %, which consistent with gross N mineralization highest in the spring and autumn and lowest in summer (Jamieson et al. 1999). It is likely because that greater soil moisture during freeze-thaw period stimulate microbial activity (we will discuss soil moisture effect in detail later). Low but significant gross N turnover in winter indicated that gross N turnover in winter should not be ignored. In the period of transition to winter, gross nitrification peaked in conjunction with sharp decline in microbial biomass likely because that some microorganism died because of low temperature and NO_3^-

released from the died microorganism.

In contrast, significant net N turnover only occurred in growing season for WG with nearly zero values in freeze-thaw period and winter. However, freeze-thaw period contributed 16% in UG for both net ammonification and nitrification. Our results suggested that estimating net N turnover only in summer seems ideal to evaluate annual net N turnover for grazed grassland, however, freeze-thaw period should not be ignored for ungrazed grassland.

3.2.4 Grazing effects on N turnover, microbial biomass and soil properties

Grazing effects were mostly pronounced in the spring freeze thaw period, but not in other periods of the year. Therefore grazing must have changed the soil and environmental conditions that determine gross N turnover during the freeze-thaw period. The aboveground biomass and vegetation height were greater and thus more snow in UG than that in WG (Wolf et al. 2010). Moreover, WG site with lower vegetation/snow cover have significantly higher freezing rates, lower winter soil temperatures and reduced soil moisture during snow melt. Study on annual N_2O emission in the same sites with ours also found enormous differences in spring freeze-thaw between UG and WG (Wolf et al. 2010) mainly because of the soil moisture. Our results showed that soil moisture was positively related with gross N mineralization, which is likely the reason that grazing effect only significant in freeze-thaw period. Grazing reduces microbial activity during spring freeze thaw period by reduced wintertime water rentention and microbial growth.

Our findings that grazing had no significant effect on gross and net N turnover in growing season, which is opposite with the result of Holst et al. (2007) though they also found small differences in soil texture, soil organic C content and bulk density between UG and WG. Most studies have proved the effect of grazing on N turnover especially net N turnover, but in this study, our WG site was only grazed in winter which has much less effect on ecosystem than summer grazing. Thus, the gross and

net N mineralization between WG and UG in growing season is similar. Our results about soil temperature, soil moisture, microbial biomass, inorganic N proved the patterns of gross and net N turnover. A study including the determination of net mineralization and net nitrification rates under grazing disturbance at the same sites of the present study also could not observe differences between grazed and ungrazed plots (Wang et al. 2006).

3.2.5 Soil temperature and moisture dominate microbial biomass and N turnover in Inner Mongolia grassland

Our results showed clearly that soil temperature and moisture appeared to be the most critical factors affecting gross and net N turnover and microbial biomass, which are basically consistent with previous studies. Gross N turnover increased with soil temperature at low temperature levels, and opposite trends appeared at high temperature. Sierra and Marban (2000) found the optimum temperature was 25 – 35 ℃ contrasting with our 6 ℃, while gross N mineralization in agroforestry systems increased with soil temperature until 40 ℃ (Zaman and Chang 2004). The optimum temperature was much lower than that of other studies may reflect the microorganism adaption to low temperature of Inner Mongolia grassland. Another possible reason is that high temperature reduced the soil moisture in growing season (except days after raining), and high soil moisture with low temperature in freeze-thaw period. Soil temperature positively related with net nitrification and mineralization while negatively related with net ammonification, which suggested that mesophilic nitrifiers (such as fungi and actinomycetes) were more active at high temperature while ammonifiers were more active at low temperature (Wang et al. 2006). The low net ammonification and high nitrification in growing season also approved that.

Soil moisture is another driven factor that control N turnover, which significantly affected gross ammonification and net N turnover, especially microbial biomass N (r^2 = 0.73). The strongly significant correlation between soil water content and microbial

biomass N over the full dataset including all investigated periods indicated that soil moisture is the dominant factor in the regulation of microbial growth. Water availability controls soil microbial activity and thus the rates of gross and net N turnover. Our findings are consistent with other studies (Zaman and Chang 2004). However, most studies found maximal net N mineralization occurs when soil moisture is close to field water holding capacity (about 40%). In contrast, we suggested that 25% is the optimal soil moisture for net mineralization and microbial biomass maybe because that microorganism in our sites have adapted to the semiarid environment. Another possible reason is that the interactive with soil temperature, the highest soil moisture occurred in freeze-thaw period when soil temperature was very low while high soil temperature was often accompanied by sever arid in summer.

The two major events of microbial decline at the beginning of July and at the end of summer 2008 were accompanied by a more than doubling of the ratio between microbial biomass C and microbial biomass N (Fig. 6E). This may indicate that bacterial communities were more negatively affected by the microbial decline in favour of fungi. Outliers of microbial biomass N in Fig. 10 at highest soil moisture are from UG at the onset of spring freeze-thaw and may be explained by a temporal delay between sudden high water availability and somewhat retarded microbial growth. Oscillation patterns of microbial biomass N in soil and gross ammonification were inversely pronounced in summer 2008 (Fig. 6 B, D). Consequently, gross ammonification was negatively correlated with soil water content in summer 2008 ($R=-0.579$, $p=0.012$, $N=18$). These interrelationships indicate that microbial residues from dieback events served as a substrate for gross ammonification. Decaying microbial biomass appears to be a major substrate for N turnover in summer and during first freeze-thaw events.

As we showed in upper, grazing effect only significant in freeze-thaw period, which indicate that moderate winter-grazing decreased gross rates of N turnover and microbial biomass, in particular due to a reduction of soil moisture during spring

freeze-thaw period. Freeze-thaw contributed to annual gross N turnover more than 50% also mainly because of the great soil moisture. However, the high N turnover in summer suggested that temperature is another key drive factor for N turnover. The great fluctuation of N turnover and microbial biomass reflected the alternation of dry and wet. Therefore, soil temperature and moisture seems the most direct and drive factor that control N turnover and microbial activity.

4. Grazing intensity effects on gross rates of N mineralization and short-term inorganic N partitioning in intact plant-soil systems of semi-arid steppe of Inner Mongolia*

4.1 Results

4.1.1 C and N pool sizes, soil parameters

Average bulk density of the whole soil cores until 20 cm depth significantly increased with increasing stocking rate (Fig. 11 A), while soil moisture decreased with increasing grazing pressure (Fig. 11 B). Over the two days incubation period, while there was no precipitation, soil moisture tended to slightly decrease at all four investigated plots with a significant decrease at the plot with low grazing intensity (Fig. 11 B). Conversely to bulk density, extractable TOC (Fig. 11 C) and DON concentrations (Fig. 11 D) decreased with increasing grazing pressure. However, soil microbial biomass carbon and nitrogen were not affected by grazing (Fig. 11 E, F). Both microbial biomass C and N significantly decreased over the two days incubation period at most of the plot (Fig.11 E, F). Extractable soil NH_4^+ concentrations significantly decrased with increasing stocking rate while NO_3^- concentrations sharply increased at moderate and heavy grazing intensity (Fig. 11 G, H). Soil NH_4^+ and NO_3^- concentrations were of similar magnitude at the control plot and under low stocking rate, while NO_3^- concentrations under moderate and heavy stocking rates

* Wu H.H., Dannenmann M., Fanselow N., Wolf B., Yao Z.S., Wu X., Brüggemann N., Zheng X.H., Han X.G., Dittert K., Butterbach-Bahl K. (2011a). Feedback of grazing on gross rates of N mineralization and inorganic N partitioning in steppe soils of Inner Mongolia. Plant & soil, 340:127-139.

I note that this chapter is mostly taken from Wu et al 2011a.

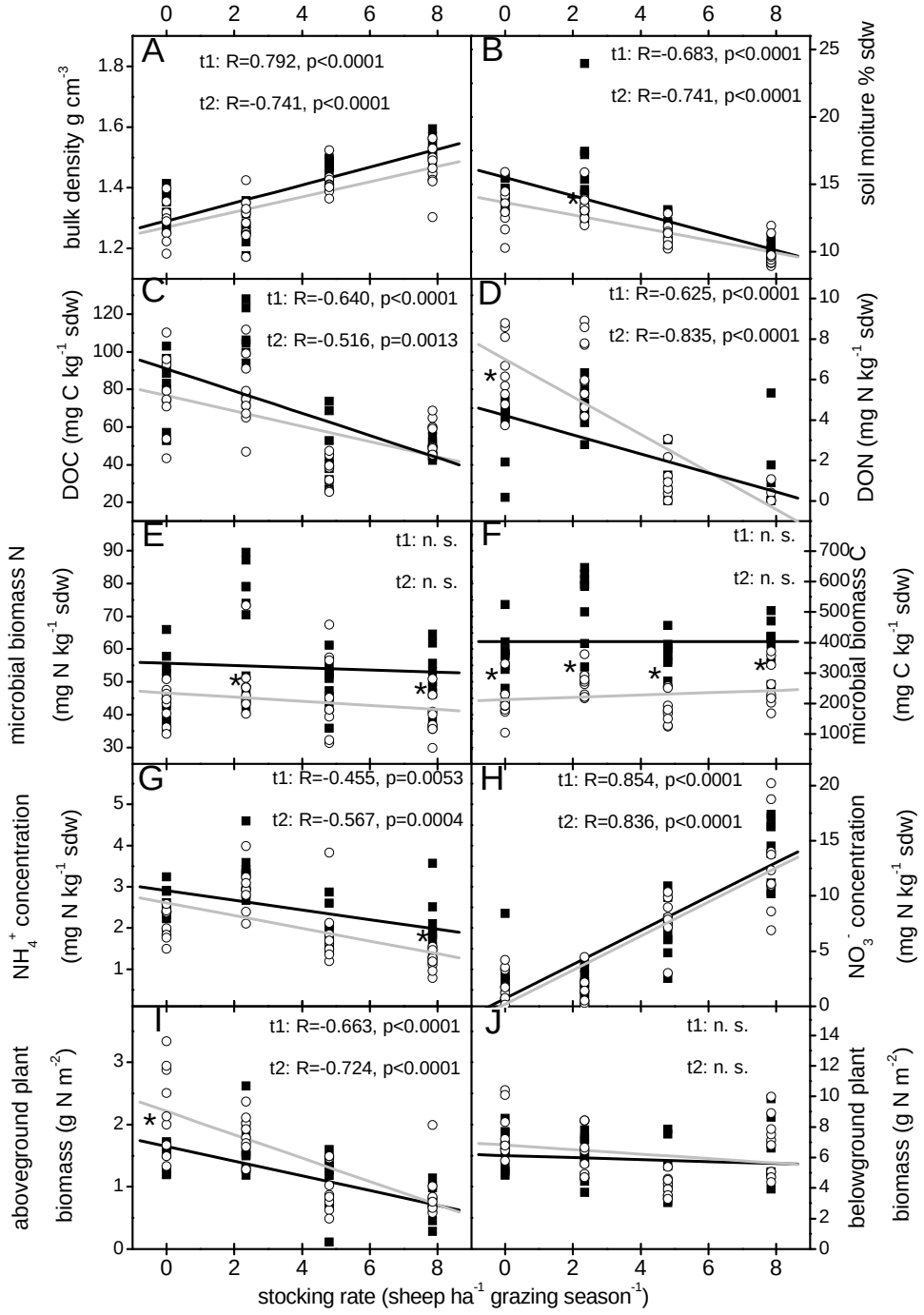

Figure 11: Soil parameters and soil and plant nitrogen pools as influenced by stocking rate. Black

squares represent values from the time 1 extraction (24 hours after ^{15}N application), open circles represent values from the time 2 extraction (72hours after ^{15}N application). Regression lines for time 1 values are in black colour while regression lines for time 2 are in light grey. Asterisks indicate significant differences between time 1 and time 2 values for a given stocking rate. DON: dissolved organic nitrogen; DOC: dissolved organic carbon (Taken from Wu et al 2011a.).

Table 3: Gross rates of N mineralization at the plots exposed to different grazing pressure**.** For uncertainty analysis see Materials and Method section .

Stocking rate [Sheep ha^{-1} grazing $season^{-1}$]	0	1.5	4.5	7. 5
Gross rate of N mineralization [mg N m^{-2} d^{-1}]	222 ± 16	125 ± 18	245 ± 60	309 ± 20

Taken from Wu et al 2011a.

were up to an order of magnitude larger than NH_4^+ concentrations (Fig. 11 G, H). Soil NH_4^+ concentrations significantly decreased over the incubation period at the heavy stocking rate plot and tended to decrease at the other plots (Fig. 11 G). The belowground biomass contained in general more nitrogen than the aboveground biomass at all plots (Fig. 11 I, J). Grazing strongly decreased the aboveground plant biomass N pool (Fig. 11 I), while belowground biomass was not significantly affected by grazing (Fig. 11 J).

4.1.2 Gross N mineralization

Gross N mineralization ranged from 125 mg N m^{-2} d^{-1} at the plot with low grazing intensity to 309 mg N m^{-2} d^{-1} at the plot with the highest grazing intensity (Table 2). Expressed on a soil dry weight basis, gross N mineralization was 0.85, 0.49, 0.85 and 1.04 mg N kg sdw d^{-1} at the control plot, lightly grazed plot, moderately grazed plot and heaviliy grazed plot. When expressed on an annual basis, these values equal 810, 455, 893 and 1128 kg N ha^{-1} $year^{-1}$. The gross N mineralization was considerably

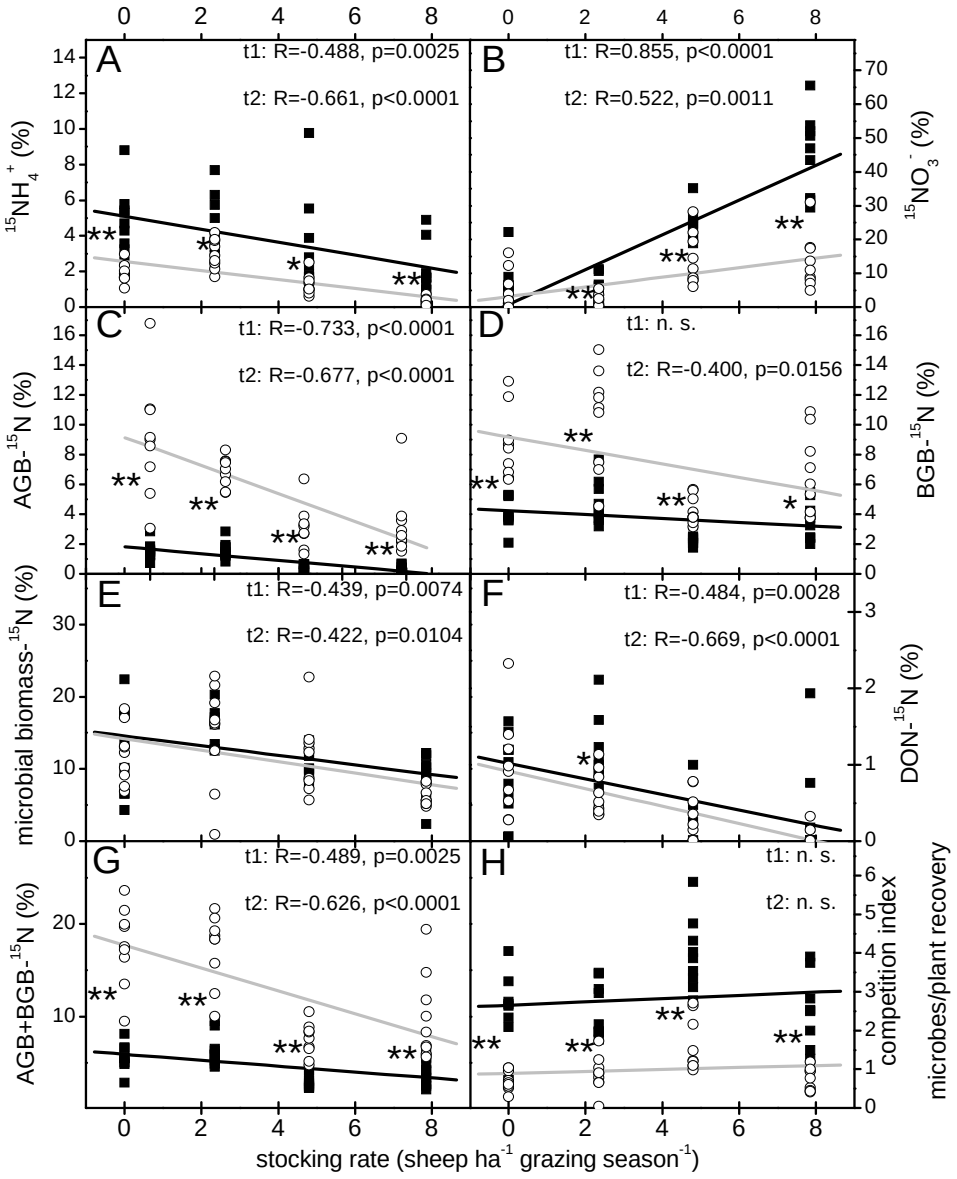

Figure 12: Recovery of 15N in the investigated pools (% of added 15N) as affected by stocking rate. Quadratic black symbols represent values from the time 1 extraction (24 hours after ^{15}N application), open circles represent values from the time 2 extraction (72 hours after ^{15}N application). Regression lines for time 1 values are in black while regression lines for time 2 are

in light grey. Asterisks indicate significant differences between time 1 and time 2 values for a given stocking rate. AGB: Aboveground plant biomass, BGB: belowground plant biomass, DON: dissolved organic nitrogen; DOC: dissolved organic carbon; competition index: ^{15}N recovery in microbial biomass divided by ^{15}N recovery in total plant biomass (aboveground plus belowground) (Taken from Wu et al 2011a.).

smaller at the lightly grazed plot than at the control plot (Table 2), but then strongly increased with increasing grazing intensity at SR 4.5 and SR 7.5 (Table 2). However, the increase of gross ammonification with grazing intensity was not significant when data from both the ungrazed control plot and the three grazed plots were included.

4.1.3 ^{15}N recovery

^{15}N recovery in the NH_4^+ pool one day after the $^{15}NH_4^+$ labelling of the soil cores was already consistently below 10% and significantly further decreased in the following 2 days at all plots (Fig. 12 A). This decline in $^{15}NH_4^+$ recovery was predominantly caused by a marked decrease of the $^{15}NH_4^+$ enrichment due to ^{15}N pool dilution via gross mineralization of unlabelled organic N substrates and a parallel consumption of NH_4^+, as indicated by the slight net decline of the NH_4^+ pool (Fig 11 G). Conversely to the ^{15}N recovery in the soil NH_4^+ pool, the recovery in the soil NO_3^- pool was several fold up to an order of magnitude larger (Fig. 12 B). At all plots except for the control plots, the ^{15}N recovery in the NO_3^- pool significantly declined between time 1 and time 2 (Fig 12 B). Both at time 1 and time 2, grazing had a significant negative effect on ^{15}N recovery in the NH_4^+ pool (Fig. 12A), while it significantly increased the ^{15}N recovery in the NO_3^- pool (Fig. 12 B).

Conversely to the ^{15}N recovery in the inorganic soil N pools, the ^{15}N recovery in aboveground (Fig. 12 C) and belowground plant biomass (Fig. 12 D) significantly increased from time 1 to time 2 at all plots. Grazing significantly decreased the ^{15}N recovery in aboveground plant biomass both at time 1 and time 2 (Fig. 12 C), while the decrease in ^{15}N recovery in belowground plant biomass with increasing stocking rate was significant only at time 2 (Fig. 12 D). Consequently, the sum of ^{15}N recovery

in aboveground and belowground plant biomass significantly increased between time 1 and time 2 and was significantly negatively correlated with stocking rate (Fig. 12 G).

In contrast to the ^{15}N recovery in plant biomass, the ^{15}N recovery in soil microbial biomass did not significantly change over the 2-days incubation period (Fig. 12 E). This was a combined effect of an increase in the ^{15}N enrichment of microbial biomass between time 1 and time 2, and, at the same time, a decrease of the total microbial biomass N pool size (see Fig. 11 E). The ^{15}N recovery in soil microbial biomass was significantly negatively correlated with stocking rate for both harvesting dates (Fig. 12 E).

^{15}N recovery in the DON pool significantly decreased with increasing grazing intensity (Fig. 12F), but was under total N mass balance considerations of minor importance as the range of mean recovery across the plots and harvesting dates was only 0.1 to 1.1 %.

As an index of plant-microbe competition for inorganic N, we divided the ^{15}N recovery in soil microbial biomass by the ^{15}N recovery in total plant biomass. Interestingly, this index was not affected by grazing intensity (Fig. 12 F). This index equalled a value of approximately three in time 1 and significantly declined to approximately one in time 2 (Fig. 12 H). Hence, microbes significantly acquired more nitrogen than plants 24h after $^{15}NH_4^+$ application, but plants overhauled microbes in nitrogen acquisition already 72 hours after the ^{15}N fertilizer had been injected into the soil cores.

The sum of ^{15}N recovery in the investigated pools ranged from 30 to 60 % (Table 3). Overall, most of the injected ^{15}N was recovered in the NO_3^-, plant biomass and soil microbial biomass pools, while recovery of ^{15}N in the NH_4^+ pools and DON pools was of minor importance.

4.1.4 Correlation of dissolved organic carbon (DOC) and C:N ratio in the soil extracts with 15N recovery

Extractable DOC concentrations were significantly negatively correlated with ^{15}N recovery in the NO_3^- pool and significantly positively correlated with ^{15}N recovery in

the NH_4^+, ABGBM, BGBM and DON pools both at time 1 and time 2 (Table 4). Furthermore, DOC concentrations were significantly positively correlated with MBN at time 1. The ratio between extractable DOC and TN was consistently significantly negatively correlated with ^{15}N recovery in the NO_3^- pool and positively correlated with ^{15}N recovery in NH_4^+, MBN, ABGBM, BGBM and DON pools both at time 1 and time 2 (Table 4)

Table 4: Sum of ^{15}N recovery ± standard deviation. The following N pools were analyzed: soil mineral N, aboveground and belowground plant tissues, microbial biomass and DON.

Stocking rate [Sheep ha^{-1} grazing $season^{-1}$]	0	1.5	4.5	7.5
Total ^{15}N recovery time 1 [%]	31 ± 6	33 ± 3	40 ± 6	60 ± 12
Total ^{15}N recovery time 2 [%]	38 ± 4	37 ± 7	35 ± 7	30 ± 6

Taken from Wu et al 2011a.

4.2 Discussion

4.2.1 Methodological uncertainties, patterns and magnitudes of N turnover

The still common procedure of excluding the plant/root compartment from experiments on soil N turnover and immobilization as well as disturbing soil before the experimental incubation begins, has the potential to dramatically alter N turnover rates (Murphy et al. 2003, Booth et al. 2006, Burger and Jackson 2004, Rennenberg et al. 2009). Gross rates of N mineralization and ^{15}N partitioning to plant and microbial pools in this study were determined in large intact plant soil microcosms where plant-microbe competition for organic and inorganic nitrogen as well as further plant-microbe interactions like substrate supply to microorganisms via root exudation persisted. Furthermore, potential bias due to non-uniform ^{15}N labelling of the intact

soil core (Davidson et al. 1991) could be minimized in this study by the labelling technique involving 38 single injections into every soil core. Additionally, potential bias by storing soil (Arnold et al. 2008) could be avoided by immediate processing.

Table 5: Pearson's coefficients for correlations between extractable soil dissolved organic carbon concentrations (DOC) and the ratio of DOC to total extractable soil N (TN) with ^{15}N recovery in the investigated plant and soil nitrogen pools (N=36).

Time 1	^{15}N rec. NH_4^+	^{15}N rec. NO_3^-	^{15}N rec. MBN	^{15}N rec. AGBM	^{15}N rec. BGBM	^{15}N rec. DON
Soil DOC	0.432**	-0.644***	0.381*	0.738**	0.448**	0.785***
DOC:TN ratio	0.472**	-0.820***	0.488**	0.786***	0.446**	0.680**
Time 2	^{15}N rec. NH_4^+	^{15}N rec. NO_3^-	^{15}N rec. MBN	^{15}N rec. AGBM	^{15}N rec. BGBM	^{15}N rec. DON
Soil DOC	0.478**	-0.375*	N. S.	0.535***	0.445**	0.626***
DOC:TN ratio	0.741***	-0.692**	0.407*	0.733***	0.656***	0.583***

MBN: microbial biomass nitrogen; AGBM: aboveground plant biomass; BGBM: belowground plant biomass; DON: dissolved organic nitrogen. N. S.: not significant; * significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p<0.001$. These correlations were calculated across all plots and grazing intensities (Taken from Wu et al 2011a.).

Another potential bias in the application of ^{15}N pool dilution techniques - internal N recycling - is commonly thought to be non-significant within periods of one week (Murphy et al. 2003), however, could occur faster in intact plant-soil-systems in the presence of roots due to internal recycling of ^{15}N via the microbial loop or via plant paths (Burger and Jackson 2004). In the present study, the slight decline of microbial biomass between time 1 and time 2 and the appearance of small amounts of ^{15}N in the extractable DON pool may indicate that indeed some internal N cycle occurred and

hence gross rates of N mineralization could be considered to be rather conservative. However, as the ^{15}N recovery in the DON pool was only approx. 1 % of the added ^{15}N (Fig. 12 F), a potential underestimation of gross N mineralization may be small. Overall, we assume that the chosen methodology represents a unique realistic approach to actual gross rates of N mineralization and short-term partitioning of inorganic N to plant-, soil- and microbial N pools.

Due to the short incubation time in the present study, plant and microbial N uptake as interpreted from ^{15}N recovery in this study does represent short-term, process-competition mediated N partitioning. In contrast, medium- to long-term plant-microbe competition for nitrogen is mainly influenced by the mean N residence time in the pools. Nitrogen acquired by microorganisms via strong short-term process competition may repeatedly enter the plant-microbe competition pools while plants sequester nitrogen for longer periods than microorganisms (Hodge et al. 2000).

The present study represents a snapshot on midsummer N mineralization and inorganic N partitioning in the plant-soil-system of typical steppe of Inner Mongolia. Due to the dynamic character of gross rates of N turnover (Dannenmann et al. 2006, Rosenkranz et al. 2009), it does neither allow to draw conclusions on N turnover and N partitioning at larger time scales nor to estimate annual N fluxes. As there is increasing evidence that winter fluxes and especially N conversion in freeze-thaw periods may be of great importance in steppe ecosystems, a major goal of future studies should be to gain full annual cycles of gross rates of N conversion, though such studies will be extremely elaborate.

4.2.2 Grazing effects on soil and plant parameters

The observed increase in bulk density (Fig. 11 A) with increasing stocking rate is in accordance with nearby conducted studies by Holst et al. 2007 and Steffens et al. 2008. The latter studies also found positive effects of grazing on bulk density, accompanied by decreases in soil organic C and soil total N. This was attributed to a combined effect of trampling, reduced aboveground organic matter input and root growth as well as grazing-induced erosion. We expect similar causes and effects in our study, e. g. by reduced aboveground biomass (Fig. 11 I), which may – in

conjunction with a decline in soil organic matter - also explain decreasing extractable DOC concentrations (Fig. 11 C), and, furthermore, decreasing soil moisture (Fig. 11 B). The negative effect of grazing on midsummer soil moisture is in accordance with another study conducted in Inner Mongolia (Xu et al. 2007).

4.2.3 Grazing effects on N turnover in the plant-soil system

Among the most obvious effects of grazing was the strong increase of soil NO_3^- concentrations with stocking rate (Fig. 11 H). High soil NO_3^- concentrations at intensively grazed plots may lead to undesired environmental consequences like increased N losses from the ecosystem in general and increased N_2O emissions at the soil-atmosphere interface in particular (Holst et al. 2007). Ecosystem N losses could have contributed to the decline in total ^{15}N recovery which was observed between time 1 and time 2 for the highest grazing intensity (Table 4), as this was caused by a decline in ^{15}N recovery in the NO_3^- pool. However, also other mechanisms could explain this decline, e. g. abiotic NO_3^- immobilization and stabilization in non-extractable organic pools (e. g. Davidson et al. 2003). Decreasing NO_3^- uptake/immobilization by plants/soil microorganisms and increased gross nitrification may have caused the increase of the NO_3^- pool with increasing grazing intensity. Smaller NO_3^- uptake by both plants and by soil microbes at intensively grazed plots is indicated by smaller ^{15}N recoveries in plant and microbial N pools (Fig. 12 C, D, E, G). The observation that the ratio of recovery in microbial and plant N pools did not change due to grazing (Fig. 12 H) implies that the competitive strength in NO_3^- acquisition of both plants and heterotrophic soil microorganisms was negatively affected by grazing. Hence, a given pool of soil NO_3^- can be expected to be less exhausted by both plants and microorganisms at increasing grazing pressure.

Postitive effects of grazing on gross rates of nitrification may also have contributed to the strong increase in soil NO_3^- as indicated by a decrease in both total NH_4^+ pool size (Fig. 11 G) and ^{15}N recovery in the $^{15}NH_4^+$ (Fig. 12 B), while the ^{15}N recovery in the soil NO_3^- pool increased with stocking rate (Fig. 12 B). Nitrification may be supported at grazed plots by increased NH_4^+ substrate supply via increased N mineralization (Table 3). It is expected that easily decomposable feces input (Tracy

and Frank 1998) represent the major organic substrate for the observed higher gross N mineralization at higher stocking rate, since the total nitrogen content of the soil is decreased by grazing in the investigated ecosystem (Steffens et al. 2008). Furthermore, in the present study, extractable organic nitrogen decreased with grazing intensity (Fig. 11 D). Interestingly, Steffens et al. 2009 found within a study conducted nearby that grazing decreased the aggregate stability in soil. Such an effect may have made the soil organic matter more available for depolymerization and mineralization and hence could also have contributed to the high gross rates of N mineralization found at the heavily grazed plot.

A key role in mediating grazing effects on changing patterns of soil N fluxes leading to the observed increase in soil NO_3^- appears to be attributed to soil C availability, as extractable DOC concentrations and the ratio of extractable DOC to extractable TN in the soil were correlated to the ^{15}N recovery in all investigated pools (Table 5). Soil DOC availability may affect both consumption and production of soil NO_3^-. First, lower C availability at intensively grazed plots (Fig. 11 C) may inhibit heterotrophic NO_3^- utilization (Booth et al. 2005, Dannenmann et al. 2006, 2007). Second, it may alter the competitive balance of microbial NH_4^+ utilization in favour of autotrophic nitrification and at the expense of heterotrophic microbial NH_4^+ immobilization (Booth et al. 2005, Dannenmann et al. 2006, 2007).

In the present study, the influence of grazing on N mineralization was not uni-directional. Gross N mineralization appeared to decrease at low grazing intensity but strongly decreased with more grazing pressure and was larger at the heavily grazed plot than at the ungrazed control plot (Table 3). Hence, there was no significant linear correlation between grazing intensity and gross N mineralization but only a positive trend. Comparably little studies investigated gross N mineralization as influenced by grazing in semi-arid grassland. In a nearby conducted study, Holst et al. (2007) found a contradictory trend that grazing decreased gross N mineralization, but lack a final conclusion due to unsufficient replication. The different grade of disturbance could have contributed to these contradictory results, as sieved soil was used by Holst et al. (2007). In the latter study, the mean rates of N mineralization at lightly to heavily grazed plots (0.6 to 1.3 mg N g^{-1}sdw) were similar with this study,

but N mineralization rates were considerably higher (1.4 to 4.1 mg N kg^{-1} sdw d^{-1}) at ungrazed plots. The results of our study are better in agreement with studies conducted in grasslands of North America, where grazing was mostly found to stimulate N mineralization and subsequent N fluxes like nitrification and denitrification (Groffman et al. 1993; Frank et al. 2000; Le Roux et al. 2003). Such effects have been attributed to increased root exudation after defoliation (Holland et al. 1996, Hamilton and Frank 2001). In particular rhizodeposition, competition- and other plant effects on microbial soil N turnover are excluded in high disturbance sieved soil ^{15}N pool dilution techniques like used by e. g. Holst et al. (2007) which could be an explanation for controversial results. In view of the limited number of studies investigating grazing effects on gross N turnover rates, in general low available temporal resolution and enormous differences in the grade of disturbance induced by the applied methods, the reasons for contradictory results of the available studies currently remain speculative.

In the present study, where sheep grazed over a time-period of three months, a series of undesirable consequences (decrease in soil moisture, soil C availability, aboveground plant biomass, plant N acquisition but increase in soil nitrate accumulation) occurred at stocking rates of 4.5 and 7.5 sheep ha^{-1}, but not at the grazing intensity of 1.5 sheep ha^{-1} (Fig. 11, 12). Hence, we assume that the critical threshold value for a sustainable summer grazing practice is around 1.5 sheep ha^{-1} for the investigated ecosystem.

5. Labile organic C and N mineralization of soil aggregate size classes in semi-arid grasslands as affected by grazing management*

5.1 Results

5.1.1 Labile organic carbon

CO_2 production after incubation ranged from 1 to 12 mg g^{-1} between all grazing intensities and ASCs (Fig. 13). HG and CG showed a much higher CO_2 production than WG, UG99 and UG79. HG was significantly ($P < 0.05$) higher than CG while there were no differences among WG, UG99 and UG79. Grazing intensity had a consistent effect on CO_2 production across all ASCs. CO_2 production was highest for cASC while fASC showed the lowest value ($P < 0.05$). CO_2 production of the bulk soil was 3.75 ± 1.60 mg g^{-1}, which was between mASC and fASC.

MBC and DOC showed similar patterns after incubation. Both MBC and DOC in CG and HG were considerably higher compared to WG, UG99 and UG79 (Fig.13). CG showed significantly ($P < 0.01$) higher value than HG both for MBC and DOC. No differences were found between WG, UG99 and UG79. Grazing intensities had a consistent effect on MBC and DOC across all ASCs (Fig. 13). Among the three ASCs, MBC and DOC of mASC showed the highest values, MBC of fASC was the lowest ($P < 0.05$), MBC of cASC was the lowest. Aggregate size, grazing and their interaction effect all influenced CO_2 production, MBC and DOC significantly (Table 6 and Fig. 13, two-way ANOVA, $P < 0.0001$).

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*Wu H.H., Wiesmeier M., Yu Q., Steffens M., Han X.G., Kögel-Knabner I. (2011b). Labile organic C and N mineralization of soil aggregate size classes in semi-arid grasslands as affected by grazing management. Biology & Fertility of Soils, 48:305-313.

I note that this chapter was mostly taken from Wu et al 2011b.

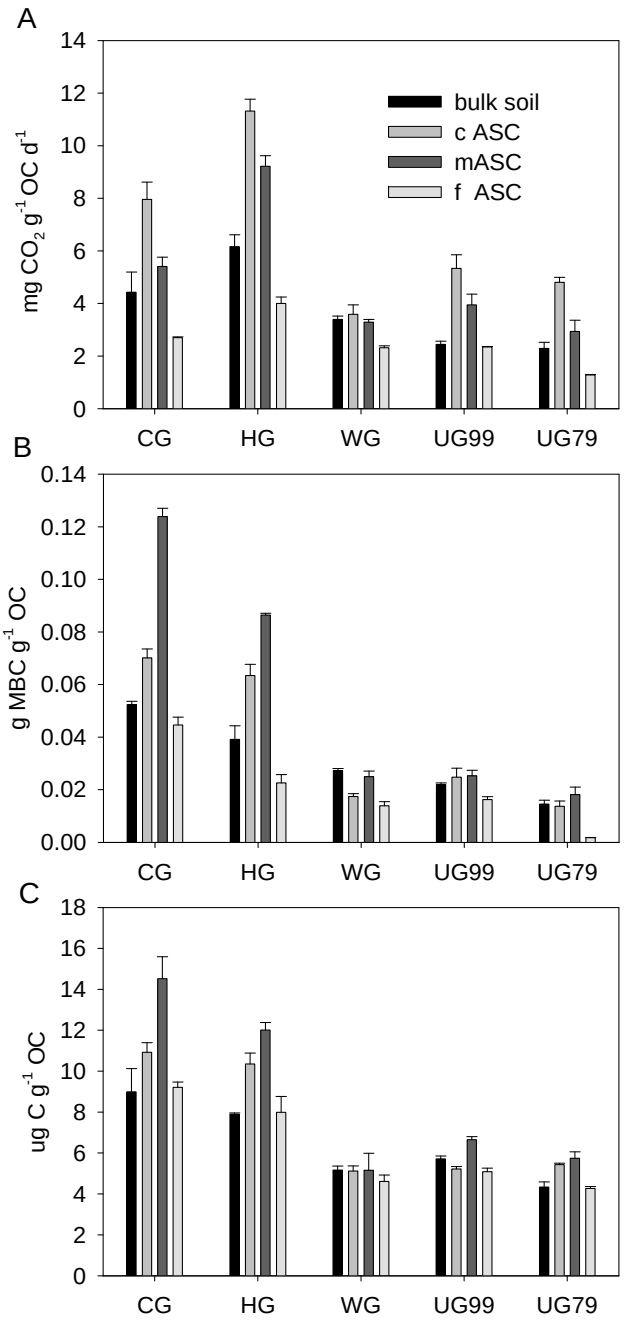

Figure 13: CO_2 production (A), microbial biomass carbon (MBC, B) and dissolved organic carbon (C) influenced by soil aggregate size and grazing. All factors were based on organic carbon (OC) (Taken from Wu et al. 2011b.).

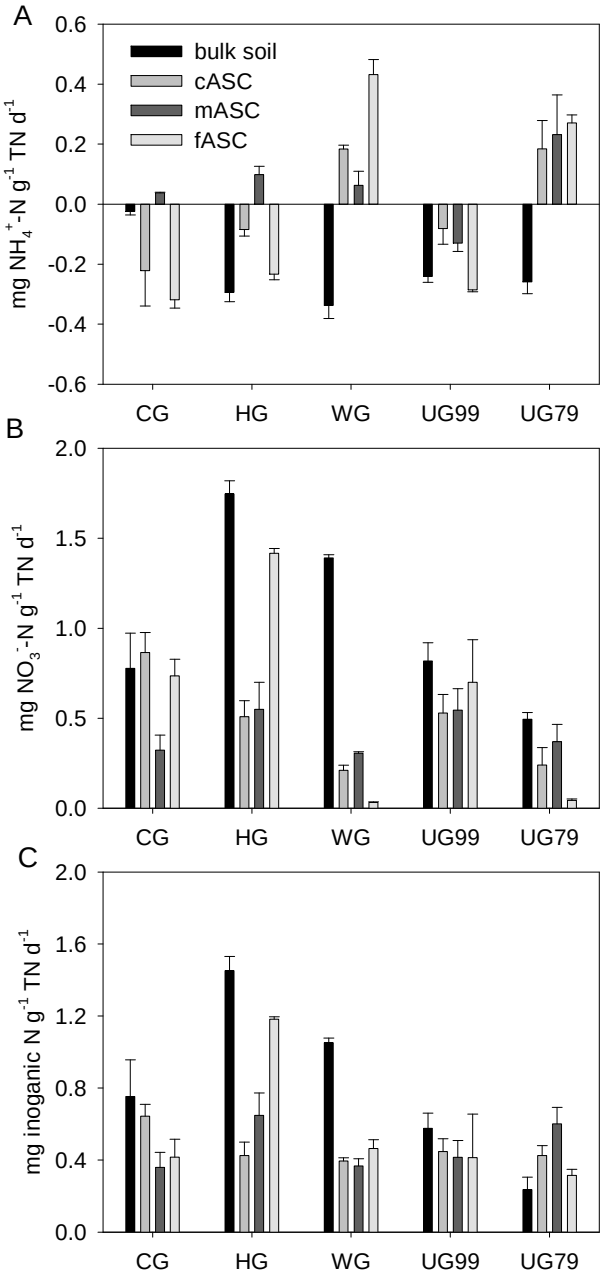

Figure 14: Ammonification (A), nitrification (B) and mineralization (C) affected by soil aggregate size and grazing. All factors were based on total nitrogen (TN) (Taken from Wu et al. 2011b.).

5.1.2 N mineralization

Land-use styles, soil aggregate sizes and their interaction effect had significant effects on ammonification, nitrification and mineralization (Table 7, $P < 0.0001$). However, there were no consistent patterns across all land-use styles and aggregate sizes.

For bulk soil, net ammonification (Fig. 14) of all experimental sites was negative. Net ammonification of CG was significantly lower ($P < 0.05$) compared to all other grazing intensities. Furthermore, net ammonification of HG and WG was significantly higher ($P < 0.05$) than that of UG99 and UG79. For mASC, all net ammonification rates were positive. For cASC and fASC, net ammonification of WG and UG79 was positive while that of UG99, CG and HG was negative.

Net nitrification revealed the highest values for HG (Fig. 14). Net nitrification in UG79 was the lowest between all experimental sites. WG showed a high net nitrification for bulk soil, but for three ASCs WG had the lowest nitrification. In General, bulk soil showed higher nitrification rates than the ASCs. fASC was significantly higher than mASC, but cASC had no difference with them.

Grazing increased net N mineralization significantly (Fig.14 and Table 7), especially in bulk soils. Net N mineralization of bulk soil was the highest among all ASCs, but no difference was found among cASC, mASC and fASC.

5.1.3 Inorganic Nitrogen

Both grazing intensities and ASC significantly influenced ammonium, nitrate and inorganic nitrogen concentrations. For bulk soils, only CG exhibited a significant effect on ammonium concentration. There was no significant difference among HG, WG, UG99 and UG79 (Fig. 15 A). There were also no clear patterns of grazing intensity effects across the three ASCs. However, the ammonium concentrations in cASC, mASC and fASC were much higher than that in bulk soil across all grazing intensities (Fig. 15 A) while nitrate and inorganic concentrations in cASC, mASC and fASC was much lower than that in bulk soil (Fig. 15 B, C). Grazing significantly increased nitrate and inorganic N in bulk soil (Fig. 15 B, C).

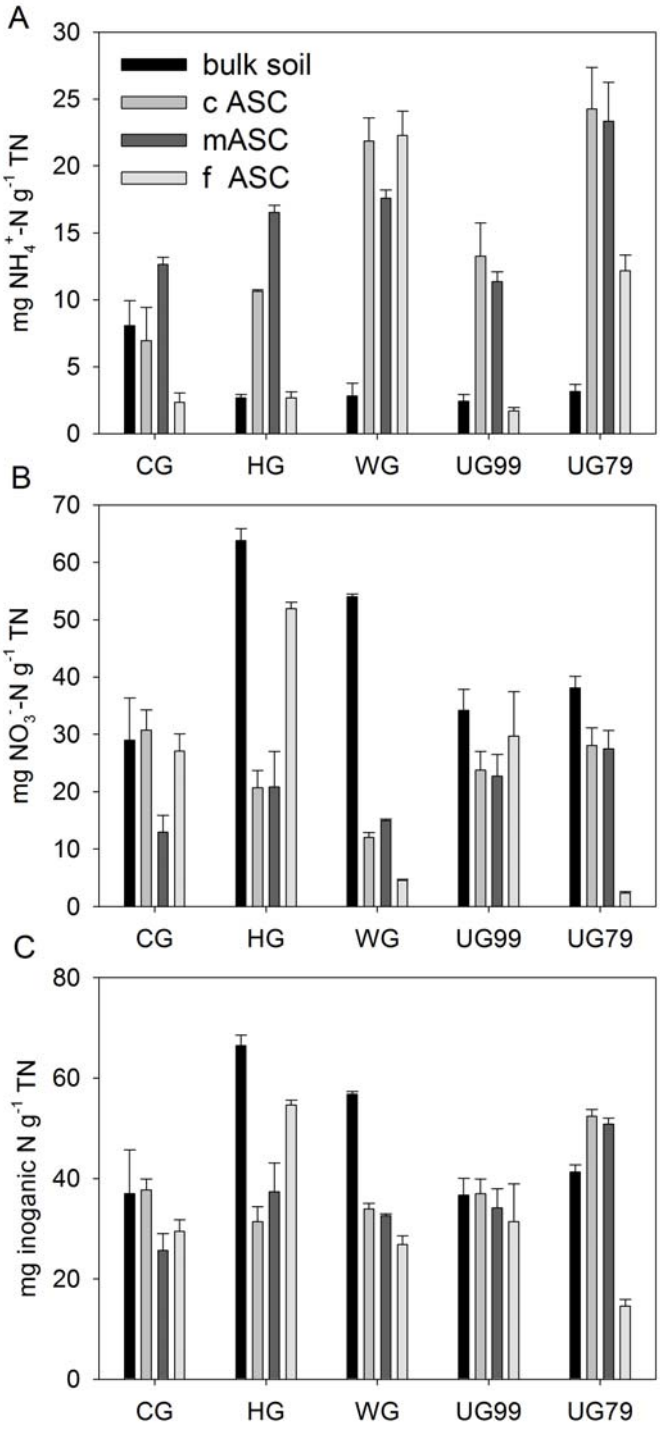

Figure 15: Ammonium (A), nitrate (B) and inorganic nitrogen (C) affected by soil aggregate size and grazing. All factors were based on total nitrogen (TN) (Taken from Wu et al. 2011b.).

Table 6: Two-way ANOVA results for the effects of soil aggregate size (SAZ) and grazing intensity (GI) on CO2 production, microbial biomass carbon (MBC) and dissolved organic carbon (DOC)**.**

	CO_2 production	MBC	DOC
CG	5.12 ± 2.14^{B}	0.073 ± 0.033^{A}	10.91 ± 2.61^{A}
HG	7.67 ± 2.99^{A}	0.053 ± 0.026^{B}	9.56 ± 1.94^{B}
WG	3.15 ± 0.59^{C}	0.020 ± 0.006^{C}	5.01 ± 0.74^{C}
UG99	3.52 ± 1.37^{C}	0.022 ± 0.005^{C}	5.66 ± 0.68^{C}
UG79	2.83 ± 1.40^{C}	0.012 ± 0.007^{D}	4.94 ± 0.75^{C}
Bulk soil	3.75 ± 1.60^{c}	0.311 ± 0.014^{c}	6.42 ± 1.96^{c}
cASC	6.60 ± 2.93^{a}	0.038 ± 0.025^{b}	7.41 ± 2.78^{b}
mASC	4.96 ± 2.43^{b}	0.056 ± 0.044^{a}	8.81 ± 3.99^{a}
fASC	2.53 ± 0.92^{d}	0.020 ± 0.015^{d}	$6{,}23 \pm 2.14^{c}$
GI	$F = 120.7^{***}$	$F = 410.77^{***}$	$F = 128.92^{***}$
SAZ	$F = 113.81^{***}$	$F = 176.5^{***}$	$F = 28.55^{***}$
GI*SAZ	$F = 8.87^{***}$	$F = 40.28^{***}$	$F = 4.72^{***}$

Different superscript letters represent statistically significant difference between treatments at $P < 0.05$. Values are expressed by mean ± SD. *** represents $P < 0.0001$ (Taken from Wu et al. 2011b.).

5.2 Discussion

5.2.1 Effect of Grazing on labile organic carbon

The results showed that CG and HG increased CO_2 production, MBC and DOC significantly across all ASCs and bulk soil, indicating overgrazing increased LOC inInner Mongolia grassland. Our results correspond well with the fact that overgrazing increased carbon loss and decreased carbon storage (He et al., 2008; He et al., 2011; Ingram et al., 2008). However, the results also showed that WG exhibited

no significant effect on LOC compared to UG99 and UG79, suggesting that moderately grazing would not increase carbon loss or even increased carbon storage (Han et al., 2008; Milchunas and Lauenroth, 1993; Schuman et al., 1999). Our results are inconsistent with the finding of a higher CO_2 production in UG99 compared to WG during freezing-thawing cycles (Holst et al., 2008) and greater microbial and enzyme activities in ungrazed compared to grazed plots in semiarid Australia (Holt, 1997). They attributed their findings to greater soil moisture and greater input of organic matter in ungrazed plots. Thus, the effects of grazing are complex because of the variations in climate, soil, landscape location, plant community type and grazing management practices (Milchunas and Lauenroth, 1993; Reeder and Schuman, 2002).

Table 7: Two-way ANOVA results for the effects of soil aggregate size (SAZ) and grazing intensity (GI) on ammonification, nitrification and mineralization.

	Ammonification	Nitrification	Mineralization
CG	-0.13 ± 0.18^{B}	0.68 ± 0.29^{B}	0.54 ± 0.25^{B}
HG	-0.13 ± 0.16^{B}	1.05 ± 0.58^{A}	0.93 ± 0.44^{A}
WG	0.09 ± 0.30^{A}	0.48 ± 0.56^{C}	0.57 ± 0.30^{B}
UG99	-0.18 ± 0.10^{B}	0.65 ± 0.26^{B}	0.46 ± 0.22^{BC}
UG79	0.11 ± 0.26^{A}	0.29 ± 0.20^{D}	0.39 ± 0.17^{C}
Bulk soil	-0.23 ± 0.12^{c}	1.04 ± 0.50^{a}	0.81 ± 0.46^{a}
cASC	0.00 ± 0.20^{ab}	0.47 ± 0.28^{bc}	0.47 ± 0.13^{b}
mASC	0.06 ± 0.15^{a}	0.42 ± 0.19^{c}	0.48 ± 0.18^{b}
fASC	-0.03 ± 0.33^{b}	0.59 ± 0.56^{b}	0.56 ± 0.37^{b}
GI	$F = 26.10^{***}$	$F = 29.99^{***}$	$F = 17.72^{***}$
SAZ	$F = 28.05^{***}$	$F = 37.93^{***}$	$F = 13.59^{***}$
GI*SAZ	$F = 13.45^{***}$	$F = 10.96^{***}$	$F = 6.88^{***}$

Different superscript letters represent statistically significant difference between treatments at $P < 0.05$. Values are expressed by mean ± SD. *** represents $P < 0.0001$ (Taken from Wu et al. 2011b.).

5.2.2 Labile organic carbon of different soil aggregate sizes

ASC had a consistent effect on CO_2 production of all grazing intensities. CO_2 production of cASC was highest while fASC was lowest among the three ASCs, suggesting that C in cASC was most labile while it was stable in fASC. This is consistent with the findings that coarse aggregates are less stable and have faster turnover times than small aggregates (Six et al., 2004; Steffens et al., 2009b). The CO_2 release of cASC is high because it contains more labile SOM and it is less protected against mineralization (Steffens et al., 2009b).

Table 8: Two-way ANOVA results for the effects of soil aggregate size (SAZ) and grazing intensity (GI) on inorganic nitrogen.

	NH_4^+	NO_3^-	DIN
CG	7.5 ± 4.52^{B}	24.94 ± 10^{BC}	32.44 ± 9.04^{C}
HG	8.13 ± 6.13^{B}	39.32 ± 20.56^{A}	47.44 ± 15.36^{A}
WG	16.14 ± 8.51^{A}	21.4 ± 20.04^{C}	37.53 ± 12.04^{BC}
UG99	7.18 ± 5.74^{B}	27.62 ± 8.75^{BC}	34.80 ± 7.39^{BC}
UG79	15.73 ± 9.64^{A}	24.03 ± 14.25^{BC}	39.76 ± 15.97^{BC}
Bulk soil	3.82 ± 2.66^{c}	43.83 ± 14.63^{a}	47.64 ± 13.88^{a}
cASC	15.39 ± 7.59^{a}	23.09 ± 7.94^{b}	38.48 ± 8.24^{b}
mASC	16.29 ± 4.82^{a}	19.8 ± 7.77^{b}	36.09 ± 9.99^{b}
fASC	8.24 ± 8.44^{b}	23.13 ± 19.67^{b}	31.36 ± 14.50^{c}
GI	$F = 37.23^{***}$	$F = 15.15^{***}$	$F = 10.85^{***}$
SAZ	$F = 78.76^{***}$	$F = 47.05^{***}$	$F = 19.09^{***}$
GI*SAZ	$F = 11.73^{***}$	$F = 13.43^{***}$	$F = 11.16^{***}$

Different superscript letters represent statistically significant difference between treatments at $P < 0.05$. Values are expressed by mean ± SD. *** represents $P < 0.0001$ (Taken from Wu et al. 2011b.).

For mASC, high values of MBC and DOC were found whereas fASC reveales much lower amounts. This can be explained by a high microbial biomass in mASC due to a high efficient usage of SOM within this fraction (Sainju et al., 2009). C mineralization of fASC was low suggesting that aggregates protect the mineralization of SOC by reducing microbial access to the substrates that bind them (Elliott, 1986; Six et al., 2000). Coarse aggregates had higher C and N concentrations than small aggregates because coarse aggregates are composed of microaggregates and organic binding agents (Elliott, 1986) while aggregate-protected C and N pools are more labile than unprotected pools because protected pools are less exposed to microbial decay (Beare et al., 1994; Cambardella and Elliott, 1993).

In WG, UG99 and UG79, ASC exhibited no significant effect on MBC and DOC while there is a significant effect on CG and HG, indicating that overgrazing increased MBC and DOC in ASCs more than those in bulk soil. It also supports the findings that small aggregates are more stable while coarse aggregates are more likely to be influenced by grazing (Cambardella and Elliott, 1993; Six et al., 2004). To reduce CO_2 emission, soil erosion, nutrient loss, and improve soil quality and productivity, the management practices should adopt moderate grazing to promote the fASC aggregation size (Sainju et al., 2009).

5.2.3 N mineralization and inorganic N of soil aggregate size classes affected by grazing and its interaction with C mineralization

The effects of grazing intensity and soil ASC on net ammonification, nitrification, N mineralization were complex. Grazing increased N mineralization significantly in bulk soils, which is consistent with other studies (Groffman et al., 1993; Le Roux et al., 2003). However grazing exhibited indefinite effects in the three soil ASCs. Most of them were not significant with the exceptions that net nitrification and mineralization of fASC in CG increased significantly compared to UG99 and UG79 and net nitrification in WG decreased significantly for the three ASCc. The inconsistent effect of bulk soil and soil ASC on N mineralization suggests the interactions of soil ASCs in bulk soil. Further research is needed in terms of N mineralization in soil ASCs, especially under the effect of grazing.

6. MAIN CONCLUSIONS AND OUTLOOK

This work shows that based on whole-year round experiment at ungrazed (UG99) and wintergrazed (WG) steppe plots, the annual net rates of ammonification is -6, -9 kg N ha^{-1} $year^{-1}$, with net nitrification of 31, 19 kg N ha^{-1} $year^{-1}$, gross ammonification of 240, 215 kg N ha^{-1} $year^{-1}$ and nitrification of 417, 362 kg N ha^{-1} $year^{-1}$ separately in the uppermost 10 cm of soil. Four different seasons with characteristic patterns of gross N dynamics could be distinguished in the investigated semi-arid continental Asian grassland. Both freeze-thaw cycles and the growing season were key periods for understanding patterns and magnitudes of gross N turnover. Various patterns of biogeochemical N turnover between seasons appeared to be closely related to microbial succession in periods of environmental stress or transition. In this context, turnover of soil microbial biomass appeared to be the major driver of gross N fluxes, and hence is of outstanding importance for nutrient retention and availability and may mitigate nutrient shortage in drought periods of the growing season. Net rates of N turnover may be of limited use in steppe ecosystem studies, since they provided only a very poor approximation to magnitude, dynamics and status of actual N turnover in soil. The observed high dynamics of N turnover within and between seasons emphasizes the necessity for high resolution gross N turnover studies as a prerequisite to infer functioning and annual budget of N turnover. Furthermore such high temporal resolution data on gross N turnover process dynamics are an indispensable prerequisite to test and improve process-oriented biogeochemical ecosystem models.

In-situ ^{15}N labeling of large intact plant-soil microcosms proved to be a valuable and realistic approach for the investigation of grazing effects on gross N mineralization and short-term inorganic N partitioning to plant and microbial pathways. Grazing negatively affected both plant and microbial N acquisition but favored nitrification and nitrate accumulation in the soil and thus negatively affected potential ecosystem N retention. These effects appear to be mediated by a decrease in soil DOC availability with increasing stocking rate. A critical threshold value for these ecologically and economically undesirable effects was found to be approximately 3 sheep ha^{-1} considering a grazing duration of three months during the

vegetation period. This threshold value is recommended to establish sustainable summer grazing in semi-arid steppe of Inner Mongolia. In view potentially high intra-annual dynamics of patterns of N turnover in the plant-soil systems, further studies investigating full annual cycles of soil gross N fluxes as influenced by grazing are required to finally verify the proposed threshold value for a sustainable grazing management in Inner Mongolia.

This study also indicated that heavy grazing (i.e. HG and CG) increased LOC significantly compared to ungrazed sites, while moderate grazing (i.e. WG) exhibited no significant effect. CO_2 production was highest in cASC, while lowest in fASC. MBC and DOC showed the highest values in mASC and were significantly lower in fASC. Grazing increased N_{min} in bulk soils while exhibited complex effects in the three ASCs. Generally, grazing increased C and N mineralization in bulk soils that is consistent with the finding that moderate grazing increases C and N sequestration (He et al., 2011), suggesting the rate of carbon mineralization was related with the rate of nitrogen accumulation (Knops and Tilman, 2000). We recommend moderate grazing as a proper way to protect C and N losses in semi-arid graslands.

Freeze-thaw period contributed significantly to the gross mineralization nearly up to 50%, while N turnover appeared evidently even at the freezing period of extremely low temperature of below -20℃ in winter. Gross N turnover in growing season is not as much as net mineralization which accured nearly totally in the growing season. Hence, to evaluate N turnover of the whole year round, the contributions of all the seasons must be included. It is very important to conduct the whole year round experiment in order to assess the real state of N turnover, particularly, in the northern arid steppe.

Gross mineralization is highly related with temperature and soil moisture, and also affected by other biotic and environmental factors such as grazing, plants community, pH, et al.. Then, in the study about the effect of one factor on the gross N turnover, all the other factors should be involved detailed to avoid the inaccurate results.

This study emphasizes the importance of the freeze-thaw period for estimatation of the impact of grazing to microbial N turnover. Grazing decreased N turnover

mainly because that grazing decrease the coverage and height of the vegetation, and reduce the snow depth and area, which in turn decrease the soil moisture and microbial activity in the freeze-thaw period. But the hypothesis shoud be approved by carrying out further experiments.

With the in-situ ^{15}N tracer method, we determined the competition of inorganic nitrogen in plants, microbes and soil in the intact plant-soil system impacted by grazing intensity. Understanding of the N turnover in the whole plant-soil system is very important since the ecosystem is indivisible. It is very interesting to develop the further research in more abundunt ecosystems to elucidate the real state of N turnover of the whole plant-soil system in the future.

This work also showed that heavy grazing caused to a considerable degradation in soil labile organic carbon with the significantly emission of CO_2 production, and decrease in soil inorganic N availability. Hence, we suggest that around 3 sheep ha-1 in the grasslands of Northern China is the critical threshold value for a sustainable summer grazing management. Grazing exclusion is effective for the restoration of the depleted grassland as inorganic N was promoted and labile organic C was decreased in the ungrazed field.

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Candidates' contribution and publications

Chapter III

Wu et al. (2012) Ecosphere

Some of this part was taken from the paper "Seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia – insights from a full year dataset of gross and net nitrogen turnover, Ecosphere", in Press.

The candidate did almost all field experiments and lab analysis, wrote the first draft of the manuscript. Michael Dannenmann contributed greatly to the manuscript and revision. Benjamin Wolf provided meteorological data, Xiangguo Han, Xunhua Zheng and Klaus Butterbach-Bahl gave comments on experiments and manuscript.

Chapter IV

Wu et al. (2011a) Plant and Soil

This part was taken from the paper "Feedback of grazing on gross rates of N mineralization and inorganic N partitioning in steppe soils of Inner Mongolia. Plant & soil, 340:127-139."

The candidate did almost all field experiments and lab analysis, wrote the first draft of the manuscript. Michael Dannenmann contributed greatly to the manuscript and revision. All coauthors gave coments on experiments or manuscript.

Chapter V

Wu et al. (2011b) Biology and Fertility of Soils

This part was taken from the paper "Labile organic C and N mineralization of soil aggregate size classes in semi-arid grasslands as affected by grazing management. Biology & Fertility of Soils, 48:305-313"

The candidate did almost all field experiments and lab analysis, wrote the manuscript. Martin Wiesmeier & Markus Steffens gave some supports for experiments. All coauthors gave coments on experiments or manuscript.

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