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Grassland Management and Diversity Effects on Soil Nitrogen Dynamics and Losses

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Ina Hoeft

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Referentin/Referent: Prof. Dr. Edzo Veldkamp

Korreferentin/Korreferent: Dr. Marife D. Corre

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike information criterion
$BaCl_2$	Barium chloride
BS	Base saturation
C	Carbon
CEC	Cation exchange capacity
CFIC	Continuous flow injection colorimetry
CO_2	Carbon dioxide
CrO_3	Chromium trioxide
DON	Dissolved organic nitrogen
EF	Emission factor
ET	Evapotranspiration
Fe	Iron
GC	Gas chromatograph
H_2O	Water
H_2O_2	Hydrogen peroxide
ICP-OES	Inductively coupled plasma - optical emission spectroscopy
IPCC	Intergovernmental Panel on Climate Change
K	Potassium
K_2SO_4	Potassium sulphate
LME	Linear mixed effects model
Mol	Mole
N	Nitrogen
^{14}N	Stable N isotope, frequency of occurrence 99.634%
^{15}N	Stable N isotope, frequency of occurrence 0.366%
N_2	Dinitrogen
N_t	Total soil nitrogen
Na	Sodium
NH_4^+	Amonium
NH_4Cl	Amonium chloride

NH_4NO_3	Ammonium nitrate
NO	Nitric oxide
NO_2	Nitrogen dioxide
NO_3^-	Nitrate
N_2O	Nitrous oxide
NPK	Mineral fertilizer with nitrogen, phosphorus and potassium
NRE	Nitrogen response efficiency
NUE	Nitrogen use efficiency
P	Phosphorus
P	Significance level
PVC	Polyvinyl chloride
R^2	Coefficient of determination
SE	Standard error
TDN	Total dissolved nitrogen
UV	Ultraviolet
WFPS	Water filled pour space

SUMMARY

Grasslands play an important role in land use, covering one third of the agriculturally utilized landscape in Europe. As a consequence of management intensification in the last 60 years, the productivity of grasslands increased and the diversity of these systems decreased. Nitrogen (N) plays a key role in these ecosystems – it limits primary productivity and affects biodiversity. Increasing N supply may alter nitrous oxide (N_2O) and nitric oxide (NO) emissions, which play a major role in atmospheric chemistry and contribute to global warming. Nitrate (NO_3^-) leaching from soils can be a threat for groundwater quality. N losses of N_2O and NO emissions and NO_3^- leaching from soils result from microbial processes denitrification and nitrification. In this study, we analyzed the effect of different management intensities and plant functional diversity on N losses and ecosystem functioning.

The study was carried out as part of the Cluster of Excellence “Functional Biodiversity Research” at the Georg-August-University of Göttingen and was funded by the State of Lower Saxony (Ministry of Science and Culture). The study was conducted in the framework of two interdisciplinary projects (BIOMIX & GRASSMAN) from 2008 – 2010 in the Solling, in Lower Saxony, Germany. We analyzed a grassland grazed by cattle or sheep (BIOMIX) and a cut grassland with different management intensities (GRASSMAN). In both projects, functional plant diversity was influenced by the use of herbicides. Our particular focus was on N losses (N_2O and NO emissions, N leaching) and N dynamics (net and gross mineralization). Within GRASSMAN, we calculated N response efficiency and N retention efficiency on ecosystem-level. N response efficiency is defined as the product of uptake efficiency (defined as plant N uptake per unit of available N) and N use efficiency (defined as productivity per unit of plant N uptake). In addition, we defined N retention efficiency as the soil available N retained in an ecosystem.

In BIOMIX, we investigated the response of N_2O and NO emissions to different grazers and sward composition. Paddocks partly pretreated with a herbicide against dicots were grazed rotationally by either cattle or sheep. Mean N_2O emissions were 38.7 µg N_2O-N m^{-2} h^{-1}, mean NO emissions 2.4 µg NO-N m^{-2} h^{-1}. Cumulative NO-N emissions were larger for sheep- than for cattle-grazed paddocks. In a controlled application experiment, plots with cattle excreta showed larger N_2O emissions than plots with sheep excreta, reaching peak emissions of 1921 µg N_2O-N m^{-2} h^{-1} on cattle urine patches compared to 556 µg N_2O-N m^{-2} h^{-}

^{1} on sheep urine patches, related to different N-inputs per excretion. Peak emissions of dung-treated plots were much smaller. The N_2O emission factors were 0.4% for cattle urine, 0.5% for sheep urine, 0.05% for cattle dung and 0.09% for sheep dung. In the main and the controlled application experiment, plant species composition was insignificant compared to the effect of grazers on N oxide emissions. N oxide emissions on the paddock scale were larger for sheep- compared to cattle-grazing, despite larger emissions per cattle excretion. We attributed this to the more even spread of sheep excreta compared to cattle excreta.

In GRASSMAN, we investigated the effects of management practices (fertilizer application and mowing frequency) and sward composition on N losses (N_2O emissions, N leaching) and N dynamics (net and gross mineralization), and calculated N response efficiency and N retention efficiency. A three-factorial design was conducted, including two fertilization treatments (180 – 30 – 100 kg NPK ha^{-1} yr^{-1} and no fertilization), two mowing frequencies (cut once and thrice per year) and three sward compositions (control, dicot-enhanced and monocot-enhanced swards) over a two-year period. In 2009, N_2O emissions were significantly influenced by both management practices (fertilization and mowing frequency). In 2010, only fertilization affected N_2O emissions. In 2009, NO_3 leaching losses were influenced by fertilization and in 2010 by both management practices (fertilization and mowing frequency) influenced NO_3 leaching. Net N mineralization rates were affected only by fertilizer application in 2009. In 2010, not only fertilization but also mowing frequency influenced net N mineralization rates. Neither management practices nor sward composition affected gross N mineralization. The N response efficiency was largely influenced by fertilization, followed by mowing frequency, explaining 41% and 3% of the variance, respectively, in 2009. In 2010, the effect of fertilization was lessened whereas the effects of mowing frequency and sward composition were more pronounced, accounting for 24%, 12% and 6% of the variance, respectively. The N response efficiency was larger in unfertilized than in fertilized plots, in plots cut thrice than once per year, and in control swards than in monocot- or dicot-enhanced swards. Fertilization decreased N response efficiency through decreases in both N uptake efficiency and N use efficiency whereas mowing frecuency and sward composition affected N response efficiency through N uptake efficiency rather than N use efficiency. N retention efficiency was calculated only for 2010 and was largely influenced by fertilization followed by sward composition explaining 22% und 17% of the variance, respectively. Unfertilized plots showed larger N retention efficiency than fertilized plots and N retention efficiency decreased in the order control > dicot-enhanced > monocot-

enhanced swards with a significant difference between control and monocot enhanced swards. N retention efficiency was highly correlated with microbial ammonium (NH_4^+) immobilization and microbial biomass and only marginally correlated with plant N uptake, underlining the importance of microbial N retention in the soil-plant system.

Our results indicated that management practices are the most important factor determining ecosystem functioning in grassland. Fertilization, mowing frequency and livestock grazing largely influenced N response efficiency, N retention efficiency and N losses. The sward botanical composition had a minor effect on N cycling or efficiency. Control sward composition of ~70% monocots and ~30% dicots, resulting from extensive long-term management showed the largest efficiencies. Deviations from these proportions - even to more balanced proportions of monocots and dicots - decreased efficiencies. N response efficiency and N retention efficiency are appropriate tools to evaluate environmental sustainability of sward compositions and management practices of grasslands.

ZUSAMMENFASSUNG

Grünland spielt eine große Rolle in der Landnutzung und nimmt ein Drittel der landwirtschaftlich genutzten Fläche von Europa ein. Als Konsequenz der Intensivierung landwirtschaftlicher Bewirtschaftungsmaßnahmen der letzten 60 Jahre nahm die Produktivität des Grünlands zu während die Diversität dieser Systeme abnahm. In Grünland-Ökosystemen spielt Stickstoff (N) eine Schlüsselrolle – N bedingt die Primärproduktion und beeinflusst die Biodiversität. Zudem kann eine steigende N-Verfügbarkeit gasförmige Emissionen, wie z.B. Distickstoffoxid (N_2O) und Stickstoffmonoxid (NO) fördern, die eine große Rolle in der Atmosphäre spielen und zur globalen Erwärmung beitragen. Eine höhere Nitratauswaschung (NO_3^-) aus Böden kann eine Gefahr für die Grundwasserqualität sein. N-Verluste durch Ausgasung von N_2O und NO sowie NO_3^--Auswaschung sind dabei die Folgen der mikrobiellen Prozesse Denitrifikation und Nitrifikation. In dieser Studie haben wir den Effekt von unterschiedlichen Bewirtschaftungsintensitäten und funktioneller Pflanzendiversität auf die N-Verluste und Ökosystemfunktionen untersucht.

Die Studie ist Teil des Excellenzclusters „Funktionelle Biodiversitätsforschung“ der Georg-August-Universität Göttingen und wurde durch das Niedersächsische Ministerium für Wissenschaft und Kultur finanziert. Die Studie wurde im Rahmen von zwei interdisziplinären Projekten (BIOMIX & GRASSMAN) von 2008 bis 2010 im Solling, Niedersachsen, Deutschland durchgeführt. Wir untersuchten von Rindern und Schafen beweidetes Grünland (BIOMIX) und gemähtes Grünland mit unterschiedlichen Bewirtschaftungsintensitäten (GRASSMAN). In beiden Projekten wurde die funktionelle Pflanzendiversität durch Herbizide eingestellt. Der Fokus unserer Arbeit lag auf den N-Verlusten (N_2O and NO Emissionen, NO_3^--Auswaschung) und der N Dynamik (Netto und Brutto Mineralisation). In GRASSMAN berechneten wir zusätzlich die N-Nutzungseffizienz und die N-Rückhalteeffizienz auf Ökosystemebene. Dabei ist die N-Nutzungseffizienz das Produkt der Aufnahmeeffizienz (definiert als N-Aufnahme der Pflanze pro verfügbares N) und der N-Nutzungseffizienz auf Pflanzenebene (definiert als Produktivität pro N-Aufnahme der Pflanze). Darüber hinaus berechnen wir N-Rückhalteeffizienz in Böden als einen Index, der das Verhältnis von N-Verlusten zu dem im Grünland verbleibenden N beschreibt.

In BIOMIX haben wir die Auswirkung von Beweidung und Pflanzenarten-zusammensetzung auf N_2O and NO Emissionen untersucht. Die mit einem Herbizid gegen Dikotyle vorbehandelten Weiden wurden mit Rindern oder Schafen Rotationsweise beweidet.

Mittlere N_2O Emissionen lagen bei 38.7 µg N_2O-N m^{-2} h^{-1}, mittlere NO Emissionen betrugen 2.4 µg NO-N m^{-2} h^{-1}. Kumulative NO-N Emissionen waren höher auf den von Schafen beweideten Flächen als auf den von Rindern beweideten Flächen. In einem kontrollierten Applikations-Experiment führte die Behandlung mit Rinderurin zu höheren N_2O Emissionen als die Behandlung mit Schafurin. Die Emissionshöchstwerte von 1921 µg N_2O-N m^{-2} h^{-1} bei Behandlung mit Rinderurin im Vergleich zu 556 µg N_2O-N m^{-2} h^{-1} bei Schafurin standen im Zusammenhang mit unterschiedlichen N-Einträgen pro Ausscheidung der Tiere. Die Emissionshöchstwerte der mit Dung behandelten Flächen waren im Vergleich mit den jeweiligen Urinbehandlungen viel geringer. Die N_2O Emissionsfaktoren betrugen 0.4% für Rinderurin, 0.5% für Schafurin, 0.05% für Rinderdung und 0.09% für Schafdung. Sowohl das Beweidungs-Experiment, als auch das kontrollierte Applikations-Experiment zeigten, dass die Pflanzenartenzusammensetzung auf N-Emissionen im Vergleich zum Einfluss der Weidetierart auf N-Emissionen unbedeutend war. Trotz höherer N-Einträge auf Rinderweiden waren die N-Emissionen aus der Schafbeweidung höher. Wir führten dies auf die gleichmäßigere Verteilung von Schafs-Exkrementen im Vergleich zu Rindern-Exkrementen zurück.

In GRASSMAN untersuchten wir die Auswirkungen von unterschiedlichen Bewirtschaftungsregimen (Düngung und Schnittintensität) und Pflanzenartenzusammensetzung auf die N-Verluste (N_2O Emissionen, NO_3^- Auswaschung) und die N-Dynamik (Netto und Brutto Mineralisation) und kalkulierten die N-Nutzungseffizienz und die N-Rückhalteeffizienz. Ein dreifaktorielles Design mit folgenden Faktoren wurde über einen Zeitraum von zwei Jahren etabliert: Düngung (180 – 30 – 100 kg NPK ha^{-1} yr^{-1} und keine Düngung), Schnittintensität (ein- und dreimal pro Jahr) und Pflanzenartenzusammensetzung (eine unbehandelte Kontrolle, eine Dikotyl-erhöhte und eine Monokotyl-erhöhte Grasnarbe). In 2009 wurden die N_2O Emissionen erheblich von beiden Bewirtschaftungsfaktoren (Düngung und Schnittintensität) beeinflusst, während in 2010 nur die Düngung die N_2O Emissionen beeinflusste. In 2009 wurden NO_3^- Auswaschungsverluste durch Düngung und in 2010 von beiden Bewirtschaftungsfaktoren (Düngung und Schnittintensität) beeinflusst. Die Netto N-Mineralisation Raten wurden in 2009 nur von der Düngung beeinflusst. In 2010, zeigte nicht nur die Düngung, sondern auch die Schnittintensität einen Einfluss auf die Netto N-Mineralisation Raten. Weder die Bewirtschaftung (Düngung) noch die Pflanzenartenzusammensetzung hatte einen Einfluss auf die Brutto N-Mineralisation. Die N-Nutzungseffizienz wurde vor allem durch die Düngung und als weiterer Faktor durch die

Schnittintensität in 2009 beeinflusst, welche 41% bzw. 3% der Varianz erklärten. In 2010 hatte die Düngung mit 24% der erklärten Varianz einen geringeren Effekt auf die N-Nutzungseffizienz, während die Auswirkungen der Schnittintensität (12%) und die Pflanzenartenzusammensetzung (6%) stärker ausgeprägt waren. Die N-Nutzungseffizienz war auf ungedüngten Flächen größer als auf gedüngten, in den dreimal geschnittenen Flächen höher als in den einmal geschnittenen, und in der unbehandelten Kontrolle höher als in der Monokotyl-erhöhte oder Dikotyl-erhöhte Grasnarbe. Düngung verringert die N-Nutzungseffizienz durch die Abnahme in der N-Aufnahmeeffizienz und der N-Nutzungseffizienz auf Pflanzenebene, während die Schnittintensität und die Pflanzenartenzusammensetzung nur durch die N-Aufnahmeeffizienz beeinflusst werden. Die N-Rückhalteeffizienz wurde nur für 2010 berechnet und wurde durch die Düngung und die Pflanzenartenzusammensetzung mit 22% und 17% der erklärten Varianz beeinflusst. N-Rückhalteeffizienz nahm in der Reihenfolge unbehandelte Kontrolle > Dikotyl-erhöhte > Monokotyl-erhöhte Grasnarbe mit einem signifikanten Unterschied zwischen der unbehandelten Kontrolle und der Monokotyl-erhöhten Grasnarbe ab. Die N-Rückhalteeffizienz ist mit dem mikrobiellen Ammonium (NH_4^+) und der mikrobiellen Biomasse hoch und mit der N-Aufnahme der Pflanzen nur gering korreliert, was die Bedeutung der mikrobiellen N Retention im System Boden-Pflanze unterstreicht.

Unsere Ergebnisse zeigen, dass die Bewirtschaftung der wichtigste und bestimmende Faktor der Ökosystemfunktionen eines Grünlands ist. Düngung, Schnittintensität und Beweidung beeinflussen die N-Nutzungseffizienz, die N-Rückhalteeffizienz und die N-Verluste. Die Zusammensetzung der botanischen Grasnarbe hat einen geringen Einfluss auf den N Kreislauf oder die N-Nutzungs- und die N-Rückhalteeffizienz. Wobei die Pflanzenartenzusammensetzung der unbehandelten Kontrolle (~70% Monokotyle und ~30% Dikotyle), die sich unter der extensiven Langzeit-Bewirtschaftung eingestellt hatte, die höchsten Effizienzen zeigte - sowohl eine Erhöhung der Monokotyledonen als auch eine Erhöhung der Dikotyledonen führte zu einer Verringerung der Effizienzen. Darüber hinaus sind N-Nutzungs- und N-Rückhalteeffizienz geeignete Werkzeuge, die sich zur Evaluierung ökologischer Nachhaltigkeit von Pflanzenartenzusammensetzungen und Management-Praktiken im Grünland eignen.

CHAPTER

1

GENERAL INTRODUCTION

1.1 TEMPERATE GRASSLANDS - MANAGEMENT AND BIODIVERSITY

Grasslands play an important role in the land use of the European Union (EU), covering more than 30% of the agriculturally utilized landscape (EUROSTAT, 2001). However, the distribution of natural European grassland is restricted to marshlands close to the coast, very dry and very wet ecosystems in the lowlands and to above the timberline in mountainous ecosystems. Since the Middle Ages, the human population increased and forests have been cleared and replaced by grassland and arable land (Ellenberg and Leuschner, 2010). In the 20th century, also semi-natural grassland was utilized agriculturally and the management was intensified by increasing mowing frequency, grazing pressure and fertilization. Grassland management shifted from extensive grazing and mowing regimes of one cut yr^{-1} to intensive grazing and mowing with three to six cuts yr^{-1}. Fertilization has been increased from no or low fertilization rates to amounts of 150 – 300 kg N ha^{-1} yr^{-1}. As a result, semi-natural grassland ecosystems were replaced by pastures and meadows or arable land where the soil was very fertile and non-profitable grassland on low fertile soils became abandoned (Isselstein et al., 2005; Krahulec et al., 2001; Tasser and Tappeiner, 2002; Vitousek et al., 1997).

Permanent grassland makes an important contribution to plant diversity (Nösberger and Rodriguez, 1996). More diverse grassland systems have a large potential in increasing the productivity and the sustainability of ecosystem services and might be advantageous compared to simplified or less species-rich communities under the conditions of climate change: Hector et al. (1999) and Naeem et al. (1994) showed that more diverse grasslands can be more productive than less diverse grasslands. Agricultural intensification in European grasslands is one of the main factors for biodiversity decline (Tscharntke et al., 2005). Grassland is managed intensively or transferred into cropland. As a result, semi-natural grassland were replaced by agriculturally grasslands with a low plant and animal diversity (Fuller, 1987; Green, 1990; Poschlod and Bonn, 1998). A large-scale vegetation survey done by Krause et al. (2011) and Wesche et al. (2009) indicated that mesic and wet grasslands in Central Europe lost 30 – 50% of its plant species richness since the mid 20th century due to changes in land use.

Biodiversity and ecosystem services in grassland have received an increasing attention. Therefore, grassland ecosystems are intensively studied. Most of the studies have so far been carried out in experimental grassland plots, sown and weeded intensively (Hector et al., 1999; Naeem et al., 1994; Tilman et al., 1996), making the results difficult to transfer to permanent grassland (Caliman et al., 2010; Petersen et al., 2012). Initiated in 2006 and 2008, the BIOMIX experiment and the GRASSMAN experiment aim to fill this knowledge gaps. Within these two experiments an untreated control sward was compared with herbicides treated swards. The swards received management treatments differing in grazing animals (BIOMIX) and in cutting frequency and fertilization (GRASSMAN).

1.2 NITROGEN IN TEMPERATE GRASSLAND ECOSYSTEMS

Nitrogen (N) is an essential plant nutrient that limits primary production in many ecosystems, making requirement for N fertilizers pervasive in agricultural systems (Vitousek and Howarth, 1991). The Haber-Bosch process was the key invention in the 20th century, which enabled the conversion of N_2 into reactive N and thus the industrial production of mineral N fertilizer (Gruber and Galloway, 2008). A large proportion of applied fertilizer N can be lost via emissions of nitrous oxides (N_2O) and nitric oxide (NO) or nitrate (NO_3^-) leaching. These N losses from soils result from the microbial processes denitrification and nitrification (Firestone and Davidson, 1989). N_2O is an important greenhouse gas contributing to global warming and the depletion of stratospheric ozone (Mosier et al., 1996; Schlesinger, 2009). It has a global warming potential that is 298 times larger than that of carbon dioxide (CO_2) and its concentration increased from 270 parts per billion (ppb) during the pre-industrial period to 319 ppb in 2005 (IPCC, 2007). The main sink of N_2O in the stratosphere is its destruction to highly reactive NO, which plays a crucial role regulating troposphere ozone concentrations (Crutzen, 1979). NO_3^- is the dominant form of N leached from soil in temperate systems. Most soils are negatively charged; therefore ammonium (NH_4^+) concentrations are usually small due to a fast conversion into NO_3^-. Leaching of NO_3^- can be a threat for groundwater quality. Taken up with drinking water, NO_3^- can be harmful to human health by causing methemoglobinemia (blue-baby syndrome) (Di and Cameron, 2002). To protect humans, the European Union (EC Directive on Drinking Water 80/788) threshold value for drinking water is 50 mg NO_3^- L^{-1}. Furthermore, NO_3^- leaching can cause eutrophication of surface waters (Di and Cameron, 2002; Howarth, 1988).

As a consequence of the increasing global population, a more intensive agricultural production and therewith increasing use of N fertilizers can be expected for the future (Galloway et al., 2008). Beside (mineral) fertilizer application, a main source of N losses results from livestock management systems (Davidson, 2009). Urine and dung patches in pastures have been identified as potential hotspots for N losses from soils (Ma et al., 2006). There are N transformation characteristics for excreta patches of different pasture animals, depending on the excreta-covered area and the N concentration (Ma et al., 2006; Williams and Haynes, 1994). Cattle excreta patches are larger than those of sheep (Bolan et al., 2004). However, sheep urinate more frequently than cattle and their urine is more evenly spread in smaller, less concentrated patches than cattle urine (Williams and Haynes, 1994). It will be necessary to comprise N losses in pastures and meadows, keeping theses losses as small as possible and ensuring soil fertility and high productivity at the same time.

N response efficiency, as a measure of ecosystem functioning, integrates biomass production and N retention (Hiremath and Ewel, 2001). N response efficiency is calculated as the product of uptake efficiency (defined as plant N uptake per unit of available N) and N use efficiency (NUE, defined as productivity per unit of plant N uptake) (Pastor and Bridgham, 1999). Experimental studies showed that the addition of species can lead to more productivity (Hector et al., 1999; Hooper and Vitousek, 1998; Naeem et al., 1994) and that greater diversity can lead to greater N retention (Tilman et al., 1996). Since increasing N response efficiency indicated increasing productivity and the associated retention of available N in plants, N response efficiency thus conversely signifies how much of available N may be lost, e.g. through gaseous N emissions and N leaching. In addition, we calculated N retention efficiency from soils as an index that describes the ratio of N losses (N_2O and NO_3^-) to the N retained in the grassland.

1.3 EXPERIMENTAL SITES

The study sites were located in the Solling Mountains, Lower Saxony, in Germany (Figure 1.1).

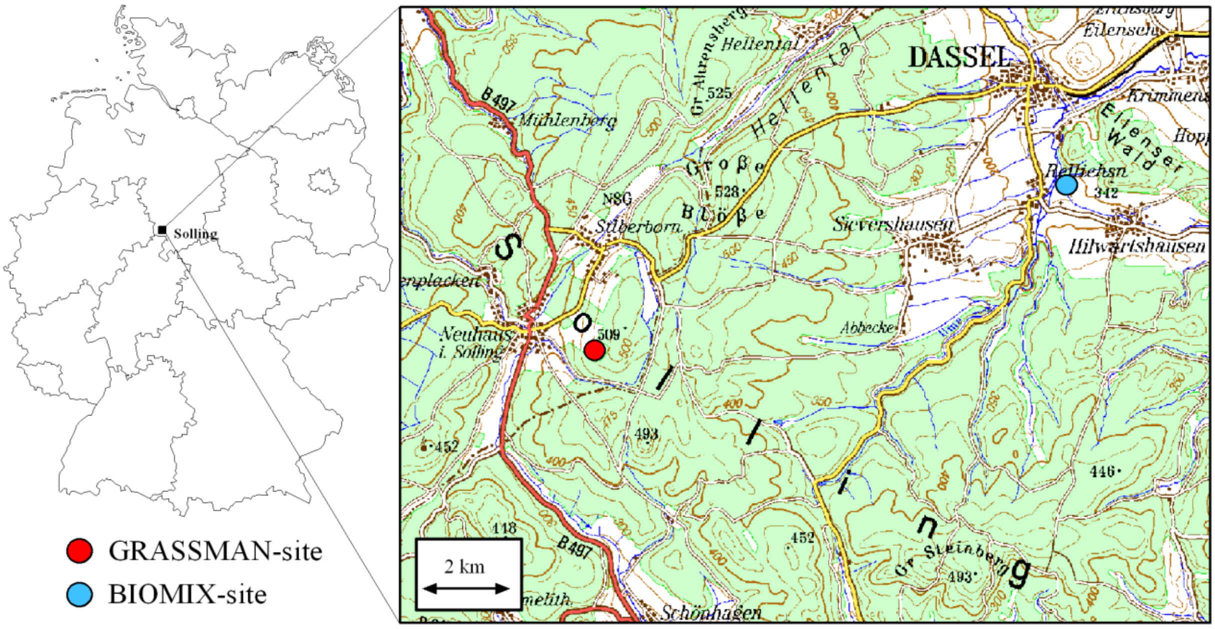


Figure 1.1 Location of the BIOMIX and GRASSMAN study sites in the Solling Mountains, Lower Saxony, Germany.

1.3.1 THE BIOMIX PROJECT

The BIOMIX pasture project was established in 2006 at the experimental farm of the University of Goettingen in Relliehausen in the Solling Mountains, Germany (51°46’47’’N, 9°42’11’’E, 184-209 m a.s.l.). Mean annual temperature is 8.2°C and the mean annual precipitation is 879 mm (Sahin Demirbag et al., 2009). The dominating soil type is a Cambisol with vertic properties developed on sediments of the Lower Triassic sandstone formation with a clay loam texture. The grassland is a 16-20 year old mesotrophic hill grassland with high productivity and moderate levels of plant diversity and is classified as a moderately species-rich *Lolio-Cynosuretum* (Seither, 2010).

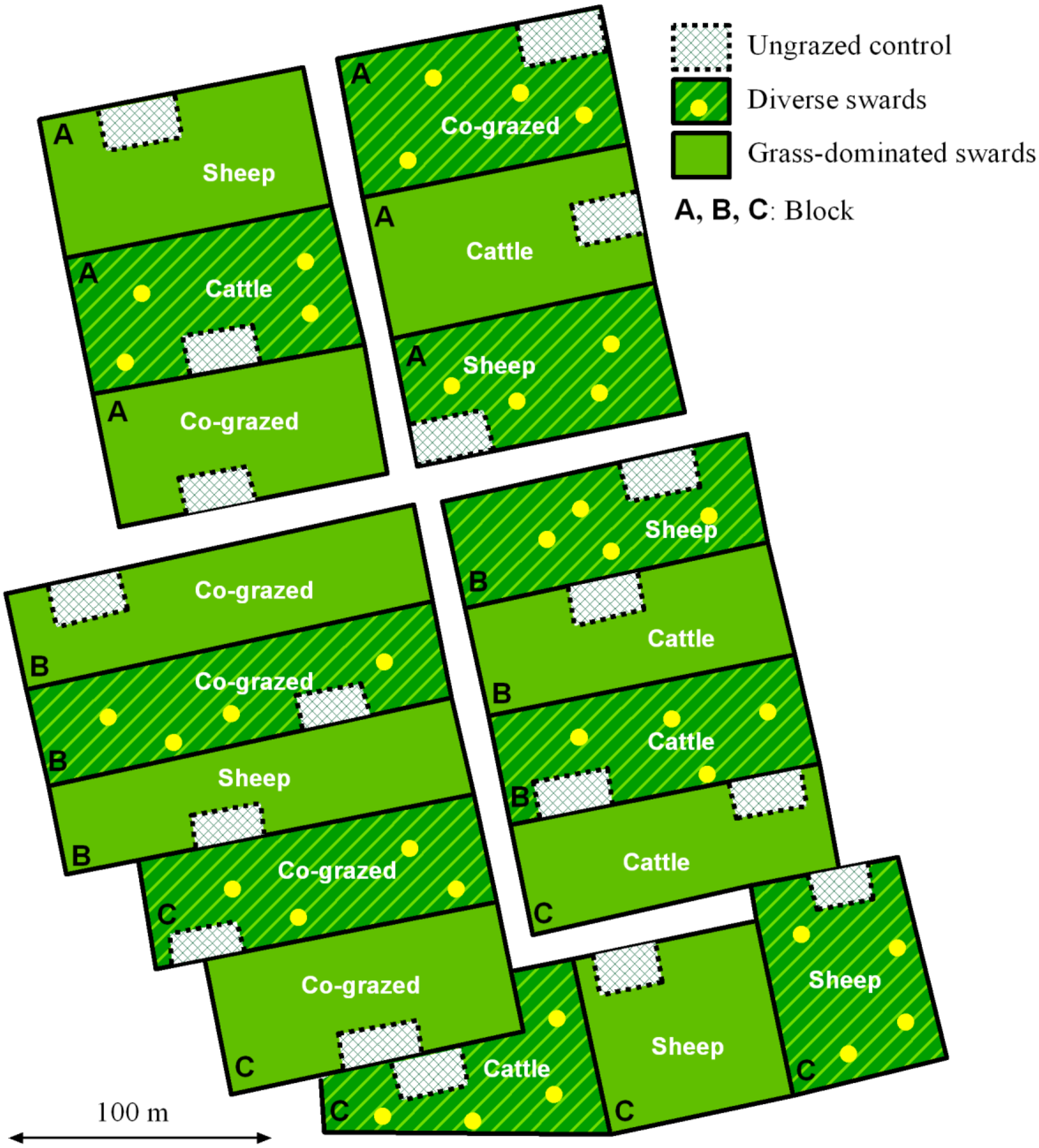
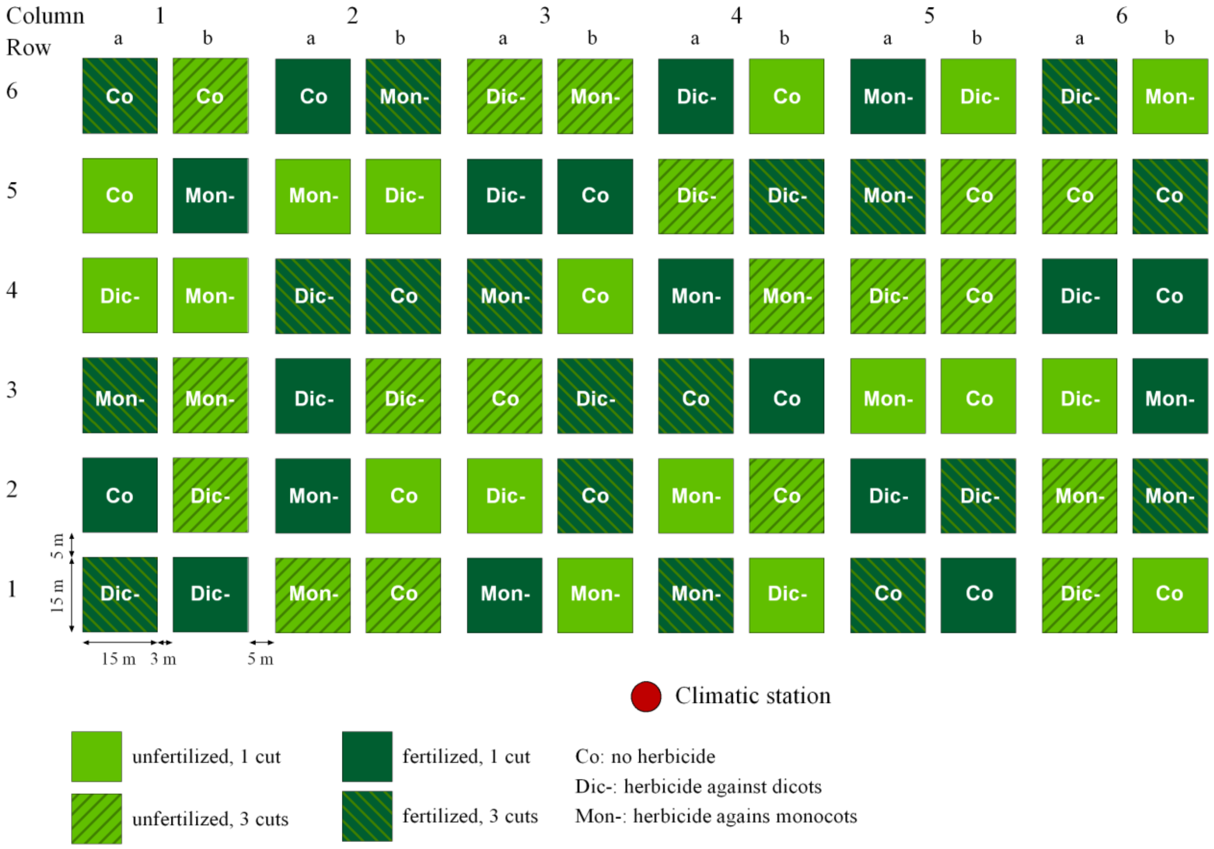


Figure 1.2 Experimental design of BIOMIX.

BIOMIX was established as a factorial experiment in a block design on 0.5 ha paddocks with three replicates presenting two sward compositions and two types of grazer (Figure 1.2). Differences in sward composition were established by applying a herbicide mixture against dicotyledons (Starane® (active ingredients: Fluroxypyr and Triclopyr) and Duplosan KV (active ingredient: Mecoprop P)), resulting in a sward dominated by grasses and an untreated control. The dominating plant species on grass swards after herbicide treatment were *Dactylis glomerata* L., *Festuca pratensis* Huds., *Lolium perenne* L., *Phleum pratense* L. and *Poa trivialis* L. On the untreated control, further dominant species were *Crepis biennis* L., *Taraxacum Sec. ruderalia* Kirschner, H. Ollg. & Stepanek, and *Trifolium repens* L. (Seither, 2010). Both sward composition treatments were grazed either by sheep or cattle or mixed grazing. Suckler cows and calves of the breed German Simmental, and ewes with lambs of Blackheaded and Leine sheep were used as grazing animals. In our study, we only considered mono-grazed treatments with either cattle or sheep. The paddocks were grazed following a rotational scheme from spring to autumn. The average target animal live weight was 3000 kg (i.e. six livestock units of 500 kg each) in the first two rotations and was reduced to an average target animal live weight per paddock of 2000 kg in the third rotation. At the beginning of the grazing season (first two rotations), cattle and sheep stayed in one block for approximately 14 days, which was reduced to about seven days at the end of the grazing season (third rotation), because less vegetation biomass was available. In July, livestock was removed from the experiment for mating for six weeks. The study site received only N inputs from direct excreta returned by livestock without additional fertilization.

1.3.2 THE GRASSMAN PROJECT

The GRASSMAN project was established in 2008 at the experimental farm of the University of Goettingen on a moderately species rich grassland in the Solling Mountains in Lower Saxony, Germany (51°44’53’’N, 9°32’42’’E, 490 m a.s.l). Mean annual temperature is 6.9°C and mean annual precipitation is 1028 mm (Deutscher Wetterdienst 1961 - 1990). The dominating soil type is a Haplic Cambisol, developed on sediments of loess on the Middle Triassic sandstone formation with a loamy silt texture. The vegetation has been classified as a montane, semi-moist *Lolio-Cynosuretum* (Petersen et al., 2012). This permanent grassland site was used for hay making or for grazing throughout the 20th century (Geological Map of Prussia 1910 (based on the topographic inventory of 1896), topographic maps of Sievershausen and Neuhaus/Solling 1924, 1956 and 1974). The study site was managed with moderate fertilization (80 kg N ha^{-1} yr^{-1}), liming, overseeding with high value forage species and cattle grazing (farm records of Relliehausen since 1966) in the last five decades. Two years before the experiment started, fertilizer application stopped.



Column / Row	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b
6	Co	Co	Co	Mon-	Dic-	Mon-	Dic-	Co	Mon-	Dic-	Dic-	Mon-
5	Co	Mon-	Mon-	Dic-	Dic-	Co	Dic-	Dic-	Mon-	Co	Co	Co
4	Dic-	Mon-	Dic-	Co	Mon-	Co	Mon-	Mon-	Dic-	Co	Dic-	Co
3	Mon-	Mon-	Dic-	Dic-	Co	Dic-	Co	Co	Mon-	Co	Dic-	Mon-
2	Co	Dic-	Mon-	Co	Dic-	Co	Mon-	Co	Dic-	Dic-	Mon-	Mon-
1	Dic-	Dic-	Mon-	Co	Mon-	Mon-	Mon-	Dic-	Co	Co	Dic-	Co

Figure 1.3 Experimental design of GRASSMAN.

Within the GRASSMAN experiment, 72 plots were arranged in a full factorial design with six replicate plots (15 m x 15 m each) per treatment presenting three sward compositions, two mowing frequencies and two fertilization treatments arranged in a Latin rectangle (Figure 1.3). Differences in sward composition were associated with three plant functional groups. We established these three sward compositions by applying a herbicide mixture against dicotyledons (Starane® (active ingredients: Fluoroxypyr and Triclopyr) and Duplosan KV (active ingredients: Mecoprop-P®)) resulting in a sward with reduced herbs and legumes, a herbicide mixture against monocotyledons (Select 240 EC® by Stähler (active ingredient: Clethodim)) resulting in a sward with reduced grasses, and an untreated control sward. Hereafter, we refer to these sward compositions as monocot-enhanced (12-13 species; 91-93% grasses, 7-9% herbs and 0% legumes) and dicot-enhanced (17 species; 40-47% grasses, 49-53% herbs and 4-9% legumes) and an untreated control sward (16-18 species; 68-76% grasses, 21-31% herbs and 1-4% legumes) (Petersen et al., 2012). Two mowing frequencies (once and thrice per year) were established. Grassland was mown in mid-July for plots with one-cut per year and in mid-May, mid-July and end of September each year for plots with three-cuts per year. A Haldrup © forage combine harvester was used with a cutting height of 7 cm. Two fertilization treatments (180 – 30 – 100 kg NPK ha^{-1} yr^{-1} and no fertilization) were established. The N fertilizer (calcium ammonium nitrate N27) was split into two equal applications per year (April and May 2009 and April and June 2010) while the combined P and K fertilizer was applied once a year (June 2009 and 2010).

1.4 METHODICAL OUTLINE OF NITROGEN LOSSES AND NITROGEN DYNAMICS

1.4.1 NITROGEN LOSSES

NO was measured using the open dynamic chamber method (Figure 1.4 a). NO was quantified with a Scintrex LMA-3 NO_2 Analyzer with chemiluminescence detector following oxidation by a CrO_3 catalyst and reaction with a Luminol solution (Figure 1.4 b). N_2O was measured using closed chamber method during the growing season (Figure 1.4 c). The gas samples were analyzed using a gas chromatograph equipped with an electron capture detector and an autosampler system (Figure 1.4 d). Additionally, for every gas sample, soil was sampled for determination of mineral N (NH_4^+ and NO_3^-), soil moisture content and temperature.

N leaching losses (NO_3^- and DON) were measured using suction cups in a depth of 0.5-0.6 m. N concentrations were measured using continuous flow injection colorimetry. The total N leaching losses were calculated by multiplying the NO_3^- concentrations with the accumulated daily drainage flux for the corresponding time interval. Daily drainage flux was modelled using the 1D hydrological model BROOK90 (Federer et al., 2003). Additionally, driving climatic data (precipitation sum, minimum/maximum air temperature, global radiation sum, average vapour pressure and average wind speed, all on a daily basis) for the model came from a weather station of the study site.

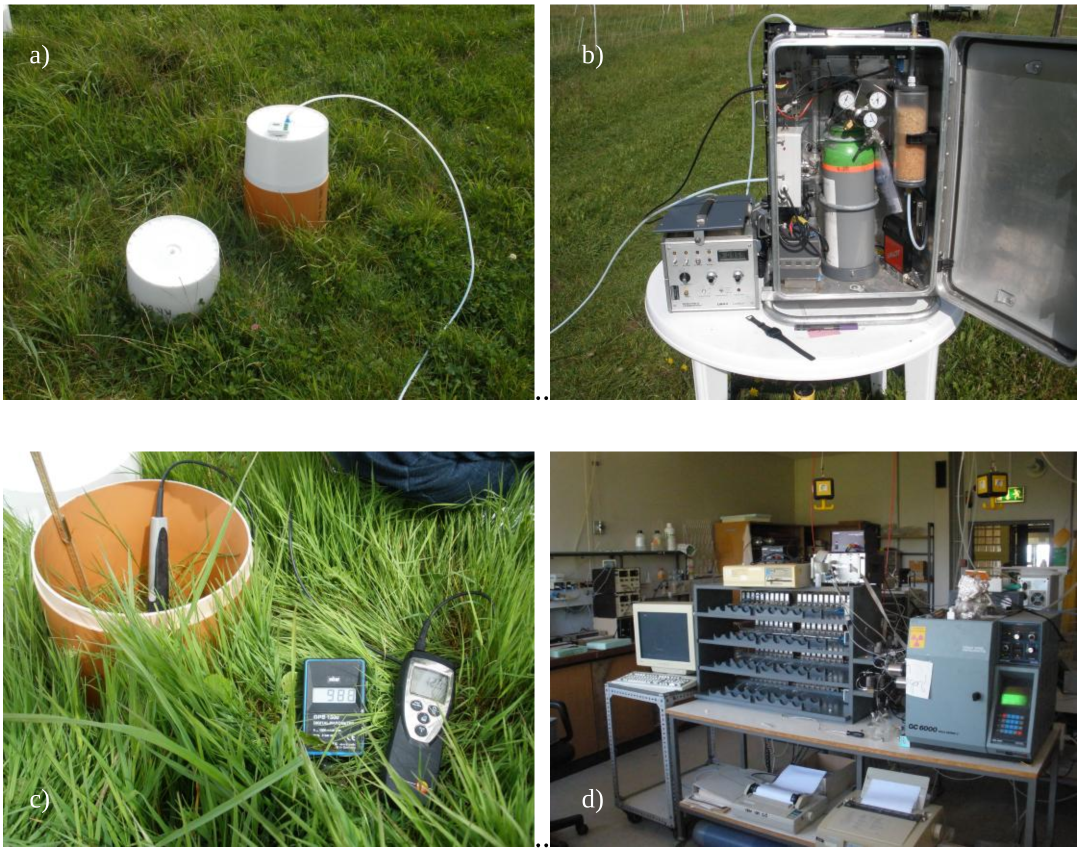


Figure 1.4 Field and laboratory equipment for soil trace gas measurements and analysis. a) Open dynamic chamber base and cover and b) NO_2 detector and calibration gas for in situ nitric oxide (NO) flux measurements; c) closed chamber method for nitrous oxide (N_2O) flux measurements and d) gas chromatograph (GC) with autosampler.

1.4.2 NITROGEN DYNAMICS

Net N mineralization rates were measured during the growing season using the buried bag method. Two intact soil cores were taken from the Ah mineral soil. The soil from one core was extruded into a plastic bag, mixed well, and extracted in 0.5 mol L^{-1} K_2SO_4 (average dry soil mass to solution ratio was 1:3). The other intact soil core was put in a plastic bag, loosely tied to permit aeration but prevent rain from entering, inserted back into the hole to incubate in-situ for ten days, and extracted in a similar manner. NH_4^+ and NO_3^- were measured using continuous flow injection colorimetry.

Gross mineralization rates of soil N cycling were measured directly in the field using ^{15}N-pool dilution technique. Two soil cores were injected with $(^{15}NH_4)_2SO_4$ solution (for gross mineralization) and two soil cores with $K^{15}NO_3$ solution (for gross nitrification) with 99% ^{15}N enrichment. After ^{15}N injection one soil core of each labeled pair was broken up, mixed well in a plastic bag and 0.5 mol L^{-1} K_2SO_4 were added (average dry soil mass to solution ratio was 1:3). The other soil core of the labeled pair was put in a plastic bag, inserted back into the soil to incubate for one day, and extracted with 0.5 mol L^{-1} K_2SO_4. ^{15}N analysis was done using isotope ratio mass spectrometry.

1.4.3 CALCULATION OF NITROGEN RESPONSE EFFICIENCY AND NITROGEN RETENTION EFFICIENCY

Data of biomass yield was reported by From et al. (2011). A Haldrup © forage combine harvester was used with a cutting height of 7 cm. Dry mass of the biomass was determined by oven-drying at 60 °C to constant mass. Oven-dried plant samples from the second cut (mid-July) were ground and analyzed for total N concentration using a CNS elemental analyser (Elementar Vario El, Hanau, Germany). Plant N uptake (kg N ha^{-1} yr^{-1}) was calculated as: N concentration (kg N kg^{-1}) * biomass yield (kg ha^{-1} yr^{-1}) (Hiremath and Ewel, 2001).

N supply of each plot is defined as the sum of cumulative net N mineralization rates of the soil, N fertilization and N deposition rates. Cumulative net N mineralization rates during a growing season (i.e. April - September) were calculated by applying the trapezoid rule on time intervals between measured rates. For N deposition, we used a value of 12.6 kg N ha^{-1} yr^{-1} from bulk precipitation measured within the scope of the EU-level II monitoring program (Keuffel-Türk et al., in press). N response efficiency was calculated for each plot as follows: N response efficiency (kg biomass kg N^{-1}) = (plant N uptake ÷ N supply) * (biomass yield ÷ plant N uptake). The ratio of plant N uptake to N supply is referred to as N uptake efficiency whereas the ratio of biomass yield to plant N uptake is the N use efficiency (Hiremath and Ewel, 2001; Pastor and Bridgham, 1999).

N retention efficiency was calculated comprising plots with three sward compositions (monocot-enhanced, dicot-enhanced and control sward), two fertilization treatments (180 - 30

- 100 kg NPK ha^{-1} yr^{-1} and no fertilization) and cut once per year, as follows: N retention efficiency = 1 – ((N losses (mg N m^{-2} d^{-1}) ÷ gross N mineralization rates (mg N m^{-2} d^{-1})) where N losses is composed of average daily N_2O (mg N m^{-2} d^{-1}) + NO_3^- (mg N m^{-2} d^{-1}) + DON (mg N m^{-2} d^{-1}); gross N mineralization rates were measured once during the growing season 2010.

1.5 STUDY OBJECTIVES

The aim of the present work was to investigate the soil N losses and soil N dynamics in a temperate pasture and a meadow differing in plant composition and management.
The specific objectives of these studies were:

I. To investigate the response of soil N oxide (N_2O and NO) emissions to grazer (cattle and sheep) and plant species composition in a pasture.
II. To investigate N response efficiency of a managed phytodiverse meadow.
III. To investigate N retention efficiency, soil N cycling and losses of a managed phytodiverse meadow.

With these aims I tested the following hypotheses:
BIOMIX:

1) On a paddock scale, sheep excreta result in larger N_2O and NO emissions than cattle excreta.
2) Grasslands with a more diverse species composition have smaller N_2O and NO emissions than grass-dominated paddocks.

GRASSMAN:

3) Unfertilized plots have larger N response efficiency than fertilized plots.
4) Plots with a high mowing frequency have larger N response efficiency than plots with a low mowing frequency.
5) N response efficiency increases with increasing number of species (i.e. monocot-enhanced swards (12-13 species) < dicot-enhanced (17 species) and control swards (16-18 species)).

6) Fertilization results to larger N losses and smaller N retention efficiency than without fertilization.
7) Intensive mowing frequency decreases N losses and increases N retention efficiency.
8) Swards with equal proportions of various plant functional groups would have small N losses and large N retention efficiency.

CHAPTER

2

RESPONSE OF NITROGEN OXIDE EMISSIONS TO GRAZER SPECIES AND PLANT SPECIES COMPOSITION IN TEMPERATE AGRICULTURAL GRASSLAND

2.1 ABSTRACT

Agriculture is an important source of the greenhouse gas nitrous oxide (N_2O) and the atmospherically-important nitric oxide (NO). We evaluated the effects of different grazers and plant species composition on N_2O and NO emissions in temperate grassland. Paddocks were grazed rotationally by either cattle or sheep. Mean N_2O emissions were 38.7 µg N_2O-N m^{-2} h^{-1}, mean NO emissions 2.4 µg NO-N m^{-2} h^{-1}. Cumulative NO-N emissions were larger for sheep- than for cattle-grazed paddocks. Plant species composition was insignificant compared to the effect of grazers on N oxide emissions. In a controlled application experiment, plots with cattle excreta showed larger N_2O emissions than plots with sheep excreta, reaching peak emissions of 1921 µg N_2O-N m^{-2} h^{-1} on cattle urine patches compared to 556 µg N_2O-N m^{-2} h^{-1} on sheep urine patches, related to different N-inputs per excretion. Peak emissions of dung-treated plots were much smaller. The N_2O emission factors were 0.4% for cattle urine, 0.5% for sheep urine, 0.05% for cattle dung and 0.09% for sheep dung. N oxide emissions on the paddock scale were larger for sheep- compared to cattle-grazing, despite larger emissions per cattle excretion. We attributed this to the more even spread of sheep excreta compared to cattle excreta.

Keywords: nitrous oxide, nitric oxide, trace gas fluxes, emission factor, dicots, monocots

2.2 INTRODUCTION

Nitrous oxide (N_2O) is an important greenhouse gas that contributes about 6% to the anthropogenic greenhouse effect. It has a global warming potential that is 298 times larger than that of carbon dioxide (CO_2) and its concentration increased from 270 parts per billion (ppb) during the pre-industrial period to 319 ppb in 2005 (IPCC, 2007). Agriculture is considered responsible for 58% of the anthropogenic N_2O production (IPCC, 2007), with main sources from livestock management systems and from (mineral) fertilizer application (Davidson, 2009). N_2O and nitric oxide (NO) emissions from soils result among other from the microbial processes denitrification and nitrification (Firestone and Davidson, 1989).

These processes are mainly controlled by available N and soil aeration status, which depend on soil water-filled pore space (WFPS), and other drivers like soil temperature (Keeney et al., 1979; Linn and Doran, 1984).

Urine and dung patches in pastures have been identified as potential hotspots for N_2O and NO emissions from soils, as large concentrations of N, usually in excess of immediate plant requirements, are deposited onto a relatively small soil area (Ma et al., 2006). Urine-N is rapidly hydrolyzed and nitrified and the resulting large concentrations of inorganic N are a major source of N losses from grazed swards. In contrast, most N in dung is in organic form, and the mineral N contents in the soil and corresponding N losses are smaller than from urine patches (Flessa et al., 1996). Although several studies have been carried out to determine how cattle urine and dung influence N oxide emissions (e.g. Flessa et al., 1996; Van Groenigen et al., 2005), much fewer studies exist on N oxide emission responses to sheep urine or dung (Shand et al., 2002; Sherlock and Goh, 1983) or on direct comparisons of both cattle and sheep excreta (Williams and Haynes, 1994). The majority of these studies was conducted under controlled laboratory conditions (Van Groenigen et al., 2005) or with artificial urine (e.g. Carter, 2007). However, there is growing concern that results from studies using artificial rather than natural urine and from the laboratory may overestimate N oxide emissions (Van Groenigen et al., 2005). Nutrient dynamics of excreta patches depend on the area covered and on nutrient concentrations (Ma et al., 2006; Williams and Haynes, 1994). Cattle excreta patches are larger than those of sheep (Bolan et al., 2004). However, sheep urinate more frequently than cattle and their urine is more evenly spread in smaller, less concentrated patches than cattle urine (Williams and Haynes, 1994). Sampling directly on an excreta patch may lead to an underestimation of paddock-scale N oxide emissions of sheep compared to cattle grazing. In contrast, random sampling on the paddock may lead to an underestimation of N oxide emissions of cattle compared to sheep grazing, as excreta patches of cattle may be missed more often than those of sheep. Additionally, soil compaction in camping areas and overlapping of excreta patches may affect N_2O emissions (Hack-ten Broeke et al., 1996; Van Groenigen et al., 2005). Beside the area influenced by excreta also the fodder or paddock size potentially affect N_2O emissions (Oenema et al., 1997).

N losses may not only be affected by grazing but also by plant diversity. There are hints in literature that N losses decrease with increasing plant community composition (Niklaus et al., 2006). This is traced back to the impact of sward composition on nitrification and denitrification processes in grassland soils and an improved efficiency in the uptake of mineral N by more diverse sward communities (Niklaus et al., 2006; Oelmann et al., 2007). Differences in N_2O and NO emissions between plots differing in sward composition may be due to interspecific discrepancies in productivity, growth rates, nutrient demands and mineral N-uptake efficiency of the distinct plant communities (Le Roux et al., 2003; Oelmann et al., 2007). Hence, more diverse swards may have a complementary and therefore overall more efficient resource use (Scherer-Lorenzen et al., 2003; Spehn et al., 2002), e.g. due to different rooting depths that enable the plants to take up nutrients from different soil horizons (Tilman et al., 1996). However, the more efficient uptake of mineral N in more diverse swards was due to plant communities dominated by legumes (Niklaus et al., 2006). Furthermore, most studies analyzing effects of plant diversity on soil nutrient dynamics have been carried out in artificial grassland plots. The short-term nature of these plots, missing agricultural management, as well as frequent weeding make the results difficult to compare with permanent agricultural grasslands (Caliman et al., 2010).

In the present study, our goal was to evaluate the effects of different grazers (cattle and sheep) and plant species composition on soil N cycling and related N_2O and NO emissions in permanent, 16-20 year-old agricultural grassland. We tested the following hypotheses: 1) on a paddock scale, sheep excreta result in larger N_2O and NO emissions than cattle excreta and 2) grasslands with a more diverse species composition have smaller N_2O and NO emissions than grass-dominated paddocks. Measurements of soil N_2O and NO emissions and supporting variables were performed throughout the grazing season on grassland differing in plant species composition due to herbicide treatment and managed according to local practices in the Solling uplands (Lower Saxony, Germany). To get a better understanding of the maximum N_2O emissions on swards differing in species composition, we also applied cattle and sheep urine and dung manually in a controlled experiment at the same field site.

2.3 MATERIAL AND METHODS

2.3.1 STUDY SITE

Our experiment was conducted at the experimental farm of the University of Goettingen in Relliehausen in the Solling uplands, Germany (51°46’47’’N, 9°42’11’’E, 184-209 m a.s.l.) on a moderately species-rich *Lolio-Cynosuretum*. The grassland is a 16-20 year old mesotrophic hill grassland with high productivity and moderate levels of plant diversity. The average annual precipitation is 879 mm, the mean annual temperature 8.2°C (Sahin Demirbag et al., 2009). The dominating soil type is a Braunerde-Pelosol (FAO: cambisol with vertic properties) developed on sediments of the Lower Triassic sandstone formation with a clay loam texture.

2.3.2 EXPERIMENTAL SETUP OF THE MAIN EXPERIMENT

We established a factorial experiment in a block design on 0.5 ha paddocks in three replicates to evaluate grazer and sward composition effects on N cycling and related N oxide emissions. In autumn 2006, we established two sward compositions by applying a herbicide mixture against dicotyledons (Starane® (active ingredients: Fluroxypyr and Triclopyr) and Duplosan KV (active ingredient: Mecoprop P)), resulting in a sward dominated by grasses (‘grass sward’; G) and an untreated control (‘diverse sward’; D). After herbicide treatment, the dominating plant species on G were *Dactylis glomerata* L., *Festuca pratensis* Huds., *Lolium perenne* L., *Phleum pratense* L. and *Poa trivialis* L. On D, further dominant species were *Crepis biennis* L., *Taraxacum Sec. ruderalia* Kirschner, H. Ollg. & Stepanek, and *Trifolium repens* L. (Seither, 2010). Both sward composition treatments were grazed either by sheep (S) or cattle (C). Grazing animals were suckler cows and calves of the breed German Simmental, and ewes and lambs of Blackheaded and Leine sheep in equal proportions. The average target animal live weight per paddock was 3000 kg (i.e. six livestock units of 500 kg each) in the first rotation and was reduced to an average target animal live weight per paddock of 2000 kg in the third rotation. The study site received only N inputs from direct excreta returned by livestock without additional fertilization. The experiment was grazed following a rotational scheme from spring to autumn (first, second and third rotation in 2008) and again in spring (fourth rotation 2009). Cattle and sheep stayed in one block for approximately 14 days at the beginning of the grazing season (first, second and fourth rotation), which was reduced to about seven days at the end (third rotation), because less vegetation biomass was available. No supplementary feeding took place while the animals were on the plots. In July, livestock was removed from the experiment for mating for six weeks. We conducted measurements of N oxide emissions, soil mineral N and WFPS shortly before and after grazing, following grazing periods from May to September 2008 and additionally for N_2O emissions in May 2009 as explained below. Further investigations were done in early spring 2009 to catch a possible peak in N_2O emissions during thawing, which we could not detect, however. Temperature and precipitation rate for the main experiment are shown in Figure 2.1 a.

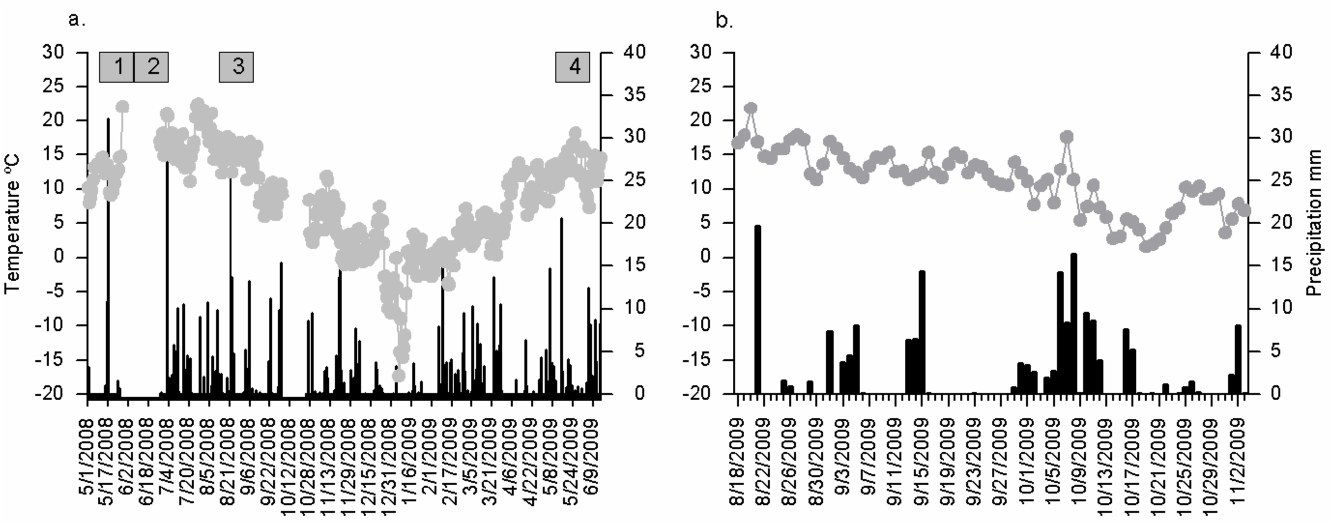


Figure 2.1 Daily air temperature in °C (●) and precipitation rate in mm in black bars of a. the main experiment in 2008 and 2009 (1, 2, 3 and 4 showed the four grazing rotations) and b. the controlled application experiment in 2009 at a grassland site in the Solling uplands, Germany. Between May 29th and June 26th, and the October 3rd and October 26th 2008 the climate stations return no values.

2.3.3 EXPERIMENTAL SETUP OF THE CONTROLLED APPLICATION EXPERIMENT

In addition to the main experiment, we conducted a controlled application experiment on four exclosures of 4 x 7 m^2 established in July 2009 on the pastures described above. Within these exclosures, we applied urine and dung inside permanent chamber bases used for gas flux measurements: urine applied on grass sward/cattle grazed (GC-U), grass sward/sheep grazed (GS-U), diverse/cattle grazed (DC-U), diverse/sheep grazed (DS-U) and dung applied on grass sward/cattle grazed (GC-D), grass sward/sheep grazed (GS-D), diverse/cattle grazed (DC-D) and diverse/sheep grazed (DS-D). Furthermore, we established on GS and DS control exclosures without excreta application. Cattle urine was collected seven days before application from dairy cattle (German Simmental and German Holstein) at a dairy farm (tiestall and extensive pasture). Sheep urine was collected five days before application from the Blackheaded and Leine sheep of the experiment. All samples were kept frozen at -18°C until used. Fresh dung was collected from the paddocks on the day of application, pooled, mixed and applied within two hours of collection. We applied the amount of one urination or defecation within one chamber base. According to own observations and published work (Flessa et al., 1996), a cow produces around 1.5 L urination and 1.5 kg defecation per event, corresponding to 18 g urine-N 1.5 L^{-1} and 6 g dung-N 1.5 kg^{-1} (Table 2.1). A sheep produces on average 70 ml urination and 60 g defecation per event (Ma et al., 2006), corresponding to 0.3 g urine-N 70 ml^{-1} and 1 g dung-N 60 g^{-1} (Table 2.1). Urine-N was determined using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands) with a dilution of 1:100 to scale down large NO_3^- and NH_4^+ peaks. NH_4^+ was determined using the Berthelot reaction method (Skalar Method 155-000) and NO_3^- was measured using the copper-cadmium reduction method (NH_4Cl buffer without ethylenediamine tetraacetic acid; Skalar Method 461-000). Dung-N was determined after air-drying on a C/N elemental analyzer (Vario MAX CN, Elementar, Hanau, Germany). The dry mass of dung was determined after air-drying to constant mass. Temperature and precipitation rate for the controlled application experiment are shown in Figure 2.1 b.

Table 2.1 Set up of the controlled application experiment showing N-concentration, applied N per chamber base and dry matter content at a grassland site in the Solling uplands, Germany. For further explanations, see text.

	Cattle-urine	Sheep-urine	Cattle-dung	Sheep-dung
N concentration (g N L^{-1})	12.2	3.88	19.4	16.7
N applied (g N $chamber^{-1}$)	18.27	0.27	6.02	0.99
Dry matter content (%)	-	-	20.7	99.2

2.3.4 SOIL ANALYSES

Soils were sampled in spring 2008 before the start of the grazing period and in summer 2009. At five sampling points per paddock, mineral soils (0-0.1 m depth) were sampled, oven-dried (40°C) for a week and sieved (2 mm). Soil texture was determined by sedimentary fractionation of the clay fraction (25°C, 21 h, 0.3 m fall height) following the Atterberg method after separation of sand and silt fraction (630 µm, 200 µm, 63 µm, 36 µm and 20 µm) by wet sieving and destruction of organic matter with 30% H_2O_2 and Fe oxides with 4% Na-dithionite-citrate solution (Schlichting et al., 1995). Soil bulk density was determined in summer 2009 by the soil core method (Blake and Hartge, 1986), assuming a particle density of 2.65 g cm^{-3} for mineral soil (Linn and Doran, 1984). Soil pH was measured as pH$_{(H2O)}$. Soil samples were ground for analysis of total C and N on a C/N elemental analyzer (Vario EL III, Elementar, Hanau, Germany). Soil characteristics are summarized in Table 2.2.

Table 2.2 Soil characteristics in the Ah horizon (0-0.1 m) of the treatments at a grassland site in the Solling uplands, Germany, sampled in spring 2008 and summer 2009.

	DC	DS	GC	GS	All paddocks
Sand (%)					6.8 ± 0.2
Silt (%)					54.4 ± 1.5
Clay (%)					38.8 ± 1.4
Bulk density (g cm^{-3}) *	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	
pH (H_2O) *	6.8 ± 0.1	6.4 ± 0.2	6.5 ± 0.1	6.8 ± 0.1	
Total carbon (C) (mg C g^{-1}) *	36.7 ± 2.4	32.6 ± 4.7	38.2 ± 9.1	34.3 ± 3.8	
Total nitrogen (N) (mg N g^{-1}) *	3.3 ± 0.2	3.3 ± 0.4	3.8 ± 0.8	3.3 ± 0.3	
C/N *	12.7 ± 0.6	11.5 ± 0.5	11.5 ± 0.4	12 ± 0.3	

DC: diverse/cattle grazed; DS: diverse/sheep grazed; GC: grass sward/ cattle grazed; GS: grass sward/sheep grazed

* Means (± SE, n=5) did not differ between treatments.

Soil chemical data were provided by the Department of Plant Ecology, University of Göttingen.

2.3.5 N OXIDE MEASUREMENTS

We used the closed chamber method for N_2O flux measurements. In the main experiment, we inserted randomly three polyvinyl chloride (PVC) chamber bases (0.04 m^2 area, 0.35-0.4 m height of chamber base and cover) in each paddock 0.02 m deep into the soil. For each measurement, the chamber base was covered with a PVC chamber hood fitted with a vent and an air sample port. Following chamber closure, gas samples were taken at 0, 12, 24, and 36 minutes and stored in pre-evacuated glass containers (100 ml) with teflon-coated stopcocks. N_2O was analyzed using a gas chromatograph (GC 6000, Carlo Erba Instruments/Thermo Fisher Scientific, Milan, Italy) equipped with an electron capture detector and an autosampler system (Loftfield et al., 1997). Gas concentrations were calculated by comparing integrated peak areas of samples with three standard gases (353, 1005, and 1592 ppb N_2O; Deuste Steiniger GmbH, Mühlhausen, Germany). NO was quantified using the same three chamber bases as for N_2O measurements with the open dynamic chamber method. Chambers were closed for five minutes and NO was quantified with a Scintrex LMA-3 NO_2 Analyzer with chemiluminescence detector (Scintrex, Ontario, Canada) following oxidation by a CrO_3 catalyst and reaction with a Luminol solution. The flow rate through the chamber was approximately 1.5 L min^{-1}. The detector was calibrated using a standard gas (3000 ppb NO; Deuste Steiniger GmbH, Mühlhausen, Germany) diluted with make-up air to the range of concentrations measured in the field. Detector signal and time were recorded every ten seconds with a CR800 series data logger (Campbell Scientific, Utah, USA). We calculated N-oxide emissions (µg N m^{-2} h^{-1}) from the linear increase of gas concentration versus time for each chamber, corrected with air temperature, chamber volume and air pressure (Ruser et al., 1998). Cumulative N oxide rates (kg N ha^{-1} $139d^{-1}$) were calculated by linear interpolation of the average N_2O and NO emissions between the measurements and adding the results over the total time period.

In the controlled application experiment, we installed per exclosure three PVC chamber bases (0.07 m^2 area, 0.35-0.4 m height) three weeks before the first measurements. Chamber bases remained at the same location for the duration of the experiment. One pre-treatment gas flux measurement was conducted on the day of application, prior to applying dung and urine. Immediately following application, another N_2O flux measurement was done. Subsequent measurements were conducted daily during the first three days of the experiment. This was followed by two measurements per week and finally, during the last four weeks, weekly measurements. Gas sampling (taken at 0, 18, 36 and 54 minutes after chamber closure) and N_2O analyses were conducted as described above. Cumulative excreta-derived N_2O emissions (kg $N_2O\text{-}N_{cum}$ m^{-2} $77d^{-1}$) were calculated by linear interpolation of the average N_2O emissions ($N_2O\text{-}N_{emitted} - N_2O\text{-}N_{control}$ in µg N m^{-2} h^{-1}) between the measurements and adding the results over the total time period. Emission factors (EF) were then calculated with Eq. 1, by using the arithmetic mean per treatment of the accumulated N_2O emissions over the experimental time of 77 days.

$$EF\% = [(N_2O\text{-}N_{emitted} - N_2O\text{-}N_{control}) / N\ applied] * 100 \qquad (Eq.\ 1)$$

Combining the data of the main and the controlled application experiment, we performed further calculations for the N input (kg N $paddock^{-1}$ d^{-1}) multiplying the number of cattle or sheep in a paddock with the frequency of urination or defecation per day, the amount and the N concentration of urine or dung per urination or defecation. The climatic conditions of both experiments were similar (Figure 2.1). For the duration of the controlled application experiment (77 days), the mean temperature was 10.7°C in 2008 and 11.2°C in 2009, and the precipitation rate was 187.0 mm in 2008 and 178.5 mm in 2009. The missing values for temperature and precipitation in 2008 were completed by a climate station nearby (Deutscher Wetterdienst). According to Williams and Haynes (1994), cattle urinate on average up to 11 times per day, while sheep urinate more frequently (18-20 times per day). Measurements of the soil surface area covered by urine (wetted area; measured by distribution of Br^- as a urine tracer) range from 0.16 to 0.49 m^2 for cattle and 0.03 to 0.05 m^2 for sheep. Cattle excreta also cover a larger area than sheep excreta: The surface area of cattle dung patches is about 0.05-0.09 m^2, while that of sheep dung patches is about 0.008-0.025 m^2 (Haynes and Williams, 1993; Williams and Haynes, 1994). For further calculations, we considered the mean value of all ranges for frequency and amount of urination and defacation as the basis for the upscaling on paddock size. Excreta-derived N_2O emissions (kg $N_2O\text{-}N_{emitt}$ $paddock^{-1}$ d^{-1}) were determined by using the EF per treatment multiplied with the calculated N input (kg N $paddock^{-1}$ d^{-1}) on the paddock scale. Assuming an even and non-overlapping distribution of the single urine and dung patches, the area covered by excreta (m^2 $paddock^{-1}$ d^{-1}) was calculated by multiplying the number of cattle or sheep in a paddock with the frequency of urinations or defecations and the size of a urine or dung patch.

2.3.6 SOIL MINERAL N

We sampled soil cores (diameter 2.2 cm; 0.1 m depth) at three points inside the chamber bases for the main experiment and at three supplementary patches each with either dung or urine application outside the chambers for the controlled application experiment. For the main experiment, soil for mineral N extraction was sampled during each N oxide measurement and for the controlled application experiment during every second measurement. Soil mineral N was determined from fresh soil samples (50-60 g) that were added to a 0.5 mol L^{-1} K_2SO_4 solution directly in the field and extracted after shaking for one hour in the laboratory. Soil extracts were filtered (Whatman 589/1) and stored at -18°C in scintillation bottles. NH_4^+ and NO_3^- were measured using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, Netherlands) as described before. Gravimetric moisture content was determined after drying at 105°C for 24 hours. Soil moisture content was expressed as WFPS.

2.3.7 STATISTICAL ANALYSIS

For the main experiment, statistical analyses were carried out on N oxide emissions averaged over the three chambers per paddock. To test the effects of grazer and plant species composition in time series data (N oxide emissions and soil mineral N from rotational grazing), we conducted a linear mixed effects model (LME) with grazer and plant species composition as fixed effects and date and block as random effects. N oxide emissions and soil mineral N were square root transformed. As negative N oxide emissions were detected, we added a constant prior to square root transformations for statistics. To test for differences between treatments of distinct plant species composition or of different species of grazer on cumulative N oxide emissions, we used a T test. To test the effects of continuous variables (soil mineral N, WFPS, temperature) on the log-transformed N_2O/NO ratio, multiple linear regression analysis were carried out.

On the controlled application experiment, we were not able to measure true replicates. With a model of nested temporal pseudoreplicates, the temporal dynamics of the experiment was included into the analysis. As we assumed that “time” could have a non-linear effect on the development of N_2O emissions, time was included into the analysis as a linear effect. We applied an LME with log-transformed N_2O emissions and soil mineral N (representing the response variable). The basic model included the experimental treatments (plant species composition, grazer, type of manure and day) as fixed effects and day and subplot as random effects.

We used LME for time series data, because they account for temporal correlation among observations on the same experimental unit (Piepho et al., 2004). For all LME-models, autocorrelation effects were tested before analysis or by modelling heteroscedasticity of residual variance by including variance function (Bliese and Ployhart, 2002). If these measures improved the relative goodness of the model fit (based on the Akaike Information Criterion), the adjustments were included into the model for further analyses (Crawley, 2007), followed by stepwise model simplification. Mean values in the text are given with standard errors. Effects were accepted as statistically significant if $P \leq 0.05$. Analyses were conducted using R version 2.11.1 (R Development Core Team, 2009).

2.4 RESULTS

2.4.1 N OXIDE EMISSIONS AND SOIL CONTROLS OF THE MAIN EXPERIMENT

As we found no differences in N oxide emissions before and after grazing, we averaged the data for N_2O or NO emissions. Over the whole experimental period, grazers and plant species composition did not significantly affect mean N oxide emissions (Table 2.3). Over the grazing season (139 days), cumulative (± standard error) N_2O-N emissions (0.6 ± 0.07 kg N_2O-N ha^{-1}) exceeded NO-N emissions (0.16 ± 0.06 kg NO-N ha^{-1}) in all treatments. There were no significant differences among treatments for cumulative N_2O emissions. Differences in cumulative NO-N emissions were found between treatments GC and GS (P = 0.022), with larger NO-N emissions on GS treatments (Table 2.3). Despite the significance, the differences were very small. Rotation also affected NO emissions: NO emissions were larger during the second grazing rotation compared to the third (with net NO uptake; $P < 0.001$; Figure 2.2 a). Average N_2O emissions were small during the first two grazing rotations but increased in the last rotation 2008 and the first 2009 ($P < 0.001$; Figure 2.2 b).

Table 2.3 Mean N oxide emissions (in µg N m^{-2} h^{-1}) and cumulative N oxide emissions (in kg N ha^{-1} and grazing season of 139 d^{-1}) in the treatments at a grassland site in the Solling uplands, Germany.

Mean*	DC	DS	GC	GS
NO-N	2.78 ± 5.1	3.98 ± 2.9	1.37 ± 3.0	1.36 ± 3.17
N_2O-N	42.9 ± 9.4	55.3 ± 26.4	28.2 ± 11.7	28.4 ± 11.6
Cumulative§				
NO-N	0.31 ± 0.16	0.19 ± 0.15	0.03 ± 0.01a	0.13 ± 0.02b
N_2O-N	0.51 ± 0.13	0.79 ± 0.13	0.65 ± 0.16	0.43 ± 0.05
NO-N+N_2O-N	0.82 ± 0.10	0.98 ± 0.30	0.68 ± 0.31	0.56 ± 0.15

DC: diverse/cattle grazed; DS: diverse/sheep grazed; GC: grass sward/cattle grazed; GS: grass sward/sheep grazed

[*] Means (±SE, n=6) did not differ between treatments (Linear mixed effects models, $P \leq 0.05$).

[§] Means (±SE, n=3) within rows followed by different letters (small letters) indicate significant differences between the treatments; compared were D versus G within grazer or C versus S within sward type (T test, $P \leq 0.05$).

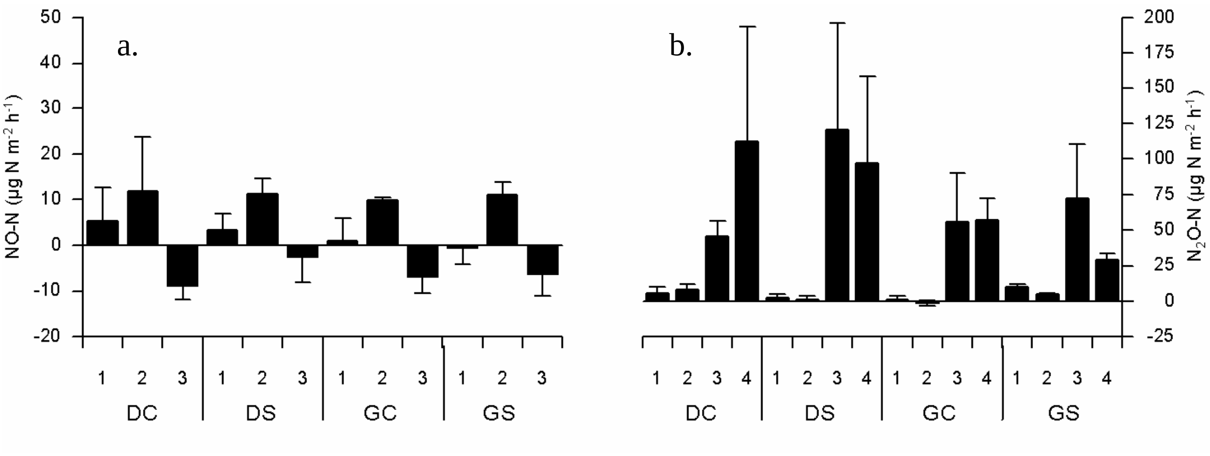


Figure 2.2 Mean (± SE, n=6) N oxide emissions in µg N m^{-2} h^{-1} in the treatments (DC: diverse/cattle grazed; DS: diverse/sheep grazed; GC: grass sward/ cattle grazed; GS: grass sward/sheep grazed) at a grassland site in the Solling uplands, Germany. a. NO-N and b. N_2O-N measurements were calculated from measurements before and after grazing and taken for three and for four grazing rotations, respectively. The fourth rotation was the first one 2009 while the others were in 2008. Please note different scales of the y-axes.

NH_4^+ was the dominant form of soil mineral N in all treatments and did not differ significantly among treatments. Contrary to N_2O emissions, soil NH_4^+ increased during the first two grazing rotations and decreased by a factor two during the last two grazing rotations ($P < 0.001$; Figure 2.3 a). Soil mineral NO_3^- was larger during rotations two and four compared to the others ($P = 0.05$). Plant species composition affected soil mineral NO_3^- ($P = 0.034$), with larger concentration in grass than in diverse swards (Figure 2.3 b). The ratio of N_2O/NO was linearly correlated to WFPS, air temperature and NH_4^+ concentration ($R^2 = 0.60$).

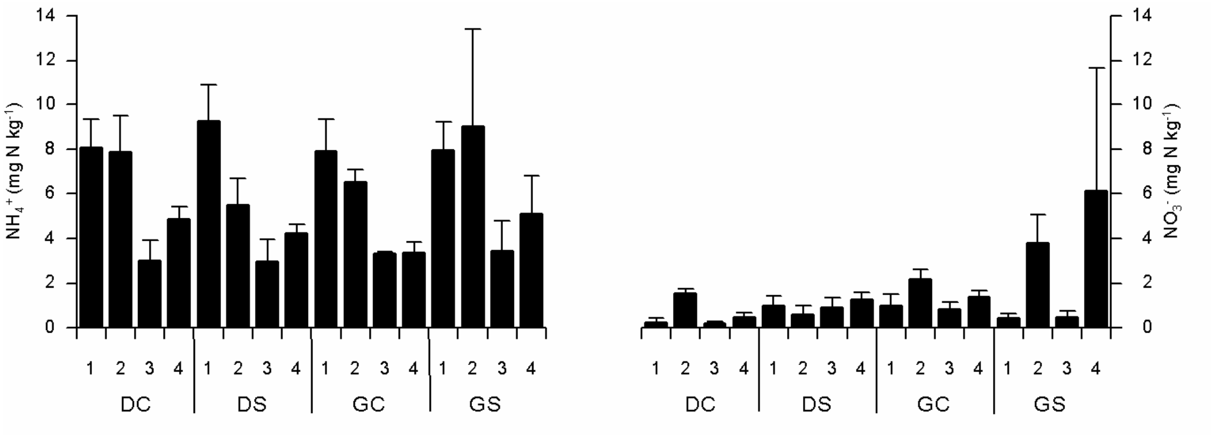


Figure 2.3 Mean (± SE, n=6) soil mineral N in mg N kg^{-1} in the treatments (DC: diverse/cattle grazed; DS: diverse/sheep grazed; GC: grass sward/ cattle grazed; GS: grass sward/sheep grazed) at a grassland site in the Solling uplands, Germany. a. NH_4^+ and b. NO_3^- concentrations were calculated from measurements before and after grazing. Shown are data for the four grazing rotations, three in 2008 and one in 2009.

2.4.2 N_2O EMISSIONS AND SOIL CONTROLS OF THE CONTROLLED APPLICATION EXPERIMENT

In the short-term controlled application experiment, patches treated with cattle urine had a 13-fold N_2O-N flux of those with sheep urine, while plots treated with cattle dung showed a 3-fold N_2O-N flux of those with sheep dung. The effects of species of grazer (P = 0.002), type of manure (P = 0.002) and the interaction between grazer species and manure (P = 0.009) on N_2O emissions were significant, while plant species composition was not (Figure 2.4). N_2O emissions of DC-U rose to a peak of 1921 µg N_2O-N m^{-2} h^{-1} immediately after the application of urine, peaking again at day 6 and 22, but decreased to the level of the control plot (6.3 µg N_2O-N m^{-2} h^{-1}) thereafter. In contrast to that, the N_2O emissions of GC-U increased slowly until day 9 (527 µg N_2O-N m^{-2} h^{-1}), with largest peaks only appearing at day 18, 24 and 51 after application (Figure 2.4 a). After application of sheep urine, N_2O emissions of GS-U and DS-U showed distinct developments: N_2O emissions increased immediately up to 556 µg N_2O-N m^{-2} h^{-1} (GS-U) and 434 µg N_2O-N m^{-2} h^{-1} (DS-U) upon application but decreased rapidly thereafter (Figure 2.4 b). After dung application, N_2O emissions of DC-D and GC-D increased steadily over the first days after application, with peaks at day 6 (145.8 µg N_2O-N m^{-2} h^{-1}) and day 11 (98 µg N_2O-N m^{-2} h^{-1}; Figure 2.4 c).

N_2O emissions of DS-D peaked with a slight time-delay at day 30 (104 µg N_2O-N m^{-2} h^{-1}). Plots of GS-D showed smallest N_2O emissions compared to all other treatments. N_2O emissions over the experimental period peaked here at day 22 (35.4 µg N_2O-N m^{-2} h^{-1}; Figure 2.4 d).

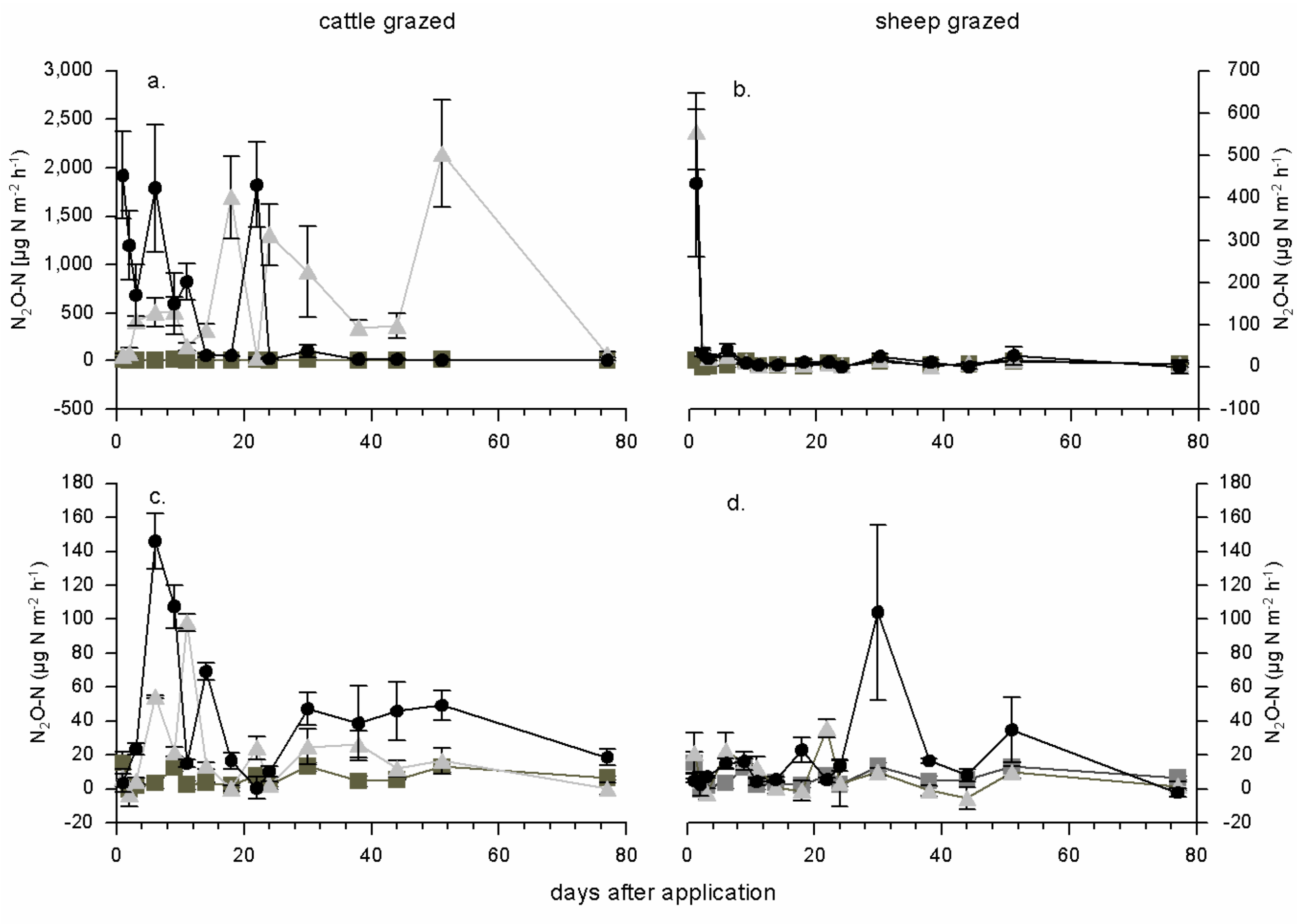


Figure 2.4 N_2O-N emissions (± SE, n=3) in µg N m^{-2} h^{-1} of the controlled application experiment (period of 77 d^{-1}) in diverse (●), grass (▲) and control (without excreta addition) (■) plots at a grassland site in the Solling uplands, Germany. Shown are results for urine-treated plots of a. cattle and b. sheep and dung-treated plots of c. cattle and d. sheep. Please note different scales of the y-axes.

The soil mineral NH_4^+ concentrations of plots treated with cattle and sheep excreta showed similar temporal dynamics, but – regarding urine treatments – at different levels: Cattle urine caused a 2-fold (diverse) to 4-fold (grass sward) increase in the concentration compared to plots treated with sheep urine (Figure 2.5 a-b). Cattle dung led to similar NH_4^+ concentrations as sheep dung, with no statistical difference to the controls (Figure 2.5 c-d). Grazer species ($P < 0.037$) and type of manure ($P < 0.001$) significantly affected NH_4^+ concentration.

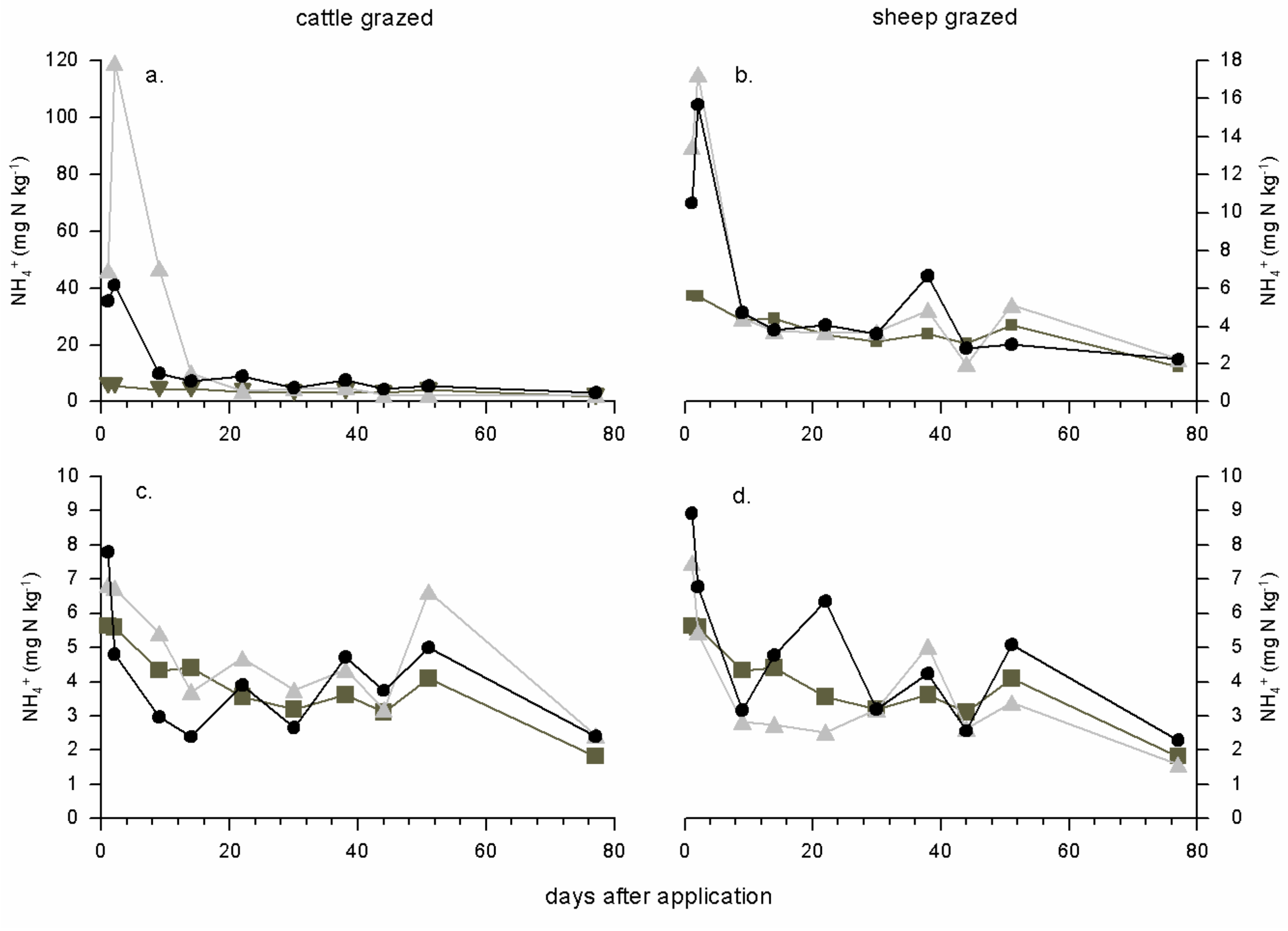


Figure 2.5 Soil mineral NH_4^+ in mg N kg^{-1} of the controlled application experiment (period of 77 d^{-1}) in diverse (●), grass sward (▲) and control (without excreta addition) (■) plots at a grassland site in the Solling uplands, Germany. Shown are results for urine-treated plots of a. cattle and b. sheep and dung-treated plots of c. cattle and d. sheep. Please note different scales of the y-axes.

Soil mineral NO_3^- concentrations of plots treated with cattle and sheep urine showed comparable dynamics to the NH_4^+ concentrations. Plots treated with cattle urine showed a 3-fold (diverse) and 5-fold (grass sward) increase in the concentration compared with plots treated with sheep urine (Figure 2.6 a-b). Plots with cattle dung again had similar concentrations as those with sheep dung (for both grass and diverse swards), with no significant differences to the controls (Figure 2.6 c-d). Different influences of urine- and dung-treatments and cattle and sheep excreta on soil NO_3^- concentrations were only detected by the significant influence of day on NO_3^- concentrations in soils ($P < 0.049$).

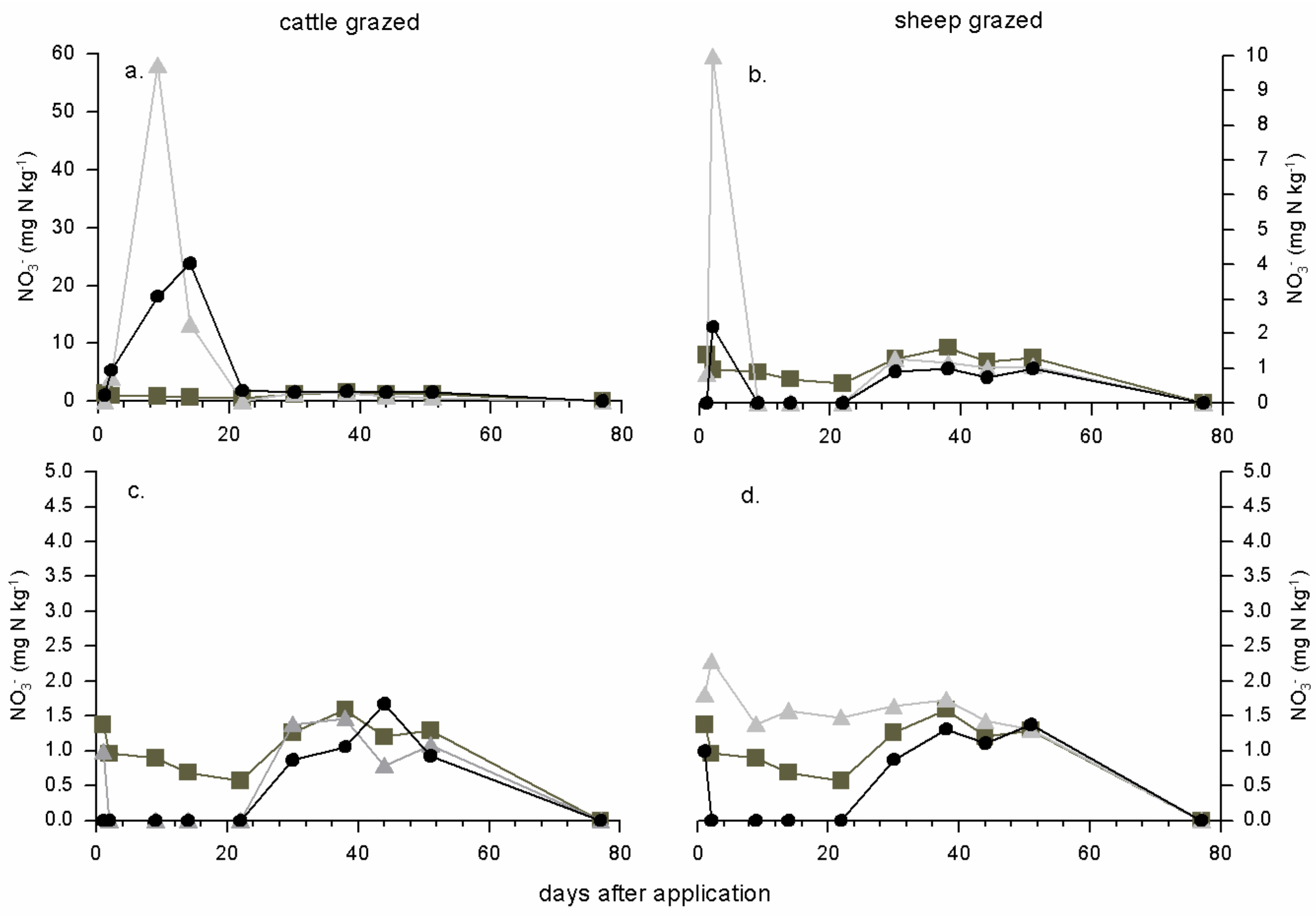


Figure 2.6 Soil mineral NO_3^- in mg N kg^{-1} of the controlled application experiment (period of 77 d^{-1}) in diverse (●), grass sward (▲) and control (without excreta addition) (■) plots at a grassland site in the Solling uplands, Germany. Shown are results for urine-treated plots of a. cattle and b. sheep and dung-treated plots of c. cattle and d. sheep. Please note different scales of the y-axes.

2.4.3 EMISSION FACTORS AND CALCULATION OF EXCRETA N INPUT AND N_2O-LOSSES ON THE PADDOCK SCALE

Over the controlled application experiment (77 days), cumulative excreta-derived N_2O-N emissions (± standard error) of plots treated with cattle urine (1.03 ± 0.55 g N_2O-N m^{-2}) exceeded those of plots treated with sheep urine (0.02 ± 0.002 g N_2O-N m^{-2}), the same applied for cattle dung (0.04 ± 0.02 g N_2O-N m^{-2}) and sheep dung (0.01 ± 0.01 g N_2O-N m^{-2}) (Table 2.4). In line with the larger N-input with cattle excreta, the emission factor was smaller for cattle than for sheep urine (0.39% and 0.48%, respectively). Emission factors for dung were also slightly smaller for cattle (0.05%) than for sheep (0.09%; Table 2.4).

Table 2.4 N_2O-N emissions and emission factors (EF) in the controlled application experiment and calculated N input with excreta (amount and area covered) and N_2O losses in the main experiment at a grassland site in the Solling uplands, Germany. For further explanations, see text.

Controlled application experiment	Cattle-urine	Sheep-urine	Cattle-dung	Sheep-dung
N_2O-N_{cum} (g m^{-2} $77d^{-1}$)*	1.03 ± 0.55	0.02 ± 0.00	0.04 ± 0.02	0.01 ± 0.01
EF (%)*	0.39 ± 0.21	0.48 ± 0.05	0.05 ± 0.03	0.09 ± 0.11
Main experiment				
N input (kg N $paddock^{-1}$ d^{-1})	1.81	0.28	1.06	0.07
Area covered by excreta (m^2 $paddock^{-1}$ d^{-1})	32.2	41.0	8.2	16.9
N_2O-N_{emitt} (kg $paddock^{-1}$ d^{-1})	0.71	0.13	0.05	0.01

[*] Means (±SE, n=2) were calculated from diverse and grass sward. Statistical analyses were not possible.

On a paddock scale (0.5 ha), the calculated N-input with cattle urine (1.81 g N m^{-2}) exceeded that with sheep urine (0.28 g N m^{-2}); the same applied for cattle dung (1.06 g N m^{-2}) and sheep dung (0.07 g N m^{-2}) (Table 2.4). Based on the emission factors calculated above, the urine-derived N_2O-N emissions were larger for cattle- than for sheep-grazed paddocks (0.71 kg N_2O-N d^{-1} and 0.13 kg N_2O-N d^{-1}, respectively). Dung-derived N_2O-N emissions were also larger in cattle-grazed than sheep-grazed paddocks (0.05 kg N_2O-N d^{-1} and 0.01 kg N_2O-N d^{-1}, respectively; Table 2.4). However, the area covered by excreta per paddock was smaller for cattle than for sheep: 32.2 m^2 d^{-1} were calculated to be covered with cattle urine, 41 m^2 d^{-1} with sheep urine, and an additional 8.2 m^2 d^{-1} with cattle dung and 16.9 m^2 d^{-1} with sheep dung (Table 2.4).

2.5 DISCUSSION

2.5.1 GRAZER EFFECTS ON N OXIDE EMISSIONS

The hypothesized larger N oxide losses on sheep-grazed paddocks compared with those grazed by cattle could only be confirmed for cumulative NO emissions in grass swards in the main experiment (Table 2.3). The observed effects for N oxide emissions in the other treatments were not consistent. N oxide losses are linked with the N-supply in the soil, which on pastures depends on the N applied with excreta. In the controlled application experiment, our finding that N_2O emissions from cattle excrement-treated plots were larger than from sheep excrement-treated plots was consistent with results of Saggar et al. (2007), who reported plots treated with cattle excreta to have an 8-fold increase and sheep excreta to result in a 2-fold increase in N_2O emissions compared to untreated plots. The aim of our experiments was to use maximum N_2O emissions of the controlled application experiment as an estimate for N_2O emissions of the main experiment. Beside available N, also soil aeration status and temperature control denitrification and nitrification processes in the soil (Keeney et al., 1979; Linn and Doran, 1984). We checked climate conditions for the duration of the controlled application experiment (77 days) of both years. Mean temperature was 10.7°C in 2008 and 11.2°C in 2009, and precipitation rate was 187.0 mm in 2008 and 178.5 mm in 2009. Thus, we carefully combined both experiments using the EF derived from the controlled application experiment and the calculated N input per paddock and day. We calculated expected N_2O emissions per paddock that were larger for cattle- than for sheep-grazing (Table 2.4). Thus, the amount of N per excretion cannot explain the missing effect of cattle and sheep on N oxide emissions in the main experiment.

In the main experiment, measured N oxide losses were dominated by the occasional measurement of urine and dung hotspots. Our first hypothesis was based on the amount and frequency of urinations and defecations and the number of cattle and sheep per plot, which led to a larger cover with excrements on sheep- than on cattle-grazed plots (1.16% of the paddock area per day for sheep grazing, 0.81% for cattle grazing; Table 2.4) and should thus lead to a larger probability of measuring on hotspots in sheep- than in cattle-grazed pastures. Other studies also concluded that the most apparent reason for differences in N_2O losses of cattle and sheep excrements are differing quantities of excrements covering the soil and different nutrient concentrations of the excrements (Ma et al., 2006; Williams and Haynes, 1994). The cover with excreta influences the aeration and the nutrient supply in the soil, and should in turn affect the rate of N_2O emissions. This should lead to larger emissions from sheep- than from cattle-grazed paddocks, which we did not observe. This could be due to the large spatial and temporal variability in emissions.

We detected larger N_2O losses of urine patches than in dung patches. Urine patches of both sheep and cattle had larger emissions than dung patches. Due to freeze-thaw events, N_2O emissions of dung-treated plots measured by Wachendorf et al. (2008) were above those of urine-treated plots, whereas studies of Yamulki et al. (2000) reported larger emissions from urine patches than from dung patches. The typical development of N_2O emissions after application of urine observed in numerous studies is a rapid increase of N_2O emissions with a successive decline thereafter, as urinary N is rapidly hydrolyzed and nitrified in pasture soils (Flessa et al., 1996; Saggar et al., 2007). Our study showed two different developments in N_2O emissions of plots treated with cattle urine, depending on the plant species composition (Figure 2.4). There are studies yielding similar results for both developments: maximum N_2O emissions appearing 12-24 h after application were e.g. measured by Sherlock and Goh (1983) and Williams et al. (1999). Studies noting a delayed maximum N_2O flux after application of urine, showing slow increases of up to 5-14 days after application as observed in our grass plots, were reported by Lovell and Jarvis (1996) or Koops et al. (1997). The observed effect of a delayed increase in N_2O emissions in the grass swards were inconsistent with results of soil mineral N, which did not show a delayed peak. Soil mineral NH_4^+ und NO_3^- were 3-fold and 2-fold in the grass swards compared to diverse plots. However, soil mineral N concentrations in all treatments were the same as in the control plots when the N_2O peaks appeared at day 18, 24 and 51 after application. The potentially higher nutrient demand of *Lolium perenne* and *Dactylis glomerata* compared to herbs or legumes (Mattsson et al., 2009) or a more efficient N use in diverse swards (Scherer-Lorenzen et al., 2003; Spehn et al., 2002) could not explain these delayed N_2O emissions in the grass paddocks. However, we observed that some of the plants in the grass sward died off after the cattle urine application, which may have played a role in the delayed N_2O peaks that we observed.

2.5.2 EFFECTS OF SWARD COMPOSITION ON N OXIDE EMISSIONS

The observed results for N oxide emissions were not consistent with our hypothesis that more diverse paddocks would have smaller N oxide emissions than grass swards. Mattsson et al. (2009) argued that high productive grass species such as *Lolium perenne* and *Dactylis glomerata* have a higher demand for nutrients from soils than other grass species, herbs or legumes. Legumes may lead to an accumulation of inorganic N in soils as they symbiotically fix atmospheric N_2, but they are generally inefficient in capturing soil extractable N. Furthermore, they may release symbiotically fixed N into the soil through turnover of root and shoot tissue, which may lead to an accumulation of NO_3^- as the substrate for denitrification in soils (Hooper and Vitousek, 1997; Niklaus et al., 2006). Legumes can therefore decrease the efficiency of capturing mineral N that normally results from species diverse grassland swards (Niklaus et al., 2006). However, these studies were not consistent with our observed findings of larger NO_3^- concentrations in grass than in diverse swards despite similar N oxide losses. Our findings were supported by the results of N yield. Regarding plant species composition no significant differences were found for N yield (Seither et al., 2012). Further research is necessary to better understand influences of plant species composition on N dynamics in permanent agriculturally managed grassland.

2.6 CONCLUSIONS

Combining the data from the two experiments, we conclude that plant species composition was insignificant compared to the effect of grazers on N oxide emissions. The response of N oxide emissions on the paddock scale were larger for sheep- compared to cattle-grazing, despite larger emissions per cattle excretion due to the larger amounts of N per excretion event. We attributed this to a presumably larger paddock surface area covered by sheep than by cattle excreta.

CHAPTER

3

NITROGEN RESPONSE EFFICIENCY OF A MANAGED AND PHYTODIVERSE TEMPERATE GRASSLAND

3.1 ABSTRACT

Aims Our goal was to assess how management and sward functional diversity affect nitrogen response efficiency (NRE), the ratio of plant biomass production to supply of available nitrogen (N) in temperate grassland.

Methods A three-factorial design was employed: three sward compositions, two mowing frequencies, and two fertilization treatments.

Results NRE was largely influenced by fertilization followed by mowing frequency and sward composition. NRE was larger in unfertilized than fertilized plots, in plots cut thrice than plots cut once per year, and in control swards than in monocot- or dicot enhanced swards. Fertilization decreased NRE through decreases in both N uptake efficiency (plant N uptake per supply of available N) and N use efficiency (NUE, biomass produced per plant N uptake) whereas mowing frequency and sward composition affected NRE through N uptake efficiency rather than NUE. The largest NRE in the control sward with 70% monocots and 30% dicots attests that these proportions of functional groups were best adapted in this grassland ecosystem.

Conclusions Optimum NRE may not be a target of most farmers, but it is an appropriate tool to evaluate the consequences of grassland management practices, which farmers may employ to maximize profit, on environmental quality.

Keywords: N uptake efficiency, N use efficiency, net N mineralization rates, functional diversity

3.2 INTRODUCTION

Nutrient response efficiency, the amount of biomass produced per unit of plant-available nutrients (Pastor and Bridgham, 1999), is a measure of ecosystem functioning that integrates productivity and the retention of nutrients (Hiremath and Ewel, 2001). In terrestrial ecosystems where nitrogen (N) is limiting net primary productivity (Vitousek and Howarth, 1991), the efficient use of available N may give communities a competitive advantage over communities that are less efficient in using N (Bridgham et al., 1995). There is no common agreement about the definitions of nitrogen response efficiency (NRE) and nitrogen use efficiency (NUE) in literature (Aerts, 1990; Vitousek, 1982; Xu et al., 2012). In our study, NRE is calculated as the product of N uptake efficiency (defined as plant N concentration x plant biomass ÷ soil available N) and NUE (defined as plant biomass ÷ plant N concentration x plant biomass), two indices that are commonly used to evaluate the role of N in ecosystem productivity (Pastor and Bridgham, 1999). However, both indices give an incomplete picture of how N availability affects biomass production. A large NUE does not necessarily reflect a large productivity because NUE can increase or decrease if the plant N concentration changes even if plant biomass stays the same. NUE is also often not well correlated with soil N availability (Iversen et al., 2010). Similarly, N uptake efficiency does not directly indicate productivity as it is influenced by changes in plant N concentration. In contrast, NRE is an index that reflects the ability of plants to acquire N from the soil and to use it for biomass production once it is taken up (Bridgham et al., 1995), and thus it is a better index if biomass production is the main goal (as is the case in most temperate grasslands). Since a large NRE indicates a large biomass production per unit of soil available N and thus a large N retention in plants, it is inversely correlated with N lost, e.g. through nitrate (NO_3^-) leaching and gaseous N emissions. A large NRE may thus contribute to the reduction of reactive N in the soil, which affects water and air pollution as well as the emission of greenhouse gases (Dobermann, 2005).

Pastor and Bridgham (1999) developed a general model of NRE showing maximum efficiency at intermediate levels of N availability. In their model, productivity ($P_{N\ supply}$) at a given level of available N (i.e. N supply in the environment) is defined as:

$$P_{N\ supply} = N\ supply * (P \div N\ supply) + 0 \qquad \text{(Eq. 2)}$$

In this model, NRE is the slope of a line from the origin to a given point of the function that describes the relationship between productivity and N supply (Pastor and Bridgham, 1999). This implies that NRE changes with N supply and depends on the shape of the function in Eq. 2. If the relationship between productivity and N supply is linear, NRE is constant across a gradient of N supply. However, a linear relationship over a wide range of N supply is unlikely given the observation that no further increase in productivity occurs at high levels of N availability (Pastor and Bridgham, 1999). Studies that investigated the relationships between NRE and soil N availability found increases in NRE as soil N availability declined in an experimental plantation of tropical trees (Hiremath and Ewel, 2001; Yuan et al., 2006). Bridgham et al. (1995) gave a mathematical proof that nutrient response efficiency has to be unimodal across broad ranges of nutrient supply. However, the unimodal efficiency has not been observed for N so far and is debatable (Yuan et al., 2006). A monotonic increase of NRE with decreasing soil N availability was observed by Yuan et al. (2006) for Mongolian grassland.

NRE can be influenced by management practices and plant diversity through their effects on N availability, uptake efficiency and NUE. Management practices commonly employed in grasslands are fertilization and mowing. Fertilization directly influences N availability. Furthermore, mowing removes available N from the ecosystem through the mown biomass. In systems with large nutrient availability, mowing may mitigate the negative effects of nutrient enrichment on plant diversity by removing excess nutrients (Ellenberg and Leuschner, 2010; Pykälä, 2000). Mowing can also lead to a denser root system in the top soil by increasing the root-to-shoot-ratio and thus causing a more effective plant N uptake (Kammann et al., 1998; Mooney and Winner, 1991). In contrast, an increase in allocation of resources to the shoots (Guitian and Bardgett, 2000) as well as a decrease in root biomass following defoliation have been reported by other studies (Dawson et al. 2000; Holland and Detling, 1990). Mikola et al. (2009) also reported that even for grazed grasslands defoliation is the most important mechanism explaining grazing effects on plant attributes while excreta return play only a minor role. To date, there are no published studies on how N fertilization, mowing frequency and their interactions affects NRE of temperate grasslands, and thus the present study is the first to report such pattern.

Apart from direct effects, management practices may also affect NRE through a change in species composition of the sward. In the past six decades, European grasslands have undergone many changes in management (e.g. increased fertilizer input and mowing frequency, or abandonment from agricultural use) that had profound consequences for biodiversity (Isselstein et al., 2005). The impact of plant diversity on nutrient uptake, productivity and NRE has been subject of several studies. Hiremath and Ewel (2001) reported an increase in N uptake efficiency with increase in life-form diversity for a tropical tree plantation. Several studies showed that more diverse grasslands were more productive (e.g. Hector et al., 1999; Tilman et al., 1996; Weigelt et al., 2009) with an increased nutrient retention from more diverse grassland ecosystems (Tilman et al., 1996). Furthermore, van Ruijven and Berendse (2005) observed an increase in productivity and NUE with increasing species richness. These studies, however, were conducted in experimentally-established plots that were weeded intensively, or in microcosms, making them difficult to compare with results from permanent grasslands where no clear effect of biodiversity on productivity has yet been demonstrated (Wrage et al., 2011). This may be due to the larger species richness in permanent grassland compared to many studies where diversity effects were largest at species richness levels smaller than five species (Wrage et al., 2011). Although Flombaum and Sala (2008) reported a larger effect of plant biodiversity on productivity in a natural grassland ecosystem compared to artificial ecosystems, their study only included a biodiversity gradient of one to six species, which is still considerably lower than managed, temperate grasslands with 10 to 60 plant species (Wrage et al., 2011). So far no studies on the impact of management practices, sward composition and their interactions on NRE in agriculturally-managed, permanent grassland have been published. As the efficiency with which grasslands use available N for biomass production is the key link between management and biogeochemical N cycling, our goal was to evaluate how NRE changes as a function of different management practices and sward compositions in an agriculturally-managed, permanent grassland site. We measured NRE of a grassland site that was managed according to local practices in the Solling Mountains (Lower Saxony, Germany). Here, we only considered the harvestable, aboveground biomass because our study focuses on agricultural management practices. Plant functional group diversity was manipulated by herbicide treatments, resulting in dicot-enhanced swards with nearly equal proportions of dicots and monocots, control swards with ~70% monocots and ~30% dicots, and monocot-enhanced swards with ~90% monocots and ~10% dicots. Species richness was significantly smaller in the monocot-enhanced swards compared to the control swards (Petersen et al., 2012). N supply (used as the measure of N availability in the environment) was defined as the sum of soil net N mineralization rates during the growing season, N deposition from bulk precipitation, and fertilizer addition rates. We tested the following hypotheses: 1) unfertilized plots have larger NRE than fertilized plots, 2) plots with high mowing frequency have larger NRE than plots with low mowing frequency, and 3) NRE increases with increasing number of species (i.e. monocot-enhanced swards (12-13 species) < dicot-enhanced (17 species) and control swards (16-18 species)).

3.3 MATERIALS AND METHODS

3.3.1 STUDY SITE

This interdisciplinary research project, grassland management experiment or GRASSMAN, was conducted at the experimental farm of the University of Goettingen on a moderately species-rich grassland in the Solling Mountains in Lower Saxony, Germany (51°44'53"N, 9°32'42"E, 490 m above sea level). This permanent grassland site has traditionally been used as a pasture for hay making or for grazing (Geological Map of Prussia 1910 (based on the topographic inventory of 1896); topographic maps of Sievershausen and Neuhaus/Solling 1924, 1956 and 1974; Braunschweigische Landesaufnahme 18th century). In the last five decades, the study site was managed with moderate fertilization (80 kg N ha-1 yr-1), liming, overseeding with high value forage species and cattle grazing (farm records of Relliehausen since 1966). Fertilization stopped two years before our experiment started. Vegetation consists of a montane, semi-moist Lolio-Cynosuretum. Mean annual precipitation is 1028 mm and mean annual temperature is 6.9°C (Deutscher Wetterdienst, 1961 – 1990, station Holzminden-Silberborn, 440 m above sea level). During the study period, mean annual temperature and annual precipitation were 8.4°C and 1001 mm in 2009 and 8.0°C and 1110 mm in 2010. The dominating soil type is a Haplic Cambisol, developed on sediments of loess on the Middle Buntsandstein formation with a loamy silt texture.

3.3.2 EXPERIMENTAL SET UP

The study had a three-factorial design: three sward compositions, two mowing frequencies and two fertilization treatments. In June 2008, we established three sward compositions by applying a herbicide mixture against dicotyledons (Starane® (active ingredients: Fluoroxypyr and Triclopyr) and Duplosan KV (active ingredients: Mecoprop-P®)) resulting in a sward with reduced proportions of herbs and legumes, a herbicide mixture against monocotyledons (Select 240 EC® by Stähler (active ingredients: Clethodim)) resulting in a sward with reduced proportions of grasses, and an untreated control sward. Hereafter, we refer to these sward compositions as monocot-enhanced (12-13 species; 91-93% grasses, 7-9% herbs and 0% legumes) and dicot-enhanced (17 species; 40-47% grasses, 49-53% herbs and 4-9% legumes) relative to the control sward (16-18 species; 68-76% grasses, 21-31% herbs and 1-4% legumes) (Petersen et al., 2012). In 2008, all experimental plots were mown, and fertilized plots received 50 kg N ha^{-1}. The experiment started in spring 2009 with two mowing frequencies (once per year in July and thrice per year in May, July and September) as well as two fertilization treatments (180 – 30 – 100 kg NPK ha^{-1} yr^{-1} and no fertilization). The N fertilizer (calcium ammonium nitrate N27) was split into two equal applications per year (April and May 2009 and April and June 2010) while the combined P and K fertilizer was applied once a year (June 2009 and 2010). The experimental treatments were set up with 6 replicates in a full factorial design (72 plots; 15 m x 15 m each) arranged in a Latin rectangle.

3.3.3 SOIL CHARACTERISTICS

Soil characteristics (Table 3.1) were determined in spring 2008 (chemical and texture analyses) and summer 2009 (bulk density). At nine sampling points per plot, mineral soils (0.01-0.1 m depth) were sampled, pooled, oven-dried (40°C) for a week and sieved (2 mm). Particle size distribution was determined by wet sieving (>20 <630 µm) and pipette methods (≤20 µm) after pre-treatment with 30% H_2O_2 and 4% Na-dithionite-citrate solution to remove organic matter and iron oxides (Schlichting et al., 1995). Soil bulk density was determined by soil core method (Blake and Hartge, 1986). Cation exchange capacity (CEC) was determined using the method of 0.1 mol $BaCl_2$ percolation (König and Fortmann, 1996) and measuring cations in percolates using Inductively Coupled Plasma - Optical Emission Spectrometry (Optima 3000 XL, Perkin Elmer, Rodgau, Germany). Base saturation was determined as the percentage exchangeable base cations of the CEC. Soil pH was measured from soil:water suspension (ratio of 1:2). Total C and N concentrations were determined from ground soils using a CN elemental analyzer (Elementar Vario EL III, Hanau, Germany).

Table 3.1 Soil characteristics in the Ah horizon (0.01-0.1 m) of a Haplic Cambisol of a grassland site in the Solling Mountains, Germany.

Physical and chemical* properties	Mean	SE	n
Sand (%)	21.62	1.2	18
Silt (%)	66.53	1.3	18
Clay (%)	11.85	0.8	18
Bulk density (g cm^{-3})	0.79	0.01	72
Cation exchange capacity ($mmol_c$ kg^{-1})	169.00	5.2	72
Base saturation (%)	37.18	1.4	72
pH (1:2 H_2O)	5.34	0.03	72
Carbon : Nitrogen ratio	12.60	0.04	72

[*] Soil chemical data were provided by the Department of Plant Ecology, University of Göttingen.

3.3.4 BIOMASS YIELD AND PLANT N UPTAKE

Data of aboveground biomass was reported by From et al. (2011) (see Appendix Table A.1). The grassland was mown in mid-July for plots with one-cut yr^{-1} and in mid-May, mid-July and end of September of each year for plots with three-cuts yr^{-1}. A Haldrup © forage combine harvester was used with a cutting height of 7 cm. Dry mass of the biomass was determined by oven-drying at 60°C to constant mass. Oven-dried plant samples from the second cut (mid-July) were ground and analyzed for total N concentration using a CNS elemental analyser (Elementar Vario El, Hanau, Germany). Plant N uptake (kg N ha^{-1} yr^{-1}) was calculated as: N concentration (kg N kg^{-1}) * biomass yield (kg ha^{-1} yr^{-1}) (Hiremath and Ewel, 2001).

3.3.5 SOIL NET N MINERALIZATION RATES, N SUPPLY, AND N RESPONSE EFFICIENCY

Net N mineralization rates were measured five times (April, May, June, August and October) in 2009 and six times (April, May, June, July, August and September) in 2010 using the buried bag method. In each plot, two intact soil cores were taken from the Ah mineral soil (0.01-0.10 m). The soil from one core was transferred into a plastic bag, crumbled, mixed well, and extracted directly in the field by taking a subsample and adding this to a prepared bottle containing 150 ml 0.5 mol L^{-1} K_2SO_4 (average dry soil mass to solution ratio was 1:3) (T_0 cores). The other soil core was put in a plastic bag that was loosely tied to permit aeration but prevent rain from entering, inserted back into the hole to incubate in-situ for ten days, and extracted in a similar manner (T_1 cores). The soil-K_2SO_4 bottles were brought to the laboratory within 6 hours, where extraction continued by shaking the bottles for one hour and filtering through K_2SO_4-prewashed filter papers (4 μm nominal pore size). Extracts were immediately frozen until analysis. Ammonium (NH_4^+) and NO_3^- were measured using continuous flow injection colorimetry (Skalar, Cenco Instruments, Breda, The Netherlands), in which NH_4^+ was determined using the Berthelot reaction method (Skalar Method 155-000) and NO_3^- was measured using the copper-cadmium reduction method (Skalar Method 461-000). Gravimetric moisture content was determined for each soil sample by oven-drying at 105°C for 24 hours. Net N mineralization was calculated as the difference between T_1- and T_0-mineral N (NH_4^+ + NO_3^-). This assay of net production of mineral N in soil under in-situ conditions in the absence of plants provides an index of plant-available N (Hart et al., 1994).

N supply of each plot is defined as the sum of cumulative net N mineralization rates of the soil, N fertilization and N deposition rates. Cumulative net N mineralization rates during a growing season (i.e. April - September) were calculated by applying the trapezoid rule on time intervals between measured rates. For N deposition, we used a value of 12.6 kg N ha^{-1} yr^{-1} from bulk precipitation measured within the scope of the EU-level II monitoring program (Keuffel-Türk et al., in press). NRE was calculated for each plot as:

$$\text{NRE (kg biomass kg N}^{-1}\text{)} = $$

$$\text{(plant N uptake} \div \text{N supply)} * \text{(biomass yield} \div \text{plant N uptake)} \qquad \text{(Eq. 3)}$$

The ratio of plant N uptake to N supply is referred to as N uptake efficiency whereas the ratio of biomass yield to plant N uptake is the NUE (Hiremath and Ewel, 2001; Pastor and Bridgham, 1999).

3.3.6 STATISTICAL ANALYSES

Effects of treatments on time series data (net N mineralization rates) were conducted using linear mixed effects models (LME) with treatments and their interactions as fixed effects and spatial replication and time as random effects. The LME model includes either 1) a variance function that allows different variances of the response variable for the fixed effects, 2) a first-order temporal autoregressive process that assumes the correlation between measurements decreases with increasing time difference, or 3) both if this improves the relative goodness of model fit based on the Akaike Information Criterion (Crawley, 2007). Treatment effects on biomass yield, plant N uptake, N supply, NRE, NUE and uptake efficiency were assessed using three-way analysis of variance (ANOVA) with Tukey's HSD test. In all tests, if residual plots revealed non-normal distribution or non-homogeneity of variance, we used either logarithmic or square root transformation (after adding a constant value if the dataset included negative values) and analyses were repeated. Effects were accepted as statistically significant if $P \leq 0.05$. All statistical analyses were conducted using the R version 2.11.1 (R Development Core Team, 2009).

3.4 RESULTS

3.4.1 BIOMASS YIELD AND PLANT N UPTAKE

In 2009, biomass yield ranged from 4048 to 14647 kg ha^{-1} yr^{-1} with an overall mean of 7758 (± 309 SE) kg ha^{-1} yr^{-1}. Fertilization as well as increasing mowing frequency increased biomass yield, and plots cut thrice per year responded stronger to fertilization than plots cut once per year (Table A.1). In 2010, biomass yield decreased to values between 1960 and 12983 kg ha^{-1} yr^{-1} with a mean of 5612 kg ha^{-1} yr^{-1}. Compared to 2009, the interaction between mowing frequency and fertilization was even more pronounced (i.e. larger explained variance). Fertilization only resulted in larger yields in plots with three cuts per year whereas no fertilization effect could be detected in plots with one cut per year (Table A.1). Apart from fertilization and mowing frequency, sward composition also influenced biomass yield in 2010 but it explained only a small fraction of the variation. Control swards had larger yields compared to monocot-enhanced swards and both did not differ from dicot-enhanced swards.

Plant N uptake in 2009 ranged from 51.37 to 340.84 kg N ha^{-1} yr^{-1} with a mean of 147.38 (± 17.37 SE) kg ha^{-1} yr^{-1}. Fertilization significantly enhanced plant N uptake and explained by far the largest part of its variance. This was followed by mowing frequency with larger plant N uptake in plots cut thrice than once a year (Table 3.2). However, an interaction between fertilization and mowing frequency showed that mowing frequency only affected plant N uptake on the fertilized plots. In 2010, plant N uptake ranged from 27.82 to 273.39 kg N kg^{-1} yr^{-1} with a mean of 98.87 (± 11.65 SE) kg ha^{-1} yr^{-1}. As before, fertilization, mowing frequency and their interaction showed significant effects with larger plant N uptake in fertilized than unfertilized plots, and in plots cut thrice than once per year. The effect of fertilization was less pronounced and that of mowing frequency was larger compared to 2009. Mowing frequency significantly affected plant N uptake on both fertilized and unfertilized plots, but fertilized plots were more strongly influenced than unfertilized plots (Table 3.2).

Table 3.2 Plant N uptake of a grassland under different management practices in the Solling Mountains, Germany.

Main factors	Treatments	n	2009				2010			
			Plant N uptake (kg N ha^{-1} yr^{-1})	SE	*P*	Variance explained (%)	Plant N uptake (kg N ha^{-1} yr^{-1})	SE	*P*	Variance explained (%)
Sward composition					0.540	0.21			0.072	1.10
	control	24	147.37 a	16.24			106.64 a	13.03		
	monocot-enhanced	24	143.20 a	15.69			94.23 a	12.26		
	dicot-enhanced	24	151.56 a	14.99			95.73 a	10.49		
Mowing frequency					<0.000	13.17			<0.000	20.78
	once per year	36	120.11 b	8.35			71.27 b	4.06		
	thrice per year	36	174.65 a	14.50			126.46 a	11.41		
Fertilization					<0.000	66.88			<0.000	56.66
	no	36	85.93 b	4.15			59.73 b	2.66		
	NPK	36	208.83 a	9.47			138.00 a	9.78		
Fertilization x mowing frequency					<0.000	5.40			<0.000	7.93
no	once per year	18	76.12 c	3.33			54.39 d	3.26		
NPK	once per year	18	164.11 b	6.91			88.15 b	4.87		
no	thrice per year	18	95.75 c	6.90			65.06 c	3.88		
NPK	thrice per year	18	253.55 a	9.28			187.85 a	8.81		

Mean values with different letter indicate significant differences among treatments within main factors or within an interaction of main factors (three-way ANOVA with Tukey HSD at $P \leq 0.05$). Treatment interactions that were not significant are not reported.

3.4.2 SOIL NET N MINERALIZATION RATES AND N SUPPLY

Net N mineralization rates showed a clear seasonal pattern with large rates in summer and small rates in spring and fall. In 2009, net N mineralization rates varied between -13 and 135 kg N ha^{-1} mo^{-1} with a mean of 29 (± 3 SE) kg N ha^{-1} mo^{-1}. NH_4^+ was the dominant form of soil mineral N before incubation. On average, NO_3^- constituted 33% of the soil mineral N but in 70% of all cases no NO_3^- was detectable. Net N mineralization rates were neither affected by sward composition nor by mowing frequency (Table 3.3). Only fertilizer application influenced net N mineralization rates. In 2010, net N mineralization rates ranged from -29 to 105 kg N ha^{-1} mo^{-1} with a mean of 19 (± 3 SE) kg N ha^{-1} mo^{-1}. Unlike in 2009, not only fertilization but also mowing frequency influenced net N mineralization rates: plots cut once per year showed larger net N mineralization rates than plots cut three times per year.

N supply in 2009 ranged from 15 to 1003 kg N ha^{-1} yr^{-1} with a mean of 278 (± 25 SE) kg N ha^{-1} yr^{-1}. In 2010, N supply ranged from 22 to 823 kg N ha^{-1} yr^{-1} with a mean of 217 (± 18 SE) kg N ha^{-1} yr^{-1}. In both years, fertilization ($P = 0.000$) was the only factor influencing N supply.

Table 3.3 Soil net N mineralization rates of a grassland under different management practices in the Solling Mountains, Germany.

Main factors	Treatments	n	2009			2010		
			Net N mineralization (kg N ha^{-1} mo^{-1})	SE	*P*	Net N mineralization (kg N ha^{-1} mo^{-1})	SE	*P*
Sward composition					0.560			0.160
	control	24	30.69 a	11.70		16.75 a	7.87	
	dicot-enhanced	24	25.56 a	8.42		24.20 a	9.47	
	monocot-enhanced	24	21.19 a	7.58		18.05 a	5.88	
Mowing frequency					0.400			0.018
	once per year	36	24.77 a	7.69		22.54 a	3.76	
	thrice per year	36	26.83 a	7.69		16.76 b	2.79	
Fertilization					<0.000			0.009
	no	36	13.50 b	3.47		15.48 b	2.58	
	NPK	36	38.12 a	9.89		23.84 a	3.97	

Mean values with different letter indicate significant differences among treatments within main factors or within an interaction of main factors (Linear mixed effects models at $P \leq 0.05$). Treatment interactions that were not significant are not reported.

3.4.3 N RESPONSE EFFICIENCY, N UPTAKE EFFICIENCY AND N USE EFFICIENCY

NRE in 2009 varied in a wide range from 10 to 488 kg biomass kg N supply^{-1} with a mean of 48 (± 7 SE) kg biomass kg N supply^{-1}. Fertilization was the main factor influencing NRE, with larger efficiency in unfertilized plots compared to fertilized plots (Table 3.4). Mowing frequency was the second important factor that explained the variance, with larger NRE in plots cut thrice than once per year. In 2010, NRE did not differ from 2009 with values ranging from 5 to 162 kg biomass kg N supply^{-1} and a mean of 42 (± 4 SE) kg biomass kg N supply^{-1}. All three factors significantly affected NRE in 2010, with fertilization explaining the largest part of the variance followed by mowing frequency and sward composition. NRE was larger in unfertilized than in fertilized plots. The interaction between sward composition and mowing frequency indicated that regardless of mowing frequency, control plots showed an NRE comparable to the monocot- and dicot-enhanced plots that were cut thrice a year and that these NRE were larger than those in monocot- and dicot-enhanced plots cut once a year (Table 3.4). Within the covered range of N supply, NRE increased monotonically with decreasing N supply (Figure 3.1 a).

Table 3.4 N response efficiency (NRE) of a grassland under different management practices in the Solling Mountains, Germany.

			2009				2010			
Main factors	Treatments	n	NRE (kg biomass kg N supply^{-1})	SE	*P*	Variance explained (%)	NRE (kg biomass kg N supply^{-1})	SE	*P*	Variance explained (%)
Sward composition					0.349	1.72			0.034	5.68
	control	24	40.19 a	6.01			50.28 a	7.02		
	monocot-enhanced	24	59.99 a	18.82			42.62 ab	7.46		
	dicot-enhanced	24	45.30 a	6.10			32.94 b	4.52		
Mowing frequency					0.047	3.29			<0.000	12.40
	once per year	36	38.67 b	4.81			35.53 b	5.68		
	thrice per year	36	58.31 a	13.30			48.37 a	5.03		
Fertilization					<0.000	41.26			<0.000	24.72
	no	36	70.97 a	12.83			55.49 a	5.29		
	NPK	36	26.02 b	2.18			28.41 b	4.63		
Sward composition x mowing frequency					n.s.	n.s.			0.036	5.54
control	once per year	12					54.02 a	12.82		
control	thrice per year	12					46.54 a	6.77		
monocot-enhanced	once per year	12					29.84 b	8.62		
monocot-enhanced	thrice per year	12					55.40 a	11.73		
dicot-enhanced	once per year	12					22.73 b	4.48		
dicot-enhanced	thrice per year	12					43.16 a	7.04		

Mean values with different letter indicate significant differences among treatments within main factors or within an interaction of main factors (three-way ANOVA with Tukey HSD at $P \leq 0.05$). Treatment interactions that were not significant are not reported. n.s. = not significant.

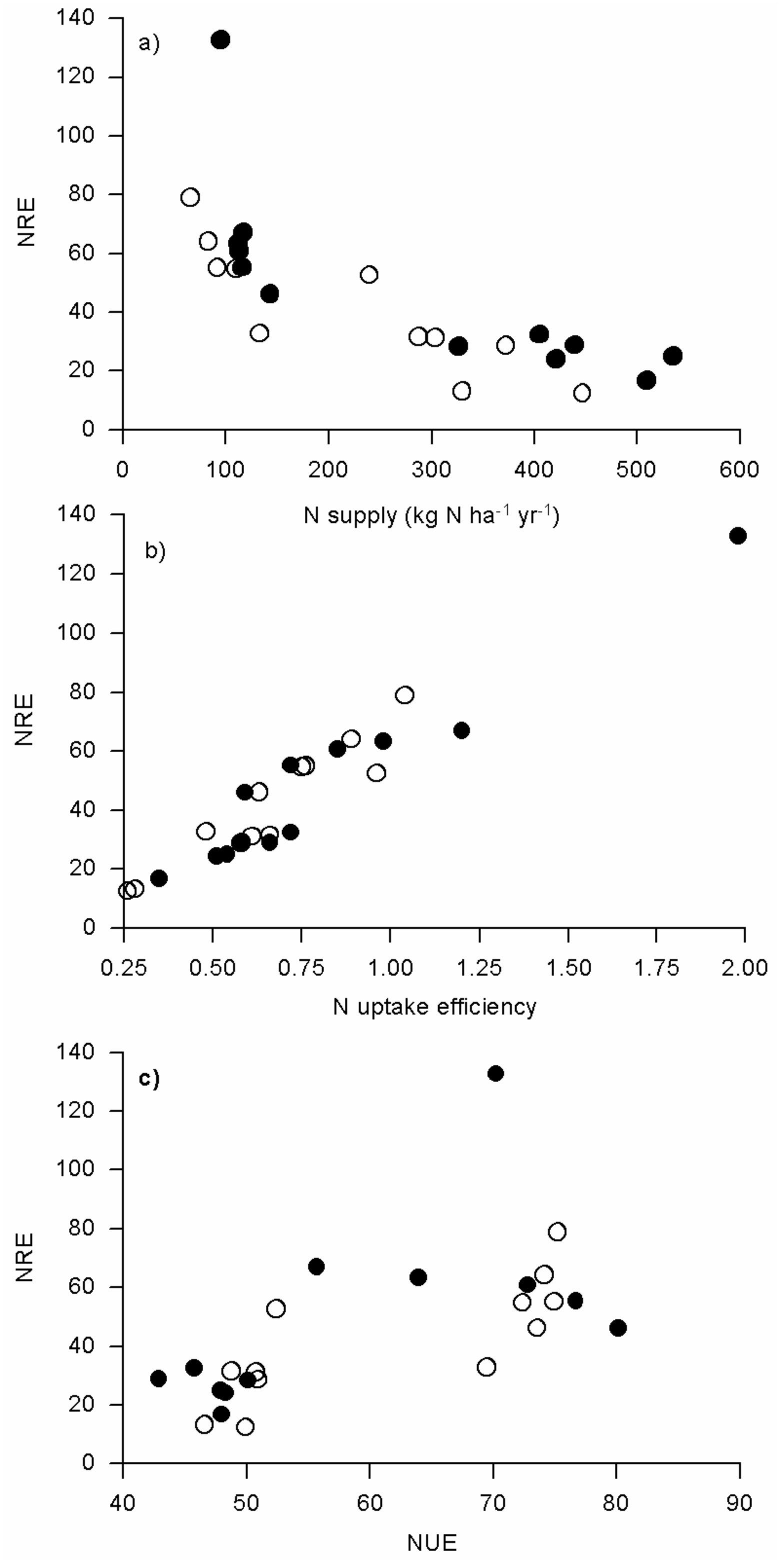


Figure 3.1 Trends between N response efficiency (NRE) and (a) N supply, (b) N uptake efficiency and (c) N use efficiency (NUE) of a grassland in the Solling Mountains, Germany. Each data point is the mean of six replicates per treatment (● for 2009, ○ for 2010). No statistical test was conducted because the X variables are components of the Y variable.

N uptake efficiency in 2009 was largely affected by fertilization with larger efficiencies in unfertilized plots compared to fertilized plots (Table 3.5). Mowing frequency explained a small part of the variance with larger efficiencies in plots cut thrice per year than plots cut only once per year. In 2010, mowing frequency explained a larger part of the variance than fertilization. In addition, sward composition marginally influenced N uptake efficiency with a larger efficiency in control swards than monocot- and dicot-enhanced swards (Table 3.5). The pattern between NRE and N uptake efficiency showed increasing NRE with increasing N uptake efficiency (Figure 3.1 b).

NUE was affected by all three factors in 2009 (Table 3.6). Fertilization was the most important factor influencing NUE. Unfertilized plots showed larger NUE than fertilized plots. Mowing was the second most important factor. Plots cut once per year showed larger NUE than plots cut thrice per year. This trend, however, was only significant for the unfertilized plots, based on fertilization and mowing interaction effect. Sward composition had the smallest influence with the largest NUE in monocot-enhanced swards, intermediate NUE in control swards and smallest NUE in dicot-enhanced swards. In 2010, fertilization was the only factor influencing NUE (Table 3.6). The trend between NRE and NUE was more scattered (Figure 3.1 c) than the pattern between NRE and N uptake efficiency (Figure 3.1 b).

Table 3.5 N uptake efficiency of a grassland under different management practices in the Solling Mountains, Germany.

Year			2009				2010			
Main factors	Treatments	n	N uptake efficiency (kg plant N uptake kg N supply^{-1})	SE	*P*	Variance explained (%)	N uptake efficiency (kg plant N uptake kg N supply^{-1})	SE	*P*	Variance explained (%)
Sward composition					0.372	2.13			0.056	6.04
	control	24	0.65 a	0.08			0.80 a	0.11		
	monocot-enhanced	24	0.97 a	0.29			0.65 a	0.10		
	dicot-enhanced	24	0.80 a	0.10			0.52 a	0.06		
Mowing frequency					0.003	9.91			< 0.001	14.97
	once per year	36	0.60 b	0.05			0.56 b	0.09		
	thrice per year	36	1.01 a	0.20			0.75 a	0.06		
Fertilization					< 0.001	16.79			0.006	7.90
	no	36	1.05 a	0.20			0.76 a	0.07		
	NPK	36	0.56 b	0.05			0.56 b	0.08		

Mean values with different letter indicate significant differences among treatments within main factors or within an interaction of main factors (three-way ANOVA at $P \leq 0.05$). Treatment interactions that were not significant are not reported.

Table 3.6 N use efficiency (NUE) of a grassland under different management practices in the Solling Mountains, Germany.

Year			2009				2010			
Main factors	Treatments	n	NUE	SE	*P*	Variance explained	NUE	SE	*P*	Variance explained
			(kg biomass yield kg plant N uptake^{-1})			(%)	(kg biomass yield kg N supply^{-1})			(%)
Sward composition					0.005	3.70			0.179	0.71
	control	24	59.10 ab	2.71			63.07 a	2.70		
	monocot-enhanced	24	61.55 a	3.39			61.01 a	2.91		
	dicot-enhanced	24	54.90 b	2.59			60.60 a	2.33		
Mowing frequency					< 0.001	8.38			0.455	0.11
	once per year	36	62.65 a	2.67			61.12 a	2.15		
	thrice per year	36	54.39 b	1.88			62.00 a	2.18		
Fertilization					< 0.001	63.42			< 0.001	83.06
	no	36	69.88 a	1.91			73.23 a	1.04		
	NPK	36	47.16 b	0.78			49.88 b	0.71		
Fertilization x Mowing frequency					0.003	3.07			n.s.	
no	once per year	18	76.51 a	2.41						
NPK	once per year	18	48.78 c	0.99						
no	thrice per year	18	63.25 b	2.01						
NPK	thrice per year	18	45.53 c	1.10						

Mean values with different letter indicate significant differences among treatments within main factors or within an interaction of main factors (three-way ANOVA with Tukey HSD at $P \leq 0.05$). Treatment interactions that were not significant are not reported. n.s. = not significant.

3.5 DISCUSSION

3.5.1 SOIL NET N MINERALIZATION, PLANT N UPTAKE AND BIOMASS YIELD

The substantial increase of net N mineralization following first fertilization in 2009 was probably caused by a priming effect. Priming effects are changes in mineralization processes caused by moderate addition of nutrients to the soil, e.g. the input of a limiting factor for microbial biomass (Kuzyakov et al., 2000). Our experimental site had been used for hay production and cattle grazing since the early 20th century. Only during the last five decades, a minimal fertilizer addition of 80 kg N ha^{-1} yr^{-1} was practiced. It is thus likely that the activity of microbial biomass was stimulated by the addition of 180 kg N ha^{-1} yr^{-1} at the beginning of our study. The reduction in net N mineralization rates in fertilized plots in the second year compared to the first year is also indicative of a priming effect, which has been suggested to be short term (Kuzyakov et al., 2000). While the addition of N fertilizer initially stimulates mineralization after a long period of N limitation, regular N additions may not increase N cycling continuously (Hassink, 1994). The decreased net N mineralization rates with increased mowing frequency in the second year could be due to reduced input of organic matter into the soil as a consequence of the removal of biomass (Holt, 1997; Mikola et al., 2001; Northup et al., 1999; Sankaran and Augustine, 2004). Alternatively, it is possible that the reduced net N mineralization rates on the plots cut thrice per year could also be caused by large N immobilization by the microbial community, which may result from increased rhizodeposition following defoliation (Holland et al., 1996). Such an explanation is corroborated by the findings of Guitian and Bardgett (2000) who observed increased soil microbial biomass caused by defoliation of grass.

Within the range of N supply covered in our study, the linear increase of plant N uptake was reflected by similar trends of increases in aboveground biomass yield and plant N concentrations (data not shown). The enhanced plant N uptake by more frequent mowing could be possibly due to 1) increased root-to-shoot ratio by intensive mowing (Kammann et al., 1998; Mooney and Winner, 1991), which may then result in large nutrient uptake by plants, and 2) defoliation-induced increase in resource allocation to shoots, as reported by Guitian and Bardgett (2000) for grazing-tolerant grasses. However, for our study site, fine-root biomass and root length density measured in the control swards in September 2009 were not affected by mowing frequency (Rose et al., 2011a). Thus, it is likely that our observed increased plant N uptake by more frequent mowing is due to increased resource allocation to shoots and overcompensatory response to defoliation (Ferraro and Oesterheld, 2002; Guitian and Bardgett, 2000).

Our finding that the dicot-enhanced swards (with equal proportions of dicots and monocots) on did not result in a larger biomass yield compared to control and monocot-enhanced swards contrasts to the findings of Hector et al. (1999) and Weigelt et al. (2009) who reported a reduction of aboveground biomass with loss of functional groups in European grasslands. Furthermore, the number of plant species was not responsible for differences in biomass yield. Dicot-enhanced and monocot-enhanced swards showed no significant differences in biomass yield although the numbers of species were different, which is not consistent to the biodiversity-productivity theory (e.g. Hector et al., 1999; Tilman et al., 1996; Weigelt et al., 2009). However, as we only considered harvestable, aboveground biomass, inclusion of belowground biomass may lead to different results.

3.5.2 N RESPONSE EFFICIENCY

To our knowledge, published information about NRE in grassland ecosystems is extremely limited and definitions of NRE are not consistent in literature (Aerts, 1990; Vitousek, 1982; Xu et al., 2012). Unlike other NRE and NUE studies that used litterfall N concentrations as an index of N availability (Vitousek, 1982) or measured mineral N concentrations in soil (Bridgham et al., 1995), we used in-situ measured net N mineralization rates as an index of soil plant-available N (Hart et al., 1994). Our measured NRE values were smaller than those reported by Yuan et al. (2006) for a semi-arid grassland in China. Apart from site-specific differences between our and their study sites, the differences in NRE could also be due to the different measures used for N supply and biomass production. On the one hand, our estimate of N supply included fertilization, N deposition and cumulative net N mineralization rates during the growing season, resulting in large values of N supply and thus small NRE. On the other hand, Yuan et al. (2006) included not only aboveground but also belowground biomass production, which would lead to large NRE.

Our finding that NRE was largely influenced by fertilization was similar with those of Hiremath and Ewel (2001) who reported that NRE (which they termed ecosystem-level NUE) was negatively correlated with mean annual net nitrification rate, used as an index of soil N supply. The smaller NRE of the fertilized than unfertilized plots was caused by decreases in both N uptake efficiency and NUE. The monotonic increase in NRE with decreasing N supply was also consistent with the findings of Yuan et al. (2006), and was unlike the unimodal curve between NRE and soil net N mineralization rates (ranging from 25-85 kg N ha^{-1} yr^{-1}) modelled by Bridgham et al. (1995) for temperate forests. This also supports the suggestion that the unimodal NRE curve may only be applicable for ecosystems with small N availability (Yuan et al., 2006). The second most important factor influencing NRE was mowing frequency. The increased NRE in plots cut thrice per year was largely due to increasing N uptake efficiency since NUE was either showing the opposite trend or not affected by mowing at all. The increased N uptake efficiency in plots cut thrice per year was caused by increased biomass yield and N concentration in biomass.

Finally, sward composition influenced NRE although to a lesser extent than fertilization and mowing frequency. Compared to the influence of sward compositions on biomass yield, where dicot-enhanced swards did not differ from control swards, the influence of sward composition on NRE was more pronounced and explained a larger part of the variance. The trends of NRE and N uptake efficiency among sward compositions were similar whereas NUE exhibited either opposite patterns or was not affected by sward composition. This emphasizes that NRE of the different sward compositions was influenced more by N uptake efficiency rather than by NUE and is contrary to the findings of van Ruijven and Berendse (2005) who observed an increasing NUE with increasing species richness in a grassland system without legumes. In our study site, legumes were only present in the control- (1-4% abundance) and dicot-enhanced (4-9%) swards. The presence of legumes however cannot support the pattern of differences in NRE among sward compositions but instead the interaction between sward composition and mowing frequency. For example, since we did not account the N input from N-fixing legumes in our NRE calculation, this could have led to an underestimation of N supply and thus an overestimation of NRE in the control and dicot-enhanced swards. Instead we found that the monocot-enhanced swards (no legumes) had comparable NRE with the control and dicot-enhanced swards all cut thrice a year and the lowest NRE was found in both monocot- and dicot-enhanced swards cut once per year (Table 3.4). The patterns of NRE cannot also be explained by the difference in plant species number. NRE of monocot-enhanced swards which had the smallest number (12-13) of species did not significantly differ from NRE of dicot-enhanced swards (17 species). According to Roy (2001) 90% of the biodiversity effect on productivity is reached at five species, implying that the difference in plant species in our experiment is larger than the threshold number and effects might not be detectable (Wrage et al., 2011). In contrast to the number of species, the proportions of plant functional groups did have a significant impact on NRE. The proportions of ~30% dicots and ~70% monocots found in the control swards showed the largest NRE. We think that over the past decades N-limited conditions and prevailing management practices have led to an equilibrium in this grassland ecosystem in which optimal proportions of monocots and dicots developed to maximize NRE. As a result, an artificial increase in the proportion of dicots as well as in the proportion of monocots would lead to a smaller NRE. Thus, our results suggest that in addition to the effect of life-form diversity (Hiremath and Ewel, 2001) also the proportions of different plant functional groups affect NRE of an ecosystem.

3.6 CONCLUSIONS

Our results show that management largely influenced NRE whereas plant functional group diversity only played a minor role. Fertilization decreased NRE due to decreases in both N uptake efficiency and NUE whereas mowing frequency and sward composition affected NRE through N uptake efficiency rather than NUE. The proportions of monocots and dicots in the control plots that were the result of long-term management practices had the largest NRE. Deviations from these proportions - even to more balanced proportions of monocots and dicots - decreased NRE. Our results show that NRE gives important insights in how different management can be evaluated to come up with a more sustainable grassland management. However, our study can only be a first step towards definite recommendations and NRE should be combined with an economic analysis before advices to farmers can be made.

CHAPTER

4

NITROGEN RETENTION EFFICIENCY AND NITROGEN LOSSES OF A MANAGED AND PHYTODIVERSE TEMPERATE GRASSLAND

4.1 ABSTRACT

Maintaining nitrogen (N) retention efficiency is crucial in minimizing N losses when intensifying management of temperate grasslands. Our aim was to evaluate how grassland management practices and sward compositions affect N retention efficiency (1 - N losses ÷ soil available N), defined as the efficiency with which soil available N is retained in an ecosystem. A three-factorial grassland management experiment was established with two fertilization treatments (no fertilization and combined nitrogen, phosphorus and potassium fertilization), two mowing frequencies (cut once and thrice per year) and three sward compositions (control, monocot- and dicot-enhanced swards). We measured N losses as leaching and nitrous oxide emissions, and soil available N as gross N mineralization rates. Fertilization was the dominant factor influencing N losses and N retention efficiency; it increased N losses due to increased nitrification and decreased microbial N immobilization, and consequently decreased N retention efficiency. Intensive mowing partly dampened high N losses following fertilization. Sward compositions influenced N retention efficiency but not N losses: control swards that have developed under decades of extensive management practices had the highest N retention efficiency whereas monocot-enhanced sward had the lowest N retention efficiency. Across treatments, N retention efficiency was highly correlated with microbial NH_4^+ immobilization and microbial biomass and only marginally correlated with plant N uptake, underlining the importance of microbial N retention in the soil-plant system. Such pathway is reflected in N retention efficiency but not in indices commonly used to reflect plant response. As N retention efficiency was able to capture the effects of sward compositions and fertilization whereas N losses were only sensitive largely to fertilization, N retention efficiency as a better index than just N losses when evaluating environmental sustainability of sward compositions and management practices of grasslands.

Keywords: nitrate leaching, dissolved organic nitrogen, nitrous oxide emissions, gross N mineralization, microbial immobilization, ^{15}N pool dilution, functional group diversity

4.2 INTRODUCTION

Nitrogen (N) is an essential plant nutrient that limits primary production in many ecosystems, making the use of N fertilizers widespread in agricultural ecosystems (Vitousek and Howarth, 1991). As a result of the growing global population, a more intensive agricultural production and hence an increase in the use of N fertilizers can be expected in the near future (Galloway et al., 2008). Agricultural intensification is, however, accompanied by negative environmental impacts: considerable amounts of applied fertilizer N can be lost through nitrate (NO_3^-) leaching or gaseous emissions, e.g. in the form of nitrous oxide (N_2O). Leaching of NO_3^- can be a threat to ground- and surface-water quality (Di and Cameron, 2002) whereas N_2O is an important greenhouse gas that also contributes to the depletion of stratospheric ozone (Schlesinger, 2009). In agriculture, maintaining soil quality and high yields while minimizing N losses can only be achieved through efficient retention of N in the plant-soil system, i.e. available mineral N should be taken up by plants or immobilized by the microbial community before it is potentially lost through the microbial processes of nitrification and denitrification. N retention efficiency, defined as the efficiency with which available N is retained in an ecosystem, is thus an important parameter to evaluate the sustainability of a land use system.

In temperate grasslands, N retention efficiency may be influenced by management practices and plant diversity (Christian and Riche, 1998; Flechard et al., 2005; Jones et al., 2005). Application of N fertilizer typically increases N losses (e.g. Christian and Riche, 1998, Jones et al., 2005) whereas mowing can lead to a more effective plant N uptake caused by overcompensatory regrowth of plants (Ferraro and Oesterheld, 2002) or by a denser root system (Kammann et al., 1998). A more diverse grassland community may have a higher N retention through complementary resource use, e.g. due to different rooting depths of various plant communities, uptake of different forms of N, or N uptake at different times of the year (e.g. Hooper and Vitousek, 1998). It has been reported that increasing plant species diversity reduces the amount of extractable soil mineral N (e.g. Ewel et al., 1991; Niklaus et al., 2001; Tilman et al., 1996). However, most studies have been carried out in artificial and intensively weeded grassland plots, making it difficult to compare with permanent, managed grassland. Furthermore, soil NO_3^- concentrations are not directly related to NO_3^- leaching, complicating their interpretation (Scherer-Lorenzen et al., 2003). To our knowledge, there are only two studies that directly quantified NO_3^- leaching losses as a function of plant diversity (Hooper and Vitousek, 1997; Scherer-Lorenzen et al., 2003) and only one study investigated biodiversity effects on leaching of dissolved organic N (DON) (Dijkstra et al., 2007). No study had been conducted on the effects of mowing on N leaching. Moreover, the impact of plant functional group diversity on N_2O emissions from soils has so far received only little research attention. In our earlier study, conducted in a ~20-year established, grazed grassland in Germany, N_2O and NO fluxes were not affected by plant species composition but by different livestock grazers (Hoeft et al., 2012). Reduced N_2O fluxes with increase in mowing frequency have been reported for a 50-year established, non-grazed grassland in Germany (Kammann et al., 1998).

In the present study, our goal was to evaluate how N retention efficiency is influenced by different management treatments (i.e. fertilization and mowing frequency) and sward compositions (differing in plant functional group compositions and plant species richness) in a temperate grassland. We were especially interested in the question whether management or manipulation of plant functional groups can compensate the high N losses that are frequently observed following fertilizer application. We tested the following hypotheses: 1) fertilization results to larger N losses and lower N retention efficiency than without fertilization; 2) intensive mowing frequency decreases N losses and increases N retention efficiency; 3) swards with equal proportions of various plant functional groups would have low N losses and high N retention efficiency due to complementary resource use.

4.3 MATERIAL AND METHODS

4.3.1 APPROACH

We first developed an index that can be used for the evaluation of the ecosystem property, N retention efficiency. Aber and collegues (1998) use the following index for N retention efficiency: 1 - (N outputs ÷ N inputs)

While this index may work well for forest ecosystems where long-term data of N input and output have been collected, it does not account for changes in soil N cycling caused by management in agricultural systems. For example, when N fertilizer is applied, large proportions of applied mineral N are immediately immobilized by the soil microbial community (Bristow et al., 1987), a process which typically exceeds plant N uptake (Jackson et al., 1989). This microbially-assimilated N will become again available through microbial turnover which occurs within about two weeks for a temperate grassland (Corre et al., 2002), and thus soil available N is usually larger than the mere external N input by fertilization. In the present study, we account for such processes by using an index of available N that is actively cycling in the soil and is available to plants and microbial biomass, and thus we used the following index for N retention efficiency:

N retention efficiency =

1 - (N losses (mg N m^{-2} d^{-1}) ÷ gross N mineralization rates (mg N m^{-2} d^{-1})) (Eq. 4)

where the N losses were the sum of average daily NO_3^- and DON leaching rates (mg N m^{-2} d^{-1}) and mean daily N_2O emissions rates (mg N m^{-2} d^{-1}); gross N mineralization rates were measured once during the growing season.

4.3.2 STUDY SITE

The study (which is part of the grassland manipulation (GRASSMAN) experiment) was conducted on a moderately species-rich grassland in the Solling uplands of Lower Saxony, Germany (51°44'53"N, 9°32'42"E, 490 m above sea level). This site presently belongs to the Relliehausen experimental farm and has been used for grazing or hay making for at least 100 years (Geological Map of Prussia 1910 (based on the topographic inventory of 1896), topographic maps of Sievershausen and Neuhaus/Solling 1924, 1956 and 1974, Braunschweigische Landesaufnahme, 18th century). Before the experiment started, the site was managed extensively by moderate application of fertilizer (80 kg N ha^{-1} y^{-1}), occasional lime application, overseeding with high value forage species and cattle grazing (farm records Relliehausen since 1966). Prior to the experiment, vegetation was classified as a montane, semi-moist *Lolio-Cynosuretum*. Mean annual precipitation is 1031 mm and mean annual temperature is 6.9°C (Deutscher Wetterdienst 1961 - 1990). The soil is a Haplic Cambisol (WRB 2006) that developed on loess sediments with a loamy silt texture overlying weathered Triassic sandstone.

4.3.3 EXPERIMENTAL SET UP

The GRASSMAN experiment was set up in a three-factorial design, including three sward compositions, two mowing frequencies (cut once and thrice a year), and two fertilization treatments (with combined N, phosphorus (P) and potassium (K) addition, and no fertilization). In 2008, three sward compositions were established: monocot-enhanced swards where herbicide mixture against dicotyledons (Starane® (active ingredients: Fluoroxypyr and Triclopyr) and Duplosan KV (active ingredients: Mecoprop-P®)) was applied and resulted in a reduced herb and legume abundance; dicot-enhanced swards where herbicide mixture against monocotyledons (Select 240 EC® by Stähler (active ingredients: Clethodim)) was applied and resulted in a reduced grass abundance: and untreated control swards. The monocot-enhanced swards had 12-13 species, 91-93% grasses, 7-9% herbs and 0% legumes. The dicot-enhanced swards had 17 species, 40-47% grasses, 49-53% herbs and 4-9% legumes. The control swards had 16-18 species, 68-76% grasses, 21-31% herbs and 1-4% legumes) (Petersen et al., 2012). In 2008, all swards were mowed once and the fertilized plots received 50 kg N ha^{-1}. Mowing and fertilization treatments started in 2009. Half of the plots were mown once per year and the other half of the plots were cut three times per year. Mowing was conducted in July for plots with one cut per year and in May, July and September for plots with three cuts per year. A Haldrup © forage combine harvester with a cutting height of 7 cm was used. Half of the plots were fertilized with 180 kg N ha^{-1} y^{-1}, 30 kg P ha^{-1} y^{-1} and 100 kg K ha^{-1} y^{-1}. Mineral N fertilizer (calcium ammonium nitrate N27) was split into two equal applications per year (April and May 2009 and April and June 2010) while the combined P and K fertilizer was applied once a year (June 2009 and 2010). The full-factorial combination of these treatments (three sward compositions, two mowing frequencies, and two fertilization treatments) led to twelve treatment combinations, each replicated six times. The resulting 72 plots (15 m x 15 m each) were arranged in a Latin rectangle.

4.3.4 SOIL CHARACTERISTICS

A summary of soil characteristics is presented in Table 4.1. In spring 2008, soil samples were taken at nine sampling points per plot (0.01-0.1 m depth), pooled, oven-dried (40°C) for a week and sieved (2 mm). Soil bulk density was measured in summer 2009 from undisturbed soil cores (0.00-0.05 m) using the soil core method (Blake and Hartge, 1986). Particle size distribution was determined by wet sieving (>20<630 µm) and pipette methods (≤20 µm) after pre-treatment with 30% H_2O_2 and 4% Na-dithionite-citrate solution. Cation exchange capacity (CEC) was determined using percolation with 0.1 mol $BaCl_2$ (König and Fortmann, 1996). Cations in percolates were measured with Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES; Optima 3000 XL, Perkin Elmer, Rodgau, Germany). Base saturation was calculated as the percentage exchangeable base cations of the CEC. Soil pH was measured from a soil-to-distilled water ratio of 1:2. Total concentrations of carbon (C) and N were determined from ground soil samples using CN elemental analyzer (Vario EL III, Elementar, Hanau, Germany).

Table 4.1 Soil characteristics in the Ah horizon (0.01-0.1 m) of a Haplic Cambisol of a grassland site in the Solling Mountains, Germany.

Physical and chemical* properties	Mean	SE	n
Sand (%)	21.62	1.2	18
Silt (%)	66.53	1.3	18
Clay (%)	11.85	0.8	18
Bulk density (g cm^{-3})	0.79	0.01	72
Cation exchange capacity ($mmol_c$ kg^{-1})	169.00	5.2	72
Base saturation (%)	37.18	1.4	72
pH (1:2 H_2O)	5.34	0.03	72
Carbon : Nitrogen ratio	12.60	0.04	72

* Soil chemical data were provided by the Department of Plant Ecology, University of Göttingen.

4.3.5 GROSS N TRANSFORMATION RATES AND MICROBIAL BIOMASS

In September 2010, gross N mineralization and nitrification rates were measured using ^{15}N pool dilution techniques (Davidson et al., 1991). These were measured from five replicates of the three sward compositions and two fertilization treatments that were all cut once per year (totalling to 5 x 3 x 2 = 30 plots). At each sampling plot, two intact soil cores (8 cm in diameter and 5 cm in height) were injected with $(^{15}NH_4)_2SO_4$ solution (for gross mineralization and NH_4^+ consumption rates) and another two intact soil cores were injected with $K^{15}NO_3$ solution (for gross nitrification rates). Each soil core received five 1-ml injections containing either 29 µg N ml^{-1} ($(^{15}NH_4)_2SO4$) or 28 µg N ml^{-1} ($K^{15}NO_3$) with 99% ^{15}N enrichment, which was equivalent to a rate of 0.78 and 0.73 µg ^{15}N g^{-1}, respectively. Ten minutes after ^{15}N injection, the soil of each labelled pair was extruded out of the core, mixed thoroughly, and part of it was added to a polyethylene bottle containing 150 ml of 0.5 mol L^{-1} K_2SO_4 (approximately 1:3 ratio of fresh soil to K_2SO_4 solution) (T_0 cores). The other soil core of the labelled pair was put in a plastic bag, inserted back into the soil to incubate in situ for one day, and extracted in the same manner (T_1 cores). The bottles containing soil and K_2SO_4 solution were shaken for one hour; the extracts were filtered through pre-washed filter papers (4 µm nominal pore size) and stored at -18°C until analysis. NH_4^+ and NO_3^- concentrations of the extracts were measured using continuous flow injection colorimetry (CFIC), in which NH_4^+ was determined by Berthelot reaction method (Skalar Method 155-000) and NO_3^- by copper-cadmium reduction method (with NH_4Cl buffer but without ethylenediamine tetraacetic acid; Skalar Method 461-000). Gravimetric moisture content was determined from all soil samples by oven-drying at 105°C for 24 hours, and these values were used to calculate the dry mass of extracted soil samples. The ^{15}N enrichment of NH_4^+ and NO_3^- pools were determined by ^{15}N diffusion with blank correction as described in details by Corre and Lamersdorf (2004). ^{15}N analysis was done using isotope ratio mass spectrometry (Finigan MAT, Bremen, Germany). Gross N mineralization, gross nitrification and NH_4^+ consumption rates were estimated using the ^{15}N pool dilution equations described by Davidson et al. (1991) of which also NH_4^+ immobilization rate was calculated as the difference between gross rates of NH_4^+ consumption and nitrification.

For microbial biomass C and N determination, we used the fumigation-extraction method (Brookes et al., 1985). Part of the soil from the T_1 cores were fumigated with chloroform for 5 days and extracted with 0.5 mol L^{-1} K_2SO_4 as described above. Organic C concentrations of the extracts from fumigated and the corresponding unfumigated soils were analyzed by UV-enhanced persulfate oxidation using a Dohrman DC-80 Carbon analyzer with an infrared detector (Rosemount Analytical Division, Santa Clara, California, USA). Similarly, total N concentrations of the extracts were determined by persulfate digestion, which involves oxidation of NH_4^+ and organic N to NO_3^- while NO_3^- remains unchanged, followed by colorimetric analysis of NO_3^- as described above. Microbial biomass C and N were calculated as the difference in organic C and total N concentrations between fumigated and unfumigated soils divided by k_C = 0.45 for microbial biomass C and k_N = 0.68 for microbial biomass N.

4.3.6 WATER BALANCE, N LEACHING LOSSES AND N_2O EMISSIONS

N leaching losses were sampled monthly. No soil water can be withdrawn from the lysimeters during January - February 2009 and 2010 (mean temperature below 0°C) and during August 2009 and 2010 (dry months). We installed one suction cup lysimeter (P80 ceramic, maximum pore size 1 µm; CeramTec AG, Marktredwitz, Germany) per plot at a depth between 0.5-0.6 m, which was beyond the rooting depth (see below), three months prior to the first sampling. Soil solution was sampled over a month by applying a suction of 600 hPa to the lysimeters and was collected into a glass bottle placed in a dark bucket that was dug in the soil. Immediately after field collection, the water samples were stored at -18°C until analysis. NH_4^+ and NO_3^- concentrations of the soil water were measured using CFIC as described above. Total dissolved N (TDN) concentrations were determined using CFIC by UV-persulfate oxidation followed by hydrazine sulphate reduction (Skalar Method 473-000). DON was calculated as the difference between TDN and NH_4^+ + NO_3^-. There was no detectable NH_4^+ in the leaching water, and thus leaching losses was mainly composed of NO_3^- and DON. Monthly NO_3^- and DON leaching losses were calculated by multiplying their concentrations in the monthly sampled soil water with the total drainage flux of the month.

Drainage flux was calculated on a daily time step using the 1D hydrological model BROOK90 (Federer et al., 2003), which was parameterized with the prevailing site conditions. Input climatic data (daily total precipitation, minimum/maximum air temperature, solar radiation, vapour pressure and wind speed) were taken from a meteorological station at the study site. Daily evapotranspiration (ET = evaporation + transpiration + interception) was calculated from the meteorological data and vegetation properties using the approach of Shuttleworth and Wallace (1985). The seasonal course of the leaf area index, used for the calculation of ET, was derived from average biomass productions according to Rose et al. (2011a). Root uptake of water from the soil was depth-partitioned by assuming that root distribution decreases exponentially within 0.3-m rooting depth with 70% of the roots in the top 0.1-m depth. The vertical water movement in the soil was simulated using the Richards equation, from which the relationships between matrix potential, water content and hydraulic conductivity for the soil texture (as described by Clapp and Hornberger, 1978) similar to that in our study site were used. To validate the model, we compared the modelled soil matrix potential with the measured matrix potential. Matrix potential was measured monthly using tensiometers (P80 ceramic, maximum pore size 1 µm; CeramTec AG, Marktredwitz, Germany), which were installed at 0.2-m, 0.5-m and 0.9-m depths. Finally, the drainage flux was calculated as the net vertical flux at the sampling depth of soil solution (0.6 m).

N_2O emissions were measured seven times in 2009 and five times in 2010 using closed chamber method. One polyvinyl chloride chamber base (0.07 m^2 area, 0.35-0.4 m height of chamber base and cover) was installed permanently in each plot. For each measurement, the chamber base was covered with a polyethylene chamber hood fitted with a vent and a sampling port. Following chamber closure, gas samples were taken four times at 15-minute interval and stored into pre-evacuated 100-ml glass bottles fitted with teflon-coated stopcocks. N_2O was analyzed using a gas chromatograph (GC 6000, Carlo Erba Instruments/Thermo Fisher Scientific, Milan, Italy) equipped with an electron capture detector and an autosampler system (Loftfield et al., 1997). Gas concentrations were calculated by comparing the sample peak integrals with the peak integrals of three standard gases (353, 1018 and 1604 ppb N_2O; Deuste Steiniger GmbH, Mühlhausen, Germany). N_2O emissions were calculated by the linear increase of N_2O concentration versus time for each chamber, corrected with the measured air temperature and pressure at the time of sampling.

4.3.8 STATISTICAL ANALYSIS

To test treatment differences of time-series parameters (NO_3^- leaching, DON leaching and N_2O emissions), we used linear mixed effects models (LME): treatments (fertilization, mowing frequency and sward composition) and row / column of the Latin rectangle design were considered fixed effects whereas sampling dates and spatial replications were included as random effects. The LME model included either 1) a variance function that allows different variances of the response variable for the fixed effects (Zuur et al., 2009), 2) a first-order temporal autoregressive process that assumes the correlation between measurements decreases with increasing time difference, or both if these improve the relative goodness of model fit based on the Akaike Information Criterion (AIC) (Crawley, 2007). Pairwise comparisons (T test with Holmes correction) were used as post-hoc tests. To test treatment differences of parameters measured one time (gross N transformation rates, microbial biomass, and N retention efficiency), we used two-way analysis of variance followed by Tukey's HSD post-hoc test. In all tests, if residual plots revealed non-normal distribution or non-homogeneity of variance we used either logarithmic or square root transformation (after adding a constant value if the dataset included negative values) and analyses were repeated. Non-significant effects of treatment interactions and of row and column were removed stepwise from the statistical models if this improved AIC (Crawley, 2007). Correlations between N retention efficiency and soil or plant parameters were assessed using Spearman's rank correlation. A significance level of $P = 0.05$ was used throughout unless stated otherwise. All statistical analyses were performed using the R version 2.11.1 (R Development Core Team, 2009).

4.4 RESULTS

4.4.1 GROSS N TRANSFORMATION RATES AND MICROBIAL BIOMASS

Gross rates of N mineralization ranged from 71 to 1440 mg N m^{-2} d^{-1} with an overall mean of 606 (± 65 SE) mg N m^{-2} d^{-1}. Neither fertilization nor sward composition affected gross N mineralization (Table 4.2). In contrast, gross nitrification rates were higher in the fertilized plots whereas sward composition showed no effect. NH_4^+ immobilization rates varied between 143 and 2356 mg N m^{-2} d^{-1} with an overall mean of 753 (± 100 SE) mg N m^{-2} d^{-1} and were marginally lower in the fertilized than unfertilized plots (Table 4.2). Across treatments, gross N mineralization rates were positively correlated with NH_4^+ immobilization rates ($P < 0.001$, rho = 0.75, n = 23) and gross nitrification rates were negatively correlated with NH_4^+ immobilization rates ($P = 0.013$, rho = -0.52, n = 22). Microbial C was not affected by any of the treatments, but fertilization resulted in marginally lower microbial N. A marginally significant interaction between fertilization and sward composition suggested that the decrease in microbial N caused by fertilization was pronounced in the dicot- and monocot-enhanced swards, which also showed in their highest microbial C:N ratios (Table 4.2).

Table 4.2 Gross rates of soil N cycling and microbial biomass (mean ± SE) of a grassland in the Solling uplands, Germany with different sward compositions and management practices.

Main factors	Treatments	n	Gross N mineralization rate (mg N m^{-2} d^{-1})	Gross nitrification rate (mg N m^{-2} d^{-1})	NH_4^+ immobilization rate (mg N m^{-2} d^{-1})	Microbial C (mg C m^{-2})	Microbial N (mg N m^{-2})	Microbial C:N
Sward composition			P = 0.478	P = 0.367	P = 0.383	P = 0.970	P = 0.736	P = 0.348
	control	10	730 (± 115) a	92 (± 26) a	978 (± 252) a	50295 (± 5707) a	6870 (± 999) a	7.53 (± 0.29) a
	dicot-enhanced	10	530 (± 114) a	112 (± 47) a	665 (± 133) a	46428 (± 4908) a	5921 (± 730) a	8.03 (± 0.21) a
	monocot-enh.	10	570 (± 113) a	123 (± 80) a	640 (± 119) a	48190 (± 3454) a	6263 (± 548) a	7.84 (± 0.33) a
Fertilization			P = 0.513	P = 0.009	P = 0.070	P = 0.203	P = 0.088	P = 0.386
	no	15	654 (± 93) a	48 (± 9) b	891 (± 133) a	51819 (± 2875) a	6797 (± 375) a	7.65 (± 0.18) a
	NPK	15	554 (± 94) a	204 (± 66) a	494 (± 95) b	44789 (± 4441) a	5906 (± 798) a	7.94 (± 0.27) a
Sward composition x Fertilization			P = 0.774	P = 0.504	P = 0.735	P = 0.103	P = 0.097	P = 0.093
control	no	5	824 (± 170) a	77 (± 25) b	1178 (± 311) a	47798 (± 3717) a	6104 (± 226) a	7.82 (± 0.48) a
control	NPK	5	612 (± 151) a	112 (± 55) a	478 (± 125) b	52791 (± 11354) a	7637 (± 2030) a	7.24 (± 0.34) a
dicot-enhanced	no	5	584 (± 168) a	49 (± 8) b	788 (± 180) a	52465 (± 6236) a	6732 (± 861) a	7.85 (± 0.20) a
dicot-enhanced	NPK	5	477 (± 167) a	190 (± 88) a	512 (± 197) b	40391 (± 7128) a	5109 (± 1148) a	8.20 (± 0.38) a
monocot-enhanced	no	5	554 (± 146) a	24 (± 7) b	707 (± 160) a	55194 (± 5166) a	7555 (± 643) a	7.29 (± 0.07) a
monocot-enhanced	NPK	5	586 (± 189) a	368 (± 224) a	473 (± 50) b	41185 (± 1508) a	4972 (± 314) a	8.40 (± 0.56) a

Means with different letter indicate significant differences within main factors (two-way ANOVA at $P \leq 0.05$). Average of the measured bulk density of 0.79 g cm^{-3} and a sampling depth of 0.00-0.05 m were used to convert rates on a dry mass basis to area-based rates.

4.4.2 WATER BALANCE AND N LOSSES

During the experimental period, total annual precipitation was 1001 mm in 2009 and 1083 mm in 2010. Modelled annual evapotranspiration was 507 mm in 2009 and 484 mm in 2010. Modelled drainage flux was 441 mm in 2009 and 609 mm in 2010. At all three soil depths, measured matrix potential was correlated with modelled matrix potential (Spearman's correlation coefficients ranged from 0.58 to 0.75 with $P < 0.02$, tested for each depth). In both years, modelled drainage flux was negligible in the summer months and strongly increased to values of more than 3 mm d^{-1} in autumn. Parallel to drainage flux, NO_3^- leaching losses were also negligible in the summer months (Figure 4.1 a, b, c). In fertilized plots, NO_3^- leaching strongly increased during autumn. The first year of treatment (2009) showed the peak of NO_3^- leaching only during the spring of the following year while the second year of treatment (2010) showed the peaks of NO_3^- leaching immediately during the fall of the same year. In 2009, fertilization was the only factor that influenced NO_3^- leaching ($P = 0.026$; unfertilized plots: 0.53 (± 0.09 SE) mg NO_3^--N m^{-2} d^{-1}; fertilized plots: 1.15 (± 0.15 SE) mg NO_3^--N m^{-2} d^{-1}). In 2010, fertilization ($P = 0.000$; unfertilized plots: 2.73 (± 1.00 SE) mg NO_3^--N m^{-2} d^{-1}; fertilized plots: 17.18 (± 2.95 SE) mg NO_3^--N m^{-2} d^{-1}) and mowing frequency ($P = 0.033$; cut once per year: 12.96 (± 2.87 SE) mg NO_3^--N m^{-2} d^{-1}; cut three times per year: 6.61 (± 1.38 SE) mg NO_3^--N m^{-2} d^{-1}) influenced NO_3^- leaching. An interaction ($P = 0.011$) between these factors showed that the increase in NO_3^- leaching losses caused by fertilization was only significant in plots cut once per year whereas plots cut three times per year were not affected. Sward composition did not affect NO_3^- leaching.

Leaching of DON ranged between 0 to 1.9 mg N m^{-2} d^{-1} with an overall mean of 0.26 (± 0.01 SE) mg N m^{-2} d^{-1} in 2009. None of the treatment factors influenced DON leaching (Figure 4.1 d, e, f). In 2010, DON leaching increased to an overall mean of 0.6 (± 0.10 SE) mg N m^{-2} d^{-1}, ranging from 0 to 13.06 mg N m^{-2} d^{-1}. We detected interaction effect between fertilization and mowing frequency ($P = 0.006$): fertilization increased DON leaching in plots cut once per year but not in plots cut three times per year. No effect of sward composition on DON leaching was detected.

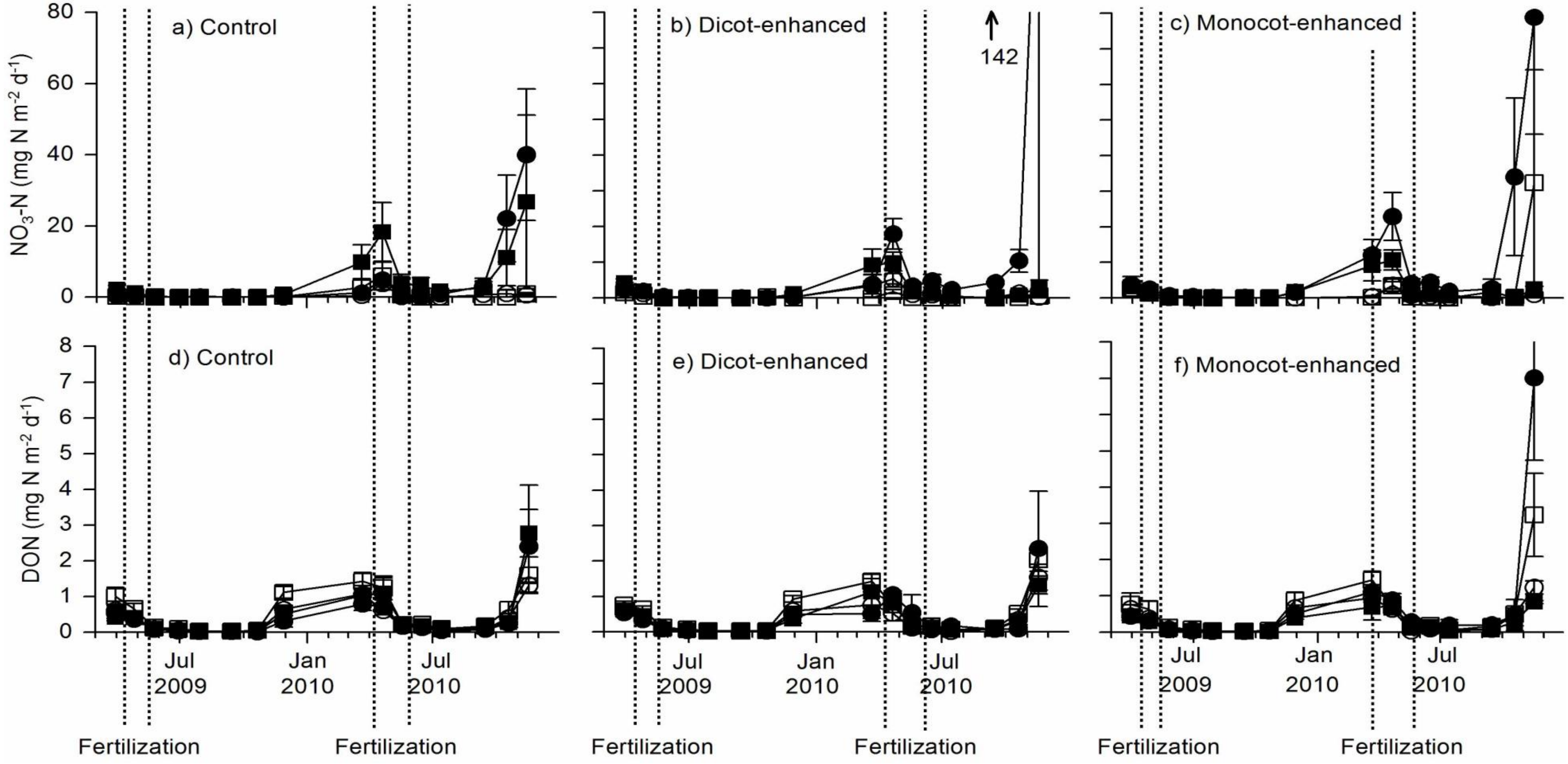


Figure 4.1 Nitrate (NO_3^-) and dissolved organic nitrogen (DON) leaching losses (± SE, n=6) at 0.5-0.6-m depth from a grassland in the Solling uplands, Germany with different sward compositions and management practices: one mowing per year without fertilization (○), one mowing per year with fertilization (●), three mowings per year without fertilization (□), three mowings per year with fertilization (■).

In 2009, fertilization ($P = 0.000$; unfertilized plots: 0.14 (± 0.05 SE) mg N_2O-N m^{-2} d^{-1}; fertilized plots: 0.50 (± 0.22 SE) mg N_2O-N m^{-2} d^{-1}), mowing frequency ($P = 0.031$; cut once per year: 0.41 (± 0.22 SE) mg N_2O-N m^{-2} d^{-1}; cut three times per year: 0.23 (± 0.07 SE) mg N_2O-N m^{-2} d^{-1}) and their interaction ($P = 0.009$) influenced N_2O emissions (Figure 4.2 a, b, c). Fertilized plots showed an increase in N_2O fluxes, especially following the second fertilizer application in May. However, this increase was only significant for the plots cut once per year. Unfertilized plots only showed a marginal increase in N_2O fluxes during the summer months. In 2010, fertilization ($P = 0.000$; unfertilized plots: 0.17 (± 0.05 SE) mg N_2O-N m^{-2} d^{-1}; fertilized plots: 0.73 (± 0.24 SE) mg N_2O-N m^{-2} d^{-1}) also affected N_2O emissions (Figure 4.2 d, e, f). Again, the increase in N_2O emissions occurred in the beginning of July after the second fertilization in June and the impact of fertilization tended to be stronger in plots cut once per year, but, unlike 2009, this treatment interaction was not significant ($P = 0.108$). In both years, N_2O uptake predominantly occurred in unfertilized plots. There was no impact of sward composition on N_2O emissions.

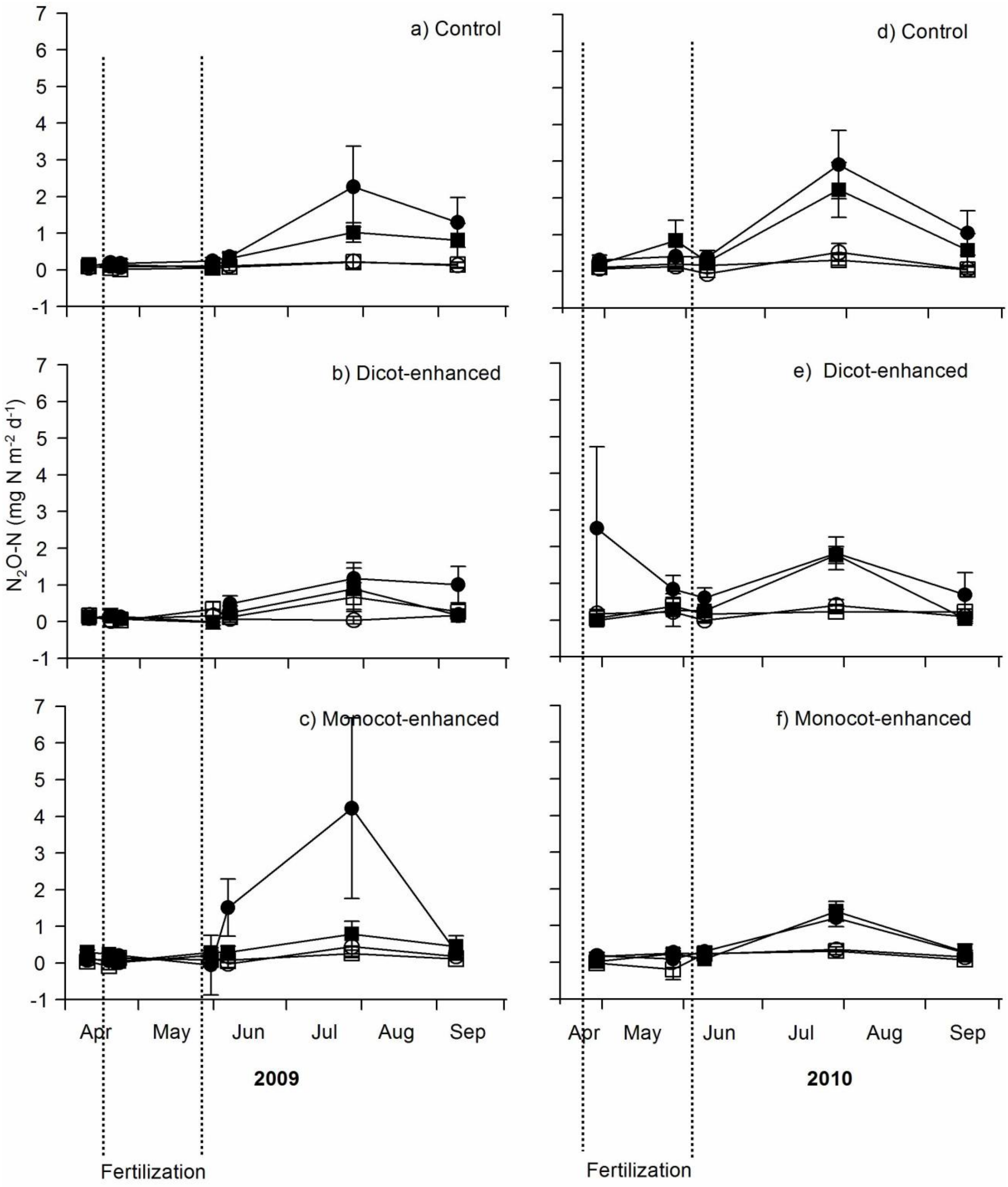


Figure 4.2 Nitrous oxide (N_2O) emissions (± SE, n=6) from a grassland in the Solling uplands, Germany with different sward compositions and management practices: one mowing per year without fertilization (○), one mowing per year with fertilization (●), three mowings per year without fertilization (□), three mowings per year with fertilization (■).

4.4.3 N RETENTION EFFICIENCY

We calculated N retention efficiency only for 2010 when gross rates of N mineralization was measured, and this ranged from 0.842 to 0.999 with an overall mean of 0.976 (± 0.02 SE). Fertilization decreased N retention efficiency and explained the variance more than the sward composition (Table 4.3). N retention efficiency was highest in the control sward followed by the dicot-enhanced and monocot-enhanced swards. Only the difference between control and monocot-enhanced swards was significant. Across all treatments, N retention efficiency was correlated with microbial NH_4^+ immobilization, microbial N and C, marginally correlated with plant N uptake, and not correlated with harvested plant biomass (Table 4.4).

Table 4.3 N retention efficiency of a grassland in the Solling uplands, Germany with different sward compositions and fertilization.

Main factors	Treatments	n	N retention efficiency [1 - (mg N losses m^{-2} d^{-1} / mg gross N mineralization m^{-2} d^{-1})]	SE	*P*	Variance explained (%)
Sward composition					0.046	16.69
	control	12	0.991 a	0.005		
	dicot-enhanced	12	0.981 ab	0.005		
	monocot-enhanced	12	0.963 b	0.016		
Fertilization					0.007	22.06
	no	18	0.995 a	0.002		
	NPK	18	0.960 b	0.011		

Means with different letter indicate significant differences among treatments within main factors (two-way ANOVA with Tukey’s HSD at $P \leq 0.05$).

Table 4.4 Spearman rank correlations between N retention efficiency and plant and soil-microbial parameters of a grassland site across different treatments of management practices in the Solling uplands, Germany.

Parameter	Spearman's rho	*P*
Plant N uptake *	0.338	0.074
Harvested plant biomass *	- 0.031	0.871
Microbial NH_4^+ Immobilization	0.560	0.006
Microbial biomass C	0.364	0.053
Microbial biomass N	0.417	0.025

* Plant N uptake was reported by Keuter et al. (2013) and harvested biomass was reported by From et al. (2011).

4.5 DISCUSSION

4.5.1 GROSS N TRANSFORMATION RATES AND MICROBIAL BIOMASS

Gross N mineralization and NH_4^+ immobilization rates were larger than those reported for temperate grasslands in northeastern USA (Corre et al., 2002) and in the UK (Jamieson et al., 1999; Ledgard et al., 1998). Gross nitrification rates were in the same range as those reported for fertilized and unfertilized temperate grasslands in Belgium (Accoe et al., 2004) and Northern Ireland (Watson and Mills, 1998). The marginally lower NH_4^+ immobilization and larger nitrification rates in the fertilized compared to the unfertilized plots demonstrated that fertilization decreased microbial retention of NH_4^+, possibly resulting to competitive advantage of the nitrifiers. This was also supported by the negative correlation between NH_4^+ immobilization rates and gross nitrification rates across treatments. A negative effect of N fertilization on microbial N immobilization in a grassland was also reported by Ledgard et al. (1998). Near to our grassland site, fertilized plots of beech forest (receiving high ambient N deposition of ≥25 kg N ha^{-1} y^{-1} and on top of this chronic N fertilization of 140 kg N ha^{-1} y^{-1} for 9 years) also showed reduced NH_4^+ immobilization rates relative to gross N mineralization rates compared to the control beech plots that showed similar NH_4^+ immobilization and gross N mineralization rates (Corre et al., 2003). The reduced NH_4^+ immobilization in fertilized plots of beech forest as well as our grassland site was paralleled by marginal reduction of microbial N, suggesting reduced N demand for build-up of microbial biomass. Other studies on chronic, high N fertilization consistently showed reduced microbial biomass (Compton et al., 2004; DeForest et al., 2004), which may be because addition of N would favored certain microbial community over the others. As to the effect of sward composition, the marginal reduction in microbial N and marginal increases in microbial C:N ratios due to fertilization that was more distinct in the dicot- and monocot-enhanced swards rather than in the control swards suggest that the control sward plots were able to take the added N without changes in microbial biomass size and composition (e.g. no change in microbial C:N ratio). Such resilience of the control swards could be due to the adaptation feedback between soil microbial biomass and plant functional group composition, which may have developed from decades of extensive management practices in this site. Conversely, modifying the plant functional diversity of this grassland was unable to counteract the effect of N addition on depressing microbial N pool and changes in microbial composition.

4.5.2 N LOSSES

Increased nitrification rates in fertilized plots had resulted to increased N losses compared to unfertilized plots. In 2009, the average rate of NO_3^- leaching was lower than those reported by other studies in fertilized temperate grasslands (e.g. Christian and Riche, 1998). The larger increase in NO_3^- leaching in 2010 than in 2009 in fertilized plots was mainly due to the larger increases in NO_3^- concentrations of the leachate rather than any change in magnitude of the drainage fluxes, which indicated the continuous nature of fertilization effect on leaching. While in the first year of treatment (2009) the grassland was still able to retain most of the soil available N (e.g. higher biomass production and plant N uptake in 2009 compared to 2010; Keuter et al., 2013), this has diminished in the second year (2010) of treatment. Baker and Johnson (1981) reported a similar pattern of increased NO_3^- concentrations in drainage water after three years of fertilization to a cropland in central Iowa, USA. Also, NO_3^- concentrations in leachate of > 60 mg NO_3-N L^{-1}, similar to our findings in 2010, have been reported for fertilized (120 kg N y^{-1}) *Miscanthuus* grassland in Great Britain (Christian and Riche, 1998). DON leaching rates from our study were an order of magnitude lower than NO_3^- leaching rates and were lower compared to values reported from several agricultural studies, which range from 0.3 to 127 kg DON ha^{-1} y^{-1} (e.g. van Kessel et al., 2009). Our low DON leaching rates may be related to the relatively low abundance of legumes, exclusive use of mineral fertilizer, and exclusion of grazing (van Kessel et al., 2009), all suggesting low possible sources of DON. Our measured N_2O fluxes were comparable to other studies conducted in temperate grasslands. Median emission rates of 0.1 mg N_2O-N m^{-2} d^{-1} (unfertilized) and 0.2 mg N_2O-N m^{-2} d^{-1} (fertilized with 200 kg N ha^{-1} y^{-1}) were reported for a grassland in Switzerland (Flechard et al., 2005), and mean emission rates of 0.1 - 1.2 mg N_2O-N m^{-2} d^{-1} were reported for a fertilized (300 kg N ha^{-1} y^{-1}) grassland in Scotland (Jones et al., 2005).

The observed high N losses following fertilizer application, independent of the pathway (NO_3^- leaching, DON leaching and N_2O emissions), is in line with our first hypothesis. Similar findings have been reported by studies on NO_3^- leaching (e.g. Christian and Riche, 1998), DON leaching (Dijkstra et al., 2007; Fang et al., 2009) and N_2O fluxes (e.g. Jones et al., 2005). Our finding that increasing mowing frequency can decrease leaching of NO_3^- and DON and reduce N_2O emissions from fertilized plots is at least partly in line with our second hypothesis that mowing reduces N losses. A decrease of N_2O emissions with increasing mowing frequency was also reported for a 50-year established non-grazed grassland in Germany, and was interpreted as a result of a higher plant N uptake and thus lower nitrification and denitrification rates (Kammann et al., 1998). In the same site as our present study, we observed increases in plant N response efficiency (i.e. plant biomass produced per unit of plant-available N) and plant N uptake efficiency (i.e. the proportion of plant-available N that is taken up by plants) with increasing mowing frequency (Keuter et al., 2013), which may be caused by overcompensatory regrowth following mowing (Guitian and Bardgett, 2000). Furthermore, microbial immobilization of N may also contribute to the influence of mowing since defoliation of plants increases rhizodeposition (Holland et al., 1996). An increased soil microbial biomass as a result of defoliation by cutting of grasses was reported by Guitian and Bardgett (2000). Thus, more frequent mowing may stimulate C input from plants to the soil which, in turn, may stimulate microbial N immobilization.

We observed no effect of sward compositions on NO_3^- and DON leaching and on N_2O fluxes, which appears to be in contrast with some other studies. Decreasing soil extractable N caused by increasing plant species or functional group diversity has been reported (e.g. Tilman et al., 1996). On the other hand, plant species diversity effects on NO_3^- and DON leaching are not consistent. Hooper and Vitousek (1998) did not observe plant diversity effects on NO_3^- leaching in artificial grassland plots in California whereas Scherer-Lorenzen et al. (2003) observed a decrease in annual NO_3^- leaching losses with increasing plant functional group richness in an artificially established grassland in Germany. This effect however was only significant for communities containing legumes and hence may have been an effect of reduction in legume abundance rather than an effect of species richness per se (Scherer-Lorenzen et al., 2003). In contrast, significant decreases in mineral N and increases in DON leaching rates with increasing grass species diversity were reported for planted grassland plots in Minnesota, USA (Dijkstra et al., 2007). This was explained by a higher productivity and consequently higher organic N pools, higher microbial activity and higher DON production at higher levels of species richness. The study of Dijkstra et al. (2007) had strongly contrasting levels of species richness (1 versus 16 species) whereas the number of species in our site was at the high level (12-18 species) with only small differences among sward compositions. A review has shown that diversity effects are most pronounced in the range of one to five species (Wrage et al., 2011). This may be the reason why we were not able to detect plant diversity effects in our study. Also, in our other study (conducted in a ~20-year established, grazed grassland in Germany) N_2O and NO fluxes were not affected by plant diversity (Hoeft et al., 2012).

4.5.3 N RETENTION EFFICIENCY

The reduced N retention efficiency in the fertilized compared to unfertilized plots supported our hypothesis. This fertilization effect was caused by increased N losses rather than changes in gross N mineralization rates as the latter were not affected by fertilization. The increased N losses from fertilized plots were the result of increased gross nitrification rates which, in turn, were due to marginal decreased in NH_4^+ immobilization rates. In the same study site for the same study years, we also observed that fertilized plots had lower plant N response efficiency and plant N uptake efficiency than unfertilized plots (Keuter et al., 2013). Thus, decrease in retention both through microbial immobilization and plant uptake caused decrease in N retention efficiency under fertilization.

Apart from fertilization, sward composition also affected N retention efficiency. The control sward had the highest N retention efficiency, which was in contrast to our hypothesis. In our earlier study, we also found higher N response efficiency and marginally higher N uptake efficiency for the control sward compared to the monocot- or dicot-enhanced swards (Keuter et al., 2013). The control sward has adapted to local soil and environmental conditions over decades of extensive management practices, such that this sward has probably developed towards a composition that is most efficient in retaining soil available N. Correlations of N retention efficiency with NH_4^+ immobilization and microbial biomass and only marginal correlation with plant N uptake (Table 4.4) corroborated that microbial assimilation may play a more important role than plant uptake on the efficiency with which N is retained in the soil-plant system. This supports the studies of Hooper and Vitousek (1997, 1998) who showed that microbial N immobilization may be a more important pathway for N retention than plant N uptake, and that indirect plant effects through microbial immobilization may equal or even exceed direct plant uptake effects on nutrient retention.

4.6 CONCLUSIONS

Fertilizer application was the dominant factor influencing N losses and N retention efficiency, of which the latter was strongly influenced by microbial NH_4^+ immobilization. Our results also show that intensive mowing can partly reduce the high N losses following N fertilization. We found indications that control swards that have developed under decades of extensive grassland management had the highest efficiency in retaining N. This high N retention efficiency was caused by high plant N response efficiency and N uptake efficiency (Keuter et al., 2013) as well as high microbial NH_4^+ immobilization in the soil. N retention efficiency responded to both sward compositions and fertilization whereas N losses were only sensitive largely to fertilization, signifying that N retention efficiency was a more sensitive index than just N losses when evaluating environmental sustainability of grasslands. Furthermore, our study underlines the importance of microbial immobilization for the retention of N. In contrast to other more commonly used indices for plant response (e.g. N response efficiency and N uptake efficiency); N retention efficiency reflected the importance of microbial immobilization as a pathway for N retention in the plant-soil system.

CHAPTER

5

SYNTHESIS

5.1 SYNTHESIS AND DISCUSSION OF THE MAJOR FINDINGS

In the synthesis, I aim to integrate the major findings obtained from our two studies (BIOMIX & GRASSMAN) and to draw more general conclusions about the importance of management and plant diversity for the functioning of grassland ecosystems. We analyzed a grazed grassland (BIOMIX) and a cut grassland (GRASSMAN) with a particular focus on N losses (N_2O and NO emissions, N leaching) and N dynamics (net and gross mineralization and nitrification); and calculated N response efficiency and N retention efficiency as a measure for ecosystem functioning. In BIOMIX, the grassland management differed in the type of livestock (cattle and sheep). In GRASSMAN, the management differed in fertilization (180 – 30 – 100 kg NPK ha^{-1} yr^{-1} and no fertilization) or in mowing frequency (once and thrice per year). In both study sites, sward botanical compositions were manipulated by specific herbicide application, i.e. by reducing the abundance of certain plant functional groups.

5.1.1 THE EFFECTS OF LIVESTOCK GRAZING ON ECOSYSTEM FUNCTIONING

In BIOMIX, the type of grazer (cattle and sheep) had an effect on gaseous N losses on pastures. The hypothesized larger N oxide losses on sheep-grazed paddocks compared with those grazed by cattle could be confirmed for cumulative NO emissions in grass swards. N losses are linked with the N supply in the soil, which on pastures mainly depend on the N applied with excreta. Our hypothesis was based on the amount and frequency of urinations and defecations and the number of cattle and sheep per paddock. The measured gaseous N losses were dominated by the occasional measurement of urine and dung hotspots, which we attributed to an apparently more even spread of sheep excreta compared to cattle excreta (1.16% of the paddock area per day for sheep grazing, 0.81% for cattle grazing). However, per excreta patch, larger N losses were found for cattle than for sheep excreta due to larger N supply in the soil.

5.1.2 THE EFFECTS OF MANAGEMENT INTENSITY (FERTILIZATION AND MOWING) ON ECOSYSTEM FUNCTIONING

A pattern similar to the effect of livestock grazing in BIOMIX was found in GRASSMAN: fertilization was the most important factor influencing N losses with hypothesized larger N losses on the fertilized plots. The determined N losses were relatively small compared to reported N_2O emissions of Flechard et al. (2005) or NO_3^- leaching losses of Christian and Riche (1998) on ungrazed and fertilized grassland sites. We hypothesized increasing N losses with decreasing mowing frequency, as our findings showed a decrease in N_2O emissions and NO_3^- leaching losses on the intensively mown and on the fertilized plots. Our results corresponded to the findings of Kammann et al. (1998) who reported a decrease of N_2O emissions with increasing mowing frequency on a German grassland site. Our findings indicated that plots cut thrice per year can counteract the negative effects of N fertilization regarding N losses. Especially the combination of fertilization and only one cut per year caused larger N losses via N_2O emissions and NO_3^- leaching.

In GRASSMAN, fertilization largely influenced N response efficiency. The hypothesized smaller N response efficiency of the fertilized compared to unfertilized plots was caused by decreases in both N uptake efficiency and N use efficiency. As we assumed, mowing frequency also influenced N response efficiency via increasing N response efficiency in plots cut thrice per year. We attributed that to increasing N uptake efficiency, because N use efficiency was either showing the opposite trend or was not affected by mowing at all. The increased N uptake efficiency in plots cut thrice per year was paralleled by increased biomass yield and N concentration in biomass. N retention efficiency was also influenced by fertilization. We hypothesized smaller efficiencies in fertilized treatments compared to unfertilized treatments. The effect of fertilization was due to larger N losses rather than gross N mineralization rates which were not affected by the N surplus. Under fertilization, the decrease in N retention efficiency was caused through microbial NH_4^+ immobilization. N retention efficiency was not calculated for the plots differing in mowing frequency. That was due to the fact that mowing frequency had no effect on net rates of mineralization and nitrification in 2009. Based on these results, we decided to sample gross rates of mineralization and nitrification only on plots cut once per year in 2010.

5.1.3 THE EFFECT OF SWARD COMPOSITION ON ECOSYSTEM FUNCTIONING

In BIOMIX, we evaluated if sward composition has an effect on N_2O and NO emissions in grazed grassland. Our findings for N oxide emissions were not consistent with our hypothesis of smaller emissions in more diverse plots, but were supported by the study of Seither et al. (2012) who also found no differences in N yield of different sward compositions in the same experiment. Combining the data from our two experiments (main and controlled application experiment) within the BIOMIX study, we can conclude that sward composition was less important compared to the effect of grazers on N oxide emissions. In GRASSMAN, neither N losses (N_2O emissions and NO_3^- leaching) nor N turnover (net and gross mineralization) was significantly affected by sward composition. This may imply that regardless of the plant functional composition in this grassland, N supply through mineralization as well as N use by the vegetation will be similar.

In GRASSMAN, sward composition influenced N response efficiency. The trends of N response efficiency and N uptake efficiency were similar whereas N use efficiency exhibited either opposite patterns or was not affected by sward composition. This emphasized that N uptake efficiency influenced N response efficiency more than N use efficiency. We assumed that N response efficiency decreases in the order dicot-enhanced swards ≥ control swards > monocot-enhanced swards, but found larger efficiencies in control compared to dicot- and monocot-enhanced swards. N retention efficiency was affected by sward composition. Contrary to our hypothesis, again untreated control swards were most efficient in N retention efficiency. We attributed the efficient N response and N retention to the ratio of ~30% dicots and ~70% monocots found in the control swards. This ratio developed in response to N limited conditions and previous management practices. In equilibrium with these conditions, this plant community would be expected to result in efficient N response and N retention. Both, an artificial increase in the proportion of dicots as well as an increase in the proportion of monocots resulted in smaller efficiencies, even if that leads to a more balanced proportion.

5.2 CONCLUDING RESULTS

The indices of N response and N retention efficiency might be suitable indices and useful as measures for ecosystem functioning in temperate managed grassland. First, we considered biomass yield per unit of plant available N and second, we measured N losses per unit of soil available N retained in an ecosystem and did not focus on N losses exclusively. At the ecosystem level, these indices can be beneficial for the assessment of grassland with regard to sustainable land-use. Optimize N response and N retention may not be a target of most farmers, but they are appropriate tools to evaluate the consequenses of grassland management practices, which farmers may employ to maximize profit, on environmental quality. Our results showed that the management of grassland is the most important factor determining ecosystem functioning. Fertilization, mowing frequency and livestock grazing largely influenced N response efficiency, N retention efficiency and N losses. Sward species composition was still a factor influencing determining ecosystem functioning but to a minor importance in this context. These trends were also found in the same experiment by Rose (2011b) for the effect of land-use intensification and biodiversity on grassland biomass, water use and plant functional traits. Grassland management determined structure, productivity and ecosystem processes while species richness was less important.

5.3 IMPLICATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

Combining all findings, we could speculate if plant diversity is important in the context of grassland management. If we consider only N losses, the management (grazing, fertilization or mowing frequency) of the grazed and the mown grasslands have a major impact, while sward composition was insignificant. Calculating the efficiencies of N response and N retention in GRASSMAN, sward composition became a significant factor. Consequently, it is important to consider not only N losses, but also evaluating available N in the soil (gross N mineralization and nitrification) and available N for the plant (net N mineralization and nitrification) and thus, calculating the efficiencies of N response and N retention. But the use of these indices on ecosystem level should be tested in future studies.

Biodiversity studies were conducted in artificial grassland, e.g. Jena Experiment, Biodepth or Cedar Creek (Marquard et al., 2009; Spehn et al., 2005; Tilman et al., 1996) or as observational studies in permanent grassland (e.g. Kahmen et al., 2005). Our grassland studies (BIOMIX & GRASSMAN) combined the advantages of artificial and observational studies - e.g. real world, all ecosystem processes, controlled experiment, interactions can be tested. Starting with natural vegetation that was manipulated by removing certain species or functional groups, it can be tested for a set of defined environmental conditions (Diaz et al., 2003). In our studies, we used the approach of plant species removal by herbicides. In our pasture experiment (BIOMIX), the herbicide treatment on forbs lasted for three years. There already was a large invasion of species from diverse into grass swards. Grassland removal experiments treated by herbicides like our studies are appropriate for short-term experiments. After this time, another herbicide treatment should be carried out and that may have an impact on the N cycling again. Further studies are required due to sensitivity of plant functional groups to different herbicides. Finally, we suggest that it might be more advantageous to reduce the N input through excreta or mineral N fertilizer than to increase plant diversity in order to reduce N losses from grassland ecosystems.

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APPENDIX

Figure A.1 BIOMIX experiment with different swards in spring 2008.

Figure A.2 BIOMIX experiment with cattle and sheep grazing in spring 2008.

Figure A.3 GRASSMAN experiment in summer 2009. Photograph: Laura Rose.

Table A.1 Biomass yield of a grassland under different management practices in the Solling Mountains, Germany.

Main factors	Treatments	n	2009				2010			
			Biomass yield * (kg ha^{-1} yr^{-1})	SE	*P*	Variance explained (%)	Biomass yield * (kg ha^{-1} yr^{-1})	SE	*P*	Variance explained (%)
Sward composition					0.648	0.28			0.007	2.34
	control	24	7922.82 a	597.58			6132.01 a	536.79		
	monocot-enhanced	24	7763.22 a	508.89			5237.22 b	498.97		
	dicot-enhanced	24	7586.48 a	482.80			5467.27 ab	467.66		
Mowing frequency					0.000	12.01			0.000	34.92
	once per year	36	6855.40 b	270.55			4146.09 b	172.92		
	thrice per year	36	8659.62 a	517.05			7078.25 a	446.14		
Fertilization					0.000	56.74			0.000	24.71
	no	36	5797.13 b	167.30			4378.75 b	210.25		
	NPK	36	9717.88 a	373.43			6845.59 a	469.71		
Mowing frequency x fertilization					0.000	9.89			0.000	16.97
once per year	no	18	5713.66 c	160.04			3934.60 b	248.95		
once per year	NPK	18	7997.14 b	349.87			4357.58 b	236.35		
thrice per year	no	18	5880.61 c	297.99			4822.89 b	311.13		
thrice per year	NPK	18	11438.62 a	321.60			9333.61 a	352.58		

* Biomass yield data was reported by From et al. (2011).

Mean values with different letter indicate significant differences among treatments within main factors or within an interaction of main factors (three-way ANOVA with Tukey HSD at $P \leq 0.05$).

Treatment interactions that were not significant are not reported.

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DECLARATION OF ORIGINALITY AND CERTIFICATE OF AUTHORSHIP

I, Ina Hoeft, hereby declare that I am the sole author of this dissertation entitled "GRASSLAND MANAGEMENT AND DIVERSITY EFFECTS ON SOIL NITROGEN DYNAMICS AND LOSSES". All references and data sources that were used in the dissertation have been appropriately acknowledged. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure. I certify that the manuscripts presented in chapters 2, 3 and 4 have been written by me as first author.

Göttingen, January 2012 ______________________________ (Ina Hoeft)

CURRICULUM VITAE

PERSONAL INFORMATION

Name	Ina Hoeft
Date of birth	10.10.1974
Place of birth	Stade

EDUCATION AND RESEARCH EXPERIENCE

03/2008 – 08/2011	GEORG-AUGUST-UNIVERSITY OF GÖTTINGEN, SOIL SCIENCE OF TROPCIAL AND SUBTROPICAL ECOSYSTEMS Scholarship in the Cluster of Excellence „Functional Biodiversity Research“
Since 09/2011	GEORG-AUGUST-UNIVERSITY OF GÖTTINGEN, SOIL SCIENCE OF TROPCIAL AND SUBTROPICAL ECOSYSTEMS Research Assistant at the Büsgen Institute

PhD/Doctorate

Since 03/2008	GEORG-AUGUST UNIVERSITY GÖTTINGEN, CENTRE FOR BIODIVERSITY AND ECOLOGY Ph.D. Program: Biological Diversity and Ecology

Academic Studies

2000- 2007	UNIVERSITY OF LÜNEBURG DIPLOMA (EQUIVALENT TO M.SC.) IN ENVIRONMENTAL SCIENCES Thesis: “Impact of land use history on soil ecological properties in Eastern Mediterranean (Israel, Galilee)” Major: Ecology (Soil Science) and Environmental Communication

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