Origin and regulation of soil N_2O and NO_x fluxes from coniferous and deciduous temperate forests exposed to chronic high N depositions

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In remembrance

dedicated to my uncle

- WOLFGANG KERSTEN -

"During the last few
decades, humans have emerged as
a new force of nature. We are modifying
physical, chemical, and biological systems
in new ways, at faster rates, and over larger
spatial scales than ever recorded on Earth. Hu-
mans have unwittingly embarked upon a grand
experiment with our planet.The outcome
of this experiment is unknown, but has
profound implications for all
of life on Earth."

— Jane Lubchenco, Science, 1998 —

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List of Abbreviations and Acronyms

A	Cross section area of a soil sample
AIC	Akaike Information Criterion
Al	Aluminium
Ar	Argon
ATF	Accumulation type forest
B	Fitting parameter
B site	Biebergemuend
BD	Bulk density
BEP	Background emission pattern
BS	Base saturation
C	Carbon
c	Gas concentration
c_A	Gas concentration in ambient air
c_C	Gas concentration in the chamber
Ca	Calcium
CEC	Cation exchange capacity
CH_4	Methane
CLRTAP	Convention on Long-Range Transboundary Air Pollution
CO_2	Carbon dioxide
CrO_3	Chromium trioxide
Cu	Copper
Da	Diffusion coefficient in air
DNRA	Dissimilatory nitrate reduction to ammonium
Ds	Apparent diffusion coefficient
Ds/Da	Relative diffusion coefficient
DTF	Degradation type forest
ECD	Electron capture detector
EEP	Event emission pattern
EF	Emission factor for N oxides from N depositions
EFF	EF calculated using the fraction method
EFF_1	EF calculated using the fraction method, mean value for the Solling beech stand for 1990–98
EFF_2	EF calculated using the fraction method, value for the Solling beech stand for 2007–08
EF_{15N}	EF calculated using ^{15}N labelling techniques
EF_{FB}	EF derived from N fertilisation studies or deposition gradients
EF_R	EF calculated using the regression approach
EF_{R1}	EF calculated using the regression approach, value for the Solling beech stand for 2007–08
EF_{R2}	EF calculated using the regression approach, value for the Solling beech stand for 2007–08, considering soil temperature
EXMAN	EXperimental MANipulation of forest ecosystems
F layer	Fermented layer of the surface organic layer
Fe	Iron
GC	Gas chromatograph
H	Hydrogen
H layer	Humified layer of the surface organic layer
H site	Huenfeld

Ha site	Haard
HIP model	"Hole in the pipe" model
HNO_2	Nitrous acid
HNO_3	Nitric acid
H_2O	Water
H_2O_2	Hydrogen peroxide
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
IPCC	Intergovernmental Panel on Climate Change
IRMS	Isotope ratio mass spectrometer
K	Potassium
k	Fitting parameter
K_2SO_4	Potassium sulphate
$K_2S_2O_8$	Potassium persulphate
L layer	Litter layer of the surface organic layer
L site	Luess
m	Mass; tortuosity parameter (chapter 5)
Mg	Magnesium
Mn	Manganese
MRT	Mean residence time
N	Nitrogen
^{14}N	Stable N isotope, frequency of occurence 99.634%
^{15}N	Stable N isotope, frequency of occurence 0.366%
N_2	Dinitrogen
N_{min}	Mineral soil nitrogen
N_{org}	Organic nitrogen
N_t	Total soil nitrogen
N site	Neuhaeusel
Na	Sodium
NaOH	Sodium hydroxide
NH_2OH	Hydroxylamine
NH_3	Ammonia
NH_4^+	Ammonium
NH_4Cl	Ammonium chloride
NITREX	NITRogen saturation EXperiment
NO	Nitric oxide
NO_2	Nitrogen dioxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_x	Nitric oxide + nitrogen dioxide
N_2O	Nitrous oxide
N_2O-NDFD	N_2O-N derived from deposition
O	Oxygen
^{16}O	Stable O isotope, frequency of occurence 99.762%
^{18}O	Stable O isotope, frequency of occurence 0.2%
O_2	Molecular oxygen
O_3	Ozone
OH·	Hydroxyl radical
PE	Polyethylene
PMQ model	Penman-Millington-Quirk model
PVC	Polyvinyl chloride
q	Rate of gas diffusion

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S site	Solling
SEP	Seasonal emission pattern
(S)OM	(Soil) organic matter
$ST_{5/10}$	Soil temperature in 5 or 10 cm soil depths
STFa	Steady state type forest with mull type humus
STFb	Steady state type forest with moder type humus
UNECE	United Nations Economic Commission for Europe
V_C	Chamber volume
WFPS	Water-filled pore space
z	Length of soil sample
$\delta^{15}N$	$^{15}N/^{14}N$ ratio expressed relative to the standard (N_2 in air)
ε	Air-filled porosity
Φ	Total soil porosity

Summary

Nitrogen (N) cycling in Europe and in many other parts of the world has been massively accelerated as a result of agricultural and industrial activities in the last decades. As a consequence, forest ecosystems have been exposed to increasing deposition rates of reactive N, which may increase soil N availability. Increased N availability in turn may alter nitrous oxide (N_2O) and nitric oxide (NO) emissions, which play a major role in atmospheric chemistry and contribute to global warming. Several studies reported that European temperate forests receiving chronic high N depositions are important sources for N_2O and NO. Despite high research efforts in the last two decades, there is still insufficient knowledge about the importance of N depositions and forest type on N oxide fluxes from temperate forest soils. Therefore, the objectives of the present study were (i) to investigate the origin of N oxide emissions from temperate forest soils exposed to chronic high N depositions and to quantify the contribution of N depositions and turnover of leaf litter N to N_2O emissions and (ii) to determine the factors that regulate N oxide fluxes in temperate forest soils with special regard to the impact of forest type and soil gas diffusivity. In the present study, for the first time ^{15}N labelling techniques were applied to calculate emission factors (EFs) for N_2O from temperate forest soils.

Research activities took place at six German Level II beech stands (*Fagus sylvatica*), at two roofed Norway spruce stands (*Picea abies* (L.) Karst.; Solling roof project), and at an adjacent unroofed spruce stand at Solling. Tracer experiments were conducted where N depositions and beech leaf litter N were labelled using the stable isotope ^{15}N. Nitrous oxide fluxes and ^{15}N isotope abundances in N_2O were measured using the closed chamber method combined with ^{15}N isotope analyses. The open dynamic chamber method was used for NO_x ($NO_x = NO + NO_2$) flux measurements. In-situ and laboratory incubation experiments were conducted to determine net N turnover rate. Soil gas diffusivity was measured on undisturbed soil columns.

The present study demonstrated that N oxide emissions from German forest soils are mainly the result of chronic atmospheric N inputs whereas turnover of total leaf litter N in the surface organic layer probably provides a minor contribution to total N_2O emissions, as shown by the long-term ^{15}N tracer experiment at the Solling beech stand. Only 6–13% of total annual N_2O emissions were derived from recent (last year) N depositions at the Solling beech and spruce stands, whereas the largest part of N_2O emissions was derived from N depositions of former years, which were accumulated in soils and remobilised more than one year after

deposition. The present study demonstrated that calculation methods of EF used in literature are not comparable and may even be incorrect. The annual EFs for N_2O from deposited N in the present study were 0.1% for the spruce stand and 0.6% for the beech stand. Emission factors for N_2O including recent depositions and remobilised accumulated depositions were approx. 0.9% for the spruce stand and approx. 11% for the Solling beech stand suggesting that the impact of N depositions on N_2O emissions strongly depends on forest type. In the beech stand, seasonal N_2O emissions with high emissions during the summer months were measured (2.6 ± 0.6 kg N ha^{-1} yr^{-1}) compared to low emissions throughout the whole year in the spruce stand (0.3 ± 0.1 kg N ha^{-1} yr^{-1}). Conditions favouring denitrification, which was shown to be the main source for N_2O in the investigated stands, are responsible for high gaseous N losses in the beech stand. Long-term reduction of N depositions turned the Solling spruce forest soil from a net source for NO_x (0.62 ± 0.24 kg N ha^{-1} yr^{-1}) into a net sink (-0.33 ± 0.01 kg N ha^{-1} yr^{-1}). Net nitrification and nitrate in the soil solution, which explained most of the variation in NO_x fluxes, were not detectable under reduced N depositions. However, long-term reduction of N depositions did not affect N_2O emissions, which were low with 0.08 kg N ha^{-1} yr^{-1} both under reduced and ambient depositions, since aerobic soil conditions limited denitrification.

A comparison of six German beech stands revealed that only the Solling beech stand exhibited high seasonal N_2O emissions of up to 113 µg N m^{-2} h^{-1} compared to fluxes of -2.4 to 7.4 µg N m^{-2} h^{-1} in the other beech stands. Fluxes of NO_x were low at all stands (-6.3 to 12.3 µg N m^{-2} h^{-1}). Fluxes of N_2O and NO_x were controlled by N turnover (net N mineralisation and net nitrification rates) and soil gas diffusivity. Fluxes of NO_x further depended on NO_x concentrations in the forest air. The hypothesis that the tightly-packed laminar beech leaf litter layer of the moder type humus generally resulted in low gas diffusivity and high N_2O emissions was rejected. However, the combination of high annual precipitation and soil moisture with a loamy soil texture and moder type humus caused anaerobicity and high N_2O losses at the Solling beech stand during times of high soil respirations. In contrast, similar conditions did not result in anaerobicity at the Solling spruce stand indicating that litter structure finally decides about soil anaerobicity. Thus, the reported forest type effect on N oxide emissions results from the influence of litter structure and quality on soil gas diffusivity and N turnover rates.

In conclusion, chronic N depositions are mainly responsible for N oxide emissions but the amount and ratio of N oxides released strongly depends on the forest ecosystem. At ecosystem level, N turnover rates and soil gas diffusivity control N oxide fluxes. However,

high seasonal N_2O emissions as observed at the Solling beech stand are probably less common in German forest soils than previously thought.

Zusammenfassung

Die Intensivierung der Landwirtschaft und Industrialisierung in den letzten Jahrzehnten hat zu einer massiven Störung des Stickstoff(N)-Kreislaufs in Europa und anderen Erdteilen beigetragen. Eine Folge stellt die erhöhte Belastung von Waldökosystemen mit N-Depositionen dar. Chronisch hohe N-Einträge können die N-Verfügbarkeit in Waldböden erhöhen. Eine erhöhte N-Verfügbarkeit kann wiederum zu einem Anstieg von Distickstoffoxid(N_2O)- und Stickstoffmonoxid(NO)-Emissionen führen, die eine wichtige Rolle in der Atmosphärenchemie spielen und zur globalen Erwärmung beitragen. Studien berichten, dass europäische temperate Wälder, die von hohen, chronischen N-Depositionen belastet werden, bedeutende Quellen für N_2O und NO darstellen. Jedoch ist das Wissen über den Einfluss der N-Deposition und des Waldtyps auf N-Oxidemissionen immer noch unzureichend. Daher war das Ziel der vorliegenden Studie (i) die Herkunft von N-Oxiden aus temperaten Waldböden, die seit Jahrzehnten von hohen N-Depositionen belastet werden, zu ermitteln und den Beitrag der N-Deposition und des N aus dem Blattstreuumsatz an N_2O-Emissionen zu quantifizieren. Des Weiteren war das Ziel (ii) die Faktoren zu bestimmen, die die N-Oxidflüsse in temperaten Waldböden steuern, wobei ein besonderer Schwerpunkt auf den Einfluss des Waldtyps und der Gasdiffusivität im Boden gelegt wurde. In dieser Studie wurde zum ersten Mal die ^{15}N-Tracermethode zur Ermittlung von N_2O-Emissionsfaktoren (EF) für temperate Wälder angewendet.

Die Untersuchungen fanden auf sechs deutschen Level II-Buchenstandorten (*Fagus sylvatica*), auf zwei überdachten Fichtenflächen (*Picea abies* (L.) Karst.; Dachprojekt im Solling) und auf einer daran angrenzenden Fichtenfläche statt. Es wurden Tracerexperimente durchgeführt, bei denen die N-Deposition und Buchenlaubstreu mit dem stabilen Isotope ^{15}N markiert wurden. Distickstoffoxidflüsse und ^{15}N-Isotopenhäufigkeiten in N_2O wurden mit der geschlossenen Haubenmethode kombiniert mit ^{15}N-Isotopenuntersuchungen ermittelt. Die offene, dynamische Haubenmethode wurde für die Messung von NO_x ($NO_x = NO + NO_2$)-Flüssen verwendet. Es wurden in-situ- und Laborinkubationsversuche zur Bestimmung der Netto-N-Umsatzraten durchgeführt. Die Gasdiffusion im Boden wurde an ungestörten Bodensäulen gemessen.

Die vorliegende Studie zeigte, dass N-Oxidemissionen aus deutschen Waldböden zum Großteil das Ergebnis hoher, chronischer N-Einträge sind, während der Umsatz von Blattstreu-N in der organischen Auflage wahrscheinlich nur einen kleinen Beitrag zu den Gesamtemissionen von N_2O leistet wie aus dem Langzeit-^{15}N-Tracerexperiments im

Buchenbestand im Solling hervorging. Nur 6–13% der gesamten N_2O-Emissionen der Buchen- und Fichtenfläche im Solling stammten von der N-Deposition des letzten Jahres. Die N_2O-Emissionen stammten zum Großteil aus der N-Deposition vorheriger Jahre, die im Boden akkumuliert und erst nach mehr als einem Jahr wieder freigesetzt wurde. Es konnte gezeigt werden, dass die Methoden, die bisher in der Literatur zur Berechnung von EF verwendet wurden, nicht vergleichbar und teilweise sogar fehlerhaft sind. Der EF für N_2O aus der N-Deposition des letzten Jahres betrug 0.1% für den Fichtenwald und 0.6% für den Buchenwald. Berücksichtigung der N-Deposition des letzten Jahres sowie der remobilisierten, akkumulierten N-Deposition vorheriger Jahre ergab einen EF von etwa 0.9% für den Fichtenwald und von etwa 11% für den Buchenwald. Der Einfluss der N-Deposition auf N_2O-Emissionen hängt somit stark von dem betrachteten Waldökosystem ab. Der Buchenwald im Solling war von saisonalen N_2O-Emissionen mit hohen Emissionen während der Sommermonate gekennzeichnet (2.6 ± 0.6 kg N ha^{-1} a^{-1}), während der Fichtenwald niedrige Emissionen über den gesamten Jahresverlauf aufwies (0.3 ± 0.1 kg N ha^{-1} a^{-1}). Hohe gasförmige N-Verluste im Buchenwald können auf Bedingungen zurückgeführt werden, die die anaerobe Denitrifikation, die die Hauptquelle für N_2O auf den untersuchten Standorten darstellte, fördern. Des Weiteren zeigten Untersuchungen im Rahmen des Dachprojektes, dass die Langzeitreduktion der N-Deposition den Fichtenwaldboden von einer Netto-Quelle für NO_x (0.62 ± 0.24 kg N ha^{-1} a^{-1}) in eine Netto-Senke (-0.33 ± 0.01 kg N ha^{-1} a^{-1}) umwandelte. Bei reduzierter N-Deposition konnte eine Netto-Nitrifikation sowie Nitrat in der Bodenlösung, die die Variation in den NO_x-Flüssen zum größten Teil erklärten, nicht mehr nachgewiesen werden. Im Gegensatz dazu, spielte die Langzeitreduktion der N-Deposition keine Rolle für N_2O-Emissionen, die sowohl bei ambienter als auch bei reduzierter N-Deposition mit 0.08 kg N ha^{-1} a^{-1} sehr niedrig waren. Aerobe Bedingungen im Boden limitierten die denitrifikative Bildung von N_2O in diesem Fichtenbestand.

Weiterhin zeigte die Untersuchung der sechs Buchenstandorte, dass nur der Buchenstandort im Solling hohe, saisonale N_2O-Emissionen von bis zu 113 µg N m^{-2} h^{-1} aufwies, während die Flüsse auf den anderen Flächen zwischen -2.4 und 7.4 µg N m^{-2} h^{-1} lagen. Außerdem zeigten alle Buchenstandorte niedrig NO_x-Flüsse (-6.3 bis 12.3 µg N m^{-2} h^{-1}). Die N-Oxidflüsse wurden von den N-Umsätzen (Netto-N-Mineralisation und Netto-Nitrifikation) und der Gasdiffusion im Boden gesteuert. Die NO_x-Konzentrationen in der Waldluft spielten zusätzlich eine Rolle für die Steuerung der NO_x-Flüsse. Die Hypothese, dass die dicht gepackte, laminare Buchenlaubschicht der Moderauflage generell zu einer niedrigen Gasdiffusion im Boden und hohen N_2O-Emissionen führt, konnte nicht

bestätigt werden. Vielmehr führte eine Kombination aus hohen Jahresniederschlagssummen und hoher Bodenfeuchte im Buchenbestand im Solling zusammen mit einer lehmigen Bodentextur und einer Moderauflage zur Anaerobie und hohen N_2O-Verlusten in Zeiten hoher Bodenrespiration. Im Gegensatz dazu führten ähnliche Bedingungen im Fichtenbestand im Solling nicht zur Anaerobie. Dies zeigt, dass letztendlich die Struktur der Streu über die Anaerobie im Boden entscheidet. Es wurde gefolgert, dass der Waldtyp-Effekt das Ergebnis von unterschiedlicher Streustruktur und Streuqualität ist, welche die Gasdiffusivität und N-Umsatzraten im Boden beeinflussen.

Zusammenfassend lässt sich sagen, dass die N-Deposition zum größten Teil für die N-Oxidemissionen verantwortlich ist. Die Höhe der Emissionen und das Verhältnis von N_2O zu NO hängt jedoch stark vom Waldökosystem ab. Auf Waldökosystemebene stellen die N-Umsatzraten und die Gasdiffusivität die Hauptsteuergrößen für die N-Oxidflüsse dar. Allerdings sind hohe, saisonale N_2O-Emissionen wie sie im Buchenbestand im Solling beobachtet wurden wahrscheinlich seltener in deutschen Wäldern als bisher angenommen wurde.

Chapter 1

General Introduction

1.   General Introduction

1.1. Anthropogenic alteration of the N cycle in European temperate forests

Nitrogen (N) is the limiting nutrient in natural temperate forest ecosystems, despite its abundance in the atmosphere (Aber et al., 1998; Fenn et al., 1998; Vitousek et al., 1997). Since N is a component of several indispensable molecular constituents, such as nucleic acids, amino acids, and chlorophyll, it is essential for all living organisms (Gruber and Galloway, 2008). However, dinitrogen (N_2), which accounts for 78% of the atmospheric air, is a largely inert gas that is not available to most organisms (Galloway et al., 2004). Mineral N further lacks input by weathering of soil minerals (Fenn et al., 1998; Parton et al., 2007). In natural ecosystems, N enters the soil system mainly by biological fixation of atmospheric N_2 and lightning (Fig. 1-1; Galloway et al., 2004). However, only a limited number of species of the domains Bacteria and Archaea is able to fix N_2 (Galloway et al., 2004). Thus, the recycling of organic N in the soil by decomposition and mineralisation of litter and soil organic matter (SOM) have a particular importance as N source for plants and micro-organisms (Aber et al., 1998; Fenn et al., 1998; Parton et al., 2007). Reactive soil N can be transformed to N_2 and returned to the atmosphere by the process of denitrification (Galloway et al., 2002). Hence, the N cycle can be subdivided into two overlying cycles (Fig. 1-1): (i) an open, gaseous cycle connecting the atmosphere and the pedosphere and (ii) a soil-bound cycle connecting the pedosphere and the biosphere, which to a large extent is closed and comprises 95% of the total N turnover (Bucher et al., 2001).

The N cycle in Europe and in many other parts of the world has been massively accelerated as a result of agricultural and industrial activities in the last decades (e.g. Galloway et al., 2008; Vitousek et al., 1997). One key event was the invention of the Haber-Bosch process, which enabled the conversion of N_2 into reactive N and thus, large-scale use of synthetic mineral N fertilisers over the past century (Gruber and Galloway, 2008). The enormous increase in food production sustained the increase in human population, which further resulted in a rapid industrialisation and traffic development (Galloway et al., 2004). However, ammonia (NH_3) volatilised from agriculture as well as NO_x ($NO_x = NO + NO_2$) emitted during fossil-fuel combustion are added to the environment (Gruber and Galloway, 2008). According to Galloway et al. (2008), world population has increased by 78% since 1970 but simultaneously reactive N creation has increased by 120%. As a consequence, forest ecosystems have been exposed to increasing deposition rates of reactive N (Berge et al., 1999; Holland et al., 2005). In particular temperate forests in Central Europe, where intensely

managed agricultural land and industry are interspersed with forested areas, have received chronic high N depositions (Holland et al., 2005; Kreutzer et al., 2009; Pilegaard et al., 2006). Forests are particularly affected by N depositions because of their huge filtering capacity due to interception (BMELV, 2009; Pilegaard et al., 2006).
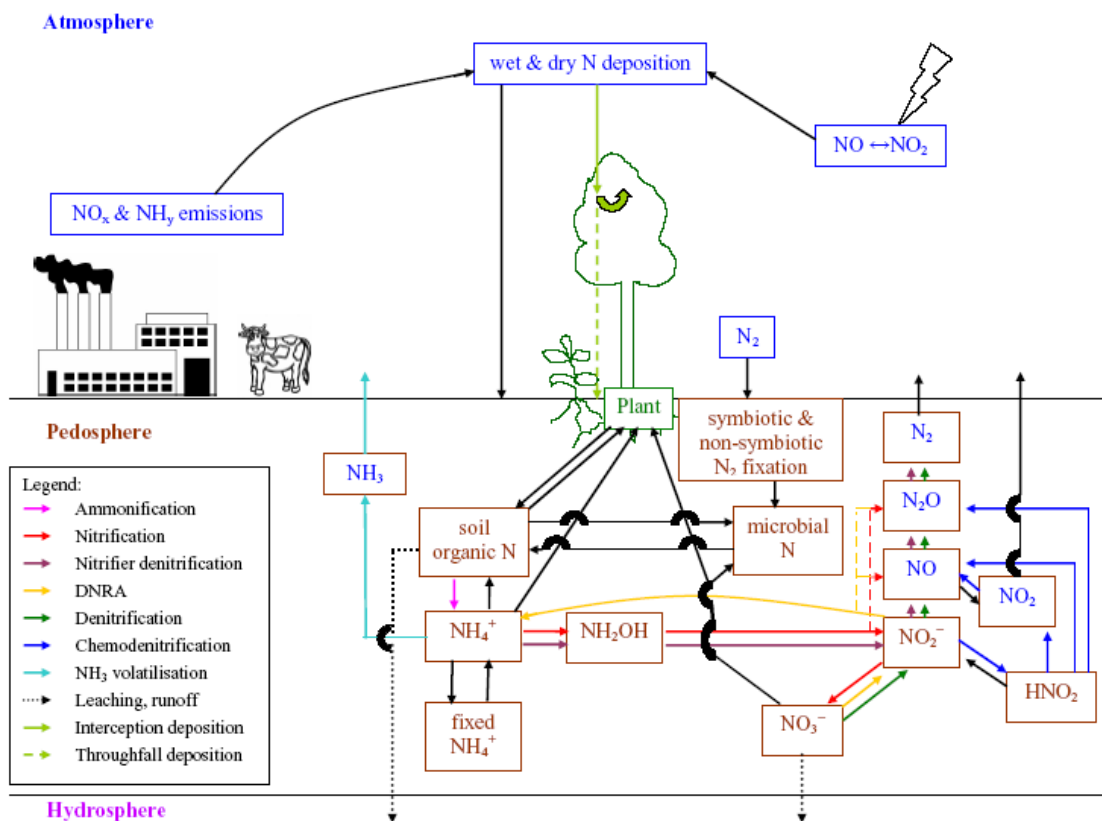
Figure 1-1: Simplified N cycle of an anthropogenically disturbed forest ecosystem. Nitrogen pools of the atmosphere are indicated by blue frames, N pools of the pedosphere are indicated by brown frames. The open, gaseous N cycle (blue lettering) connects the atmosphere and the pedosphere. The soil-bound N cycle is indicated by brown lettering. Fluxes of N and their corresponding processes involved in N cycling and N losses are indicated by coloured arrows (see legend). Total N depositions include wet deposition and dry (gaseous and particulate) depositions. DNRA is dissimilatory nitrate reduction to ammonium.

International conventions on prevention of air pollution, such as the Geneva Convention on Long-Range Transboundary Air Pollution (CLRTAP) of 1979 and its Multi-Effect (Gothenburg) Protocol to Abate Acidification, Eutrophication, and Ground-Level Ozone of 1999, defined emission limits for the European Union. In Germany, sulphur dioxide emissions, which were mainly responsible for acid depositions, peaked in the mid 80ies and were significantly reduced by 90% from 1990 to 2005 (UBA, 2007; Wochele and Kiese, 2010). Emissions of NO_x also experienced a strong reduction by 50% during this time period

whereas NH_3 emissions were only reduced by 16% (UBA, 2007). Despite these reductions, total N depositions in German forests are currently estimated to be on average 40 kg N ha^{-1} yr^{-1} (Fig. 1-2) compared to 26 kg N ha^{-1} yr^{-1} in open land (Wochele and Kiese, 2010). According to UBA (2007), critical loads of N are still exceeded in more than 90% of the German forests. Therefore, N **enrichment of temperate forests from air pollution has become one of the major concerns of recent decades** (Zechmeister-Boltenstern et al., 2002).
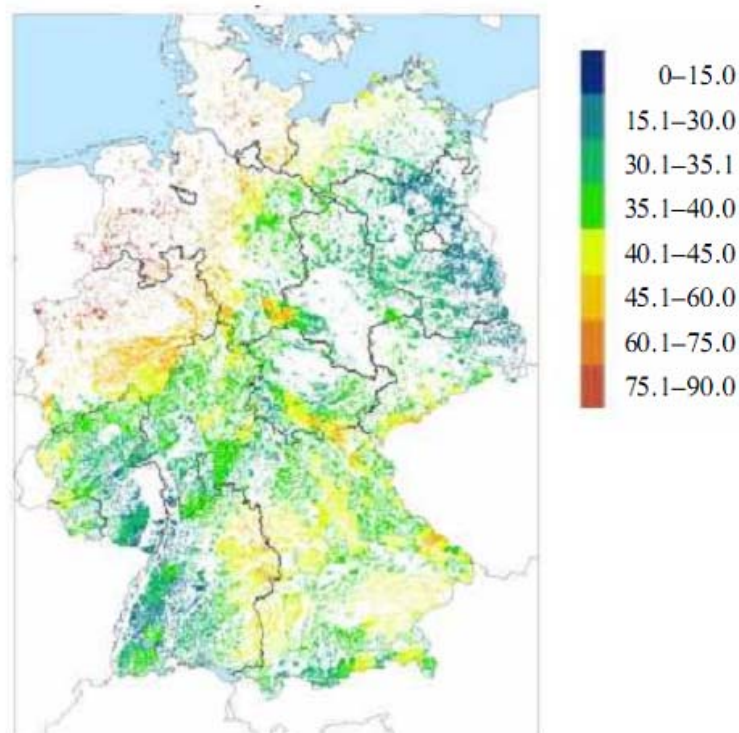
Figure 1-2: Total N deposition [kg N ha^{-1} yr^{-1}] in German forest ecosystems in 2000 (Wochele and Kiese, 2010; modified).

1.2. Consequences of chronic N depositions in European forest ecosystems

According to the N saturation hypothesis by Aber et al. (1998), pre-industrial European forests were N-limited. However, widespread increase in N depositions has caused a shift in the N status of many forests, which under chronic high N depositions may lead to N-saturated conditions (Aber et al., 1998). A change to N saturation is a serious matter of concern, as surplus N exceeds the storage capacity of the soil or the N demand by microbes and plants. Nitrogen losses from the ecosystem may increase and cause a cascade of environmental and human health problems (Galloway et al., 2008; Kreutzer et al., 2009). The major sink for N inputs is biotic and abiotic immobilisation of N in soils followed by plant N uptake (Feng et

al., 2008; Fenn et al., 1998). The response of the ecosystem to increasing N depositions is continuous but mostly non-linear (Aber et al., 1998). The major consequences of increasing N depositions and N availability in forest ecosystems are listed in the following.

(i)   Growth of forests that were previously N-limited is initially enhanced as was observed across Europe (e.g. Binkley and Högberg, 1997; Hunter and Schuck, 2002). However, increased ratios of N to other nutrients in tree foliage cause nutrient imbalances that may finally result in forest decline (Magill et al., 1997; Schulze, 1989; Ulrich, 1995). A shift in plant community composition and loss in biodiversity may be further consequences (Vitousek et al., 1997).

(ii)   Increased litter production due to enhanced forest growth may result in litter accumulation in the surface organic layer (Fenn et al., 1998). Although, higher N concentrations in litter have been found to stimulated early phase litter decomposition, later stages of decomposition are retarded (Berg and McClaugherty, 2003). Soil and surface organic matter accumulation has frequently been reported from European forests receiving chronic N depositions (e.g. Berg and Matzner, 1997).

(iii)   Initially enhanced mineralisation and nitrification rates have been observed, which, however, peaked at intermediate N depositions (Aber et al., 1998; Corre et al., 2007; Fenn et al., 1998). Suggested reasons for the decline at N-enriched sites are changes in the chemical bond structure of SOM and suppression of humus-degrading enzymes by high N availability in soils (Aber et al., 1998; Berg and Matzner, 1997).

(iv)   Chronic high N inputs are further attended by soil acidification, base cation depletion, and aluminium mobility as a result of enhanced nitrification and nitate (NO_3^-) leaching (Fenn et al., 1998; Meesenburg et al., 2009).

(v)   Nitrate leaching is a primary symptom of N excess (Fenn et al., 1998) and is negatively related to the C/N ratio of the surface organic layer and upper mineral soil (Borken and Matzner, 2004; Tietema et al., 1998). Associated negative consequences for the environment and public health are e.g. eutrophication of aquatic ecosystems and methemoglobinemia after water consumption by humans ("blue baby syndrome") (Vitousek et al., 1997). Mean NO_3^- leaching rates from German forests were estimated to be 7.5 kg N ha^{-1} yr^{-1} in 2000 (Wochele and Kiese, 2010).

(vi)   Increased nitrification and denitrification rates may also dramatically alter **nitrous oxide (N_2O) and nitric oxide (NO) fluxes** from soils. Several studies reported that European temperate forests exposed to high N depositions were considerable sources

for N_2O and NO (e.g. Brumme and Beese, 1992; Kreutzer et al., 2009; Papen and Butterbach-Bahl, 1999; Skiba et al., 1999; van Dijk and Duyzer, 1999).

1.3. Impact of N oxides on the environment and human health

The anthropogenic amplification of **global warming**, the **depletion of the** stratospheric **ozone (O_3) layer**, "**summer smog**", and atmospheric **acid and N depositions** are severe environmental threads of recent decades which can in part be ascribed to the increase in atmospheric N oxide (N_2O, NO, nitrogen dioxide (NO_2)) concentrations.

Despite its relatively low concentration in the atmosphere, **N_2O** belongs to the three main "anthropogenic" greenhouse gases (IPCC, 2007). Two attributes make N_2O a potent greenhouse gas: (i) it is a chemically relatively inert gas with an average lifetime of 114 years in the atmosphere and (ii) it has a high radiative forcing potential (IPCC, 2007). The long-term impact of N_2O on global warming becomes evident when considering that the global warming potential of N_2O on a per-molecule basis is 298 times that of carbon dioxide over a 100-year time horizon (IPCC, 2007). Currently, the atmospheric concentration of N_2O increases linearly at a rate of 0.26% per year (Fig. 1-3; IPCC, 2007). The increase during the industrial era is in particular attributed to enhanced microbial production of N_2O in agricultural soils due to land-use change and fertilisation (IPCC, 2007). In 2005, N_2O reached a concentration of 319 ppbv compared to the pre-industrial level of 270 ppbv (IPCC, 2007). The major sink for N_2O is photolysis to N_2 and excited O (^1D) in the stratosphere. However, reaction of N_2O with excited O (^1D) is the major source for ozone-depleting NO and NO_2 in the stratosphere (Crutzen 1979). Thus, N_2O contributes to the catalytic decomposition of stratospheric O_3, which protects the biosphere from the harmful effects of ultraviolet radiation (IPCC, 2007).

The highly reactive trace gases **NO and NO_2** are secondary greenhouse gases (IPCC, 2007) and play a crucial role for the oxidising capacity of the troposphere (Crutzen 1979; Logan et al., 1983). Nitrogen oxides are involved in a sequence of photochemical reactions, which may lead to the formation of the greenhouse gas O_3 and hydroxyl radicals (OH·). However, due to the short lifetime (hours to days) and complex nonlinear chemistry, a global warming potential for NO_x was not calculated in the IPCC reports (IPCC, 2007). Due to the net production of O_3 and OH·, NO_x is responsible for "summer smog" affecting human health and plant productivity (Crutzen 1979). Furthermore, NO_x is a precursor in the photochemical formation of gaseous nitric acid (HNO_3), which represents a major component of acid and N depositions (Crutzen, 1979; Logan, 1983). Fossil fuel combustion is primarily responsible for

accelerated release of NO_x to the atmosphere. However, biogenic production in soils further contributes to total NO_x emissions with approx. 15% considering the redeposition of NO_x to the canopy (Gasche and Papen, 1999).
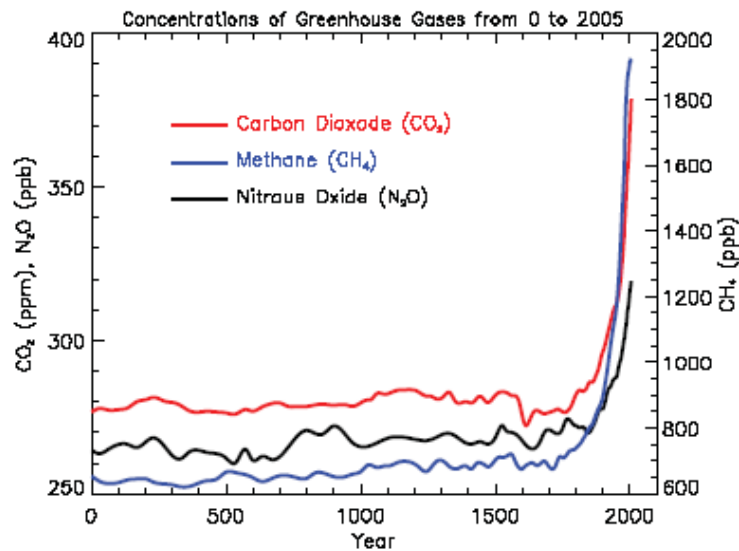
Figure 1-3: Atmospheric concentrations of the three main „anthropogenic" greenhouse gases carbon dioxide, methane, and nitrous oxide over the last 2,000 years (IPCC, 2007).

1.4. Processes responsible for N oxide production in forest soils

Nitrogen oxides emitted from soils are primarily produced via the two biological processes of nitrification and denitrification (Conrad, 1996; Granli and Bøckman, 1994). **Nitrification** is the oxidation of ammonium (NH_4^+) or NH_3 to NO_3^- via hydroxylamine (NH_2OH) and nitrite (NO_2^-; Wrage et al., 2001; Fig. 1-1). Autotrophic as well as heterotrophic microorganisms have the ability to nitrify (Wrage et al., 2001). Autotrophic nitrification is carried out by a group of aerobic bacteria together addressed as Nitrobacteriaceae (Wrage et al., 2001). Fungi, which prevail in soils with low pH values, play a major role for heterotrophic nitrification. Nitrous oxide and nitric oxide may be released as by-products of nitrification. **Denitrification** is the stepwise anaerobic reduction of NO_3^- to N_2 via NO_2^-, NO, and N_2O (Russow et al., 2009; Stevens et al., 1998; Wrage et al., 2001; Fig. 1-1). Thus, N_2O and NO are regular intermediates of denitrification. Both gases are not only produced but also consumed during this process and can be reduced to N_2 by the enzyme N_2O reductase (Butterbach-Bahl et al., 2004; Ludwig et al., 2001). The N_2O reductase is the most O_2 and pH sensitive enzyme of denitrification (Wrage et al., 2001). The N_2O/N_2 ratio increases at low pH

values, high NO_3^- concentrations and presence of some O_2 (Simek and Cooper, 2002; Wrage et al., 2001). Denitrifiers are common among several bacterial taxa. These bacteria are predominantly heterotrophs and facultative anaerobes that can use NO_3^- as electron acceptor instead of O_2 (Skiba and Smith, 2000; Wrage et al., 2001). However, in the presence of O_2, several denitrifiers are able to nitrify. In acid soils, **chemodenitrification** can be a further source of N_2O and NO_x (Fig. 1-1). During this abiotic process, biologically produced nitrite is protonated to nitrous acid (HNO_2), which can spontaneously decompose or react with organic (e.g. amines, phenols) or inorganic (e.g. Fe^{2+}) compounds (van Cleemput and Baert, 1984; van Cleemput and Samater, 1996; Venterea et al., 2005). Several other processes, such as nitrifier denitrification and dissimilatory NO_3^- reduction to NH_4^+ (DNRA), have been reported as N oxide sources (Conrad, 1996; Stevens et al., 1998; Wrage et al., 2001; Fig. 1-1). Different processes may occur simultaneously in aerobic and anaerobic microsites in soils contributing to N oxide production and consumption (Stevens et al., 1998).

There is evidence that denitrification is the main process for N_2O production in European forests. Ambus et al. (2006) observed that 62% of N_2O emissions in 11 European forests were derived from NO_3^-. Wolf and Brumme (2002) reported that almost 100% of N_2O derived from NO_3^- in a beech stand at the Solling, Germany. However, in a spruce and beech forest in South-Germany nitrification contributed to 70% to N_2O emissions (Papen and Butterbach-Bahl, 1999). These forest soils also displayed high NO emissions (Gasche and Papen, 1999).

1.5. Mechanisms and factors regulating N oxide fluxes in forest ecosystems

N oxide fluxes are regulated by soil environmental (temperature, moisture), chemical (pH, N and organic C contents), physical (soil texture), and biological (microbial community, plant species) variables (e.g. Dupont et al., 2008; Ludwig et al., 2001). Firestone and Davidson (1989) proposed the conceptual "hole-in-the-pipe" (HIP) model, which postulates two levels of regulation of N oxide emissions from soils: (i) the sum of total N oxide production is a function of **N availability**, specifically of nitrification and denitrification rates (rate of N moving 'through the pipe') and (ii) the ratio of released gases (NO, N_2O, N_2) is a function of environmental factors such as **O_2 availability**, which define the size of 'holes in the pipe' (Davidson et al., 2000). Whether nitrification or denitrification prevails, depends on O_2 availability, which is controlled by soil gas diffusivity and O_2 consumption by micro-organisms and roots (Bollmann and Conrad, 1998; Brumme et al., 1999; Smith et al., 2003). Due to its effect on O_2 availability, soil moisture has frequently been described as one of the key drivers for N oxide flux regulation (e.g. Skiba and Smith, 2000). Maximum N_2O

emissions occur at water filled pore spaces (WFPS) between 60% and 95% (Davidson et al., 2000; Schindlbacher et al., 2004; Skiba and Smith, 2000). For maximum NO release, the optimum can substantially vary between 15 and 85% WFPS depending on the soil type (Gasche and Papen, 1999; Ludwig et al., 2001; Schindlbacher et al., 2004; van Dijk and Duyzer, 1999). Since enzymatic processes increase with increasing temperature as long as other factors have no limiting effect, soil temperature is a further main factor controlling N oxide fluxes (Schaufler et al., 2010; Schindlbacher et al., 2004; Skiba and Smith, 2000). Hence, N oxide fluxes strongly depend on current weather conditions.

Chronic high N depositions may increase N availability and N turnover rates in forest soils (see chapter 1.2.). In highly N-affected European temperate forests, significantly higher N_2O and NO emissions were measured compared to temperate forests which were less affected by N depositions (Brumme and Beese, 1992; Castro et al., 1993; Johansson et al., 1984; Kreutzer et al., 2009; van Dijk and Duyzer, 1999). Some studies reported a significant positive relationship between N deposition rates and N oxide fluxes (e.g. Butterbach-Bahl et al., 1998; Skiba et al., 2004). However, Liu and Greaver (2009) and Pilegaard et al. (2006) did not find a clear relationship between N depositions and N_2O emissions at a large scale, most likely due to the predominant effect of ecosystem type. Thus, the role of atmospheric N depositions on N oxide emissions is still highly unclear. The IPCC (2006) assumes that 1% of N deposited in temperate forests is emitted as N_2O. But the uncertainity of this emission factor (EF) is huge with a reported range of EF from 0.2% to 5%. The higher values seem to be mainly derived from deciduous forests and the lower values from coniferous forests (Brumme et al., 1999; Denier van der Gon and Bleeker, 2005). Hence, the impact of tree species on soil properties may control the amount of N oxides and ratio of NO to N_2O emitted (Butterbach-Bahl et al., 1997). Several studies have reported that in general N_2O emissions from deciduous forests were higher than from coniferous forests, whereas this seems to be opposite for NO emissions (Denier van der Gon and Bleeker, 2005; Papen and Butterbach-Bahl et al., 1999; Pilegaard, 2006; van Dijk and Duyzer, 1999). Brumme et al. (1999) defined three emission patterns of N_2O from forests: (i) the seasonal emission pattern (SEP) with high N_2O emissions in wet summer, (ii) the background emission pattern (BEP) with low N_2O emissions throughout the whole year, and (iii) the event emission pattern (EEP), e.g. during freeze-thaw events.

Tree species related differences in litter quality, litter structure, soil moisture, and N depositions may be responsible for the reported variations (Brumme et al., 1999; Papen and Butterbach-Bahl, 1999; Pilegaard, 2006). Despite higher N deposition rates in coniferous

forests (Borken and Matzner, 2004; Meesenburg et al., 1995), soil N turnover rates are typically higher in deciduous forests than in coniferous forests as a result of higher litter quality (e.g. Ambus et al., 2006). A literature review revealed that deciduous forests with mull type humus and acid coniferous forests with moder type humus showed low non-seasonal emissions whereas deciduous forest with moder type humus showed high and seasonal emissions (Ball et al., 1997; Brumme et al., 1999; Brumme and Borken, 2009). Brumme et al. (1999) proposed that **molecular gas diffusion** of the surface organic layer may be responsible for these differences. The tightly-packed laminar leaf litter layer of moder type humus of beech forests on acid soils can induce a diffusion barrier creating anaerobic soil conditions (Fig. 1-4) compared to the well-aerated needle litter layer of moder type humus of coniferous forests (Ball et al., 1997; Brumme and Borken, 2009). Therefore, NO release by nitrification may be favoured in coniferous forests compared to N_2O release and NO consumption by denitrification in deciduous forests.
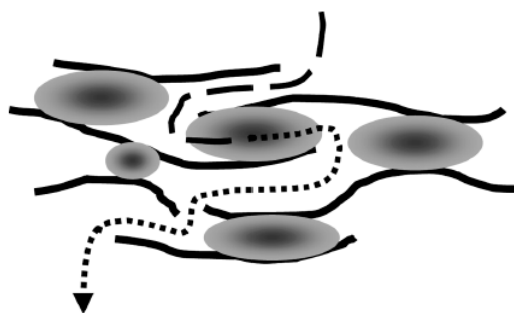
Figure 1-4: Diffusion pathway of oxygen around beech leaves in the air and water-filled (ellipse) pore space of a moder type humus (Brumme and Borken, 2009).

1.6. Nitrogen oxide emissions from German forest soils

Germany belongs to the most densely wooded countries of the European Union with a forest area of 31% of total land area (BMELV, 2009). Wochele and Kiese (2010) estimated mean annual N_2O fluxes from German forest soils to be 1.4 kg N yr^{-1} ha^{-1} (15.5 Gg N yr^{-1} assuming a forest area of 11,075,798 ha (BMVEL, 2004)) for 2000 by using a process-oriented modelling approach. Schulte-Bisping et al. (2003) stratified forests according to the N_2O emission patterns. The estimated annual fluxes were 2.05 kg N yr^{-1} ha^{-1} for deciduous forests with SEP, 0.37 kg N yr^{-1} ha^{-1} for deciduous forests with BEP, and 0.17 kg N yr^{-1} ha^{-1} for coniferous forests with BEP. The mean estimated N_2O flux for German forests was 0.32 kg N yr^{-1} ha^{-1} (3.3 Gg N yr^{-1}). This value, however, may represent the lower boundary of

N_2O emissions, since N deposition rates and event emissions from frozen soils were not considered (Schulte-Bisping et al., 2003). Therefore, N_2O emissions from forests contribute 2–9% to total German N_2O emissions (170 Gg N yr^{-1}; Schulte-Bisping et al., 2003; Wochele and Kiese, 2010). In addition, German forest soils contributed 4–17% to total N_2O emissions from European forest soil (EU 15) in 2000 (81.6 Gg N yr^{-1}; Kesik et al. 2005). Simulated NO emissions ranged from 1.1 to 1.5 kg N ha^{-1} yr^{-1}in German forests for the year 2000 (12.1–16.6 Gg N yr^{-1}; Butterbach-Bahl et al., 2009; Wochele and Kiese, 2010). Mean annual NO emissions from European forest soils were calculated to be 75.1–99.2 Gg N yr^{-1} (Butterbach-Bahl et al., 2009; Kesik et al., 2005). Therefore, German forests account for approx. 15% of total European forest NO emissions. Thus, **German forests have a significant importance for European N_2O and NO budgets**. However, **great uncertainties** in regional as well as in global N oxide budgets arise from high spatial and temporal variability of N oxide fluxes (e.g. Brumme and Borken, 2009; Groffman et al., 2000; Rosenkranz et al., 2006) and from **insufficient process understanding of the importance of N deposition and forest type** on N oxide fluxes (Ambus et al., 2006; Pilegaard et al., 2006).

1.7. Long-term forest monitoring in Germany – the study sites

The impact of air pollution on European forests has been investigated in the framework of the programme of "Intensive and Continuous Monitoring of Forest Ecosystems". This programme is based on two programmes, the "European Scheme on the Protection of Forests against Atmospheric Pollution" and the "International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests", which emerged from the Convention on LRTAP of the UNECE (Baumgarten, 2006). Large-scale European forest monitoring started with the so called Level I sites in 1984. In the 90ies, the 6000 Level I sites were supplemented by 860 intensive monitoring **Level II sites** (Baumgarten, 2006). At the Level II sites, the interaction between forest status and environmental variables has been investigated by permanent and continuous data acquisition. In Germany, 89 Level II sites were established representing the main German forest ecosystems (Fig. 1-5). A beech and spruce stand at the **Solling** plateau in Central Germany are part of the Level II monitoring programme and have the longest continuous record of deposition and ecosystem flux measurements worldwide, which already started in 1968 (Meesenburg et al., 2009; Ulrich et al., 1973). The Solling plays a special role regarding chronic high N depositions due to its location and elevation. The Solling forests have been exposed to long-range transported air

pollutants from the Rhein/Ruhr industrial area and from the industrial centres of Eastern Germany (Walse et al., 1998).
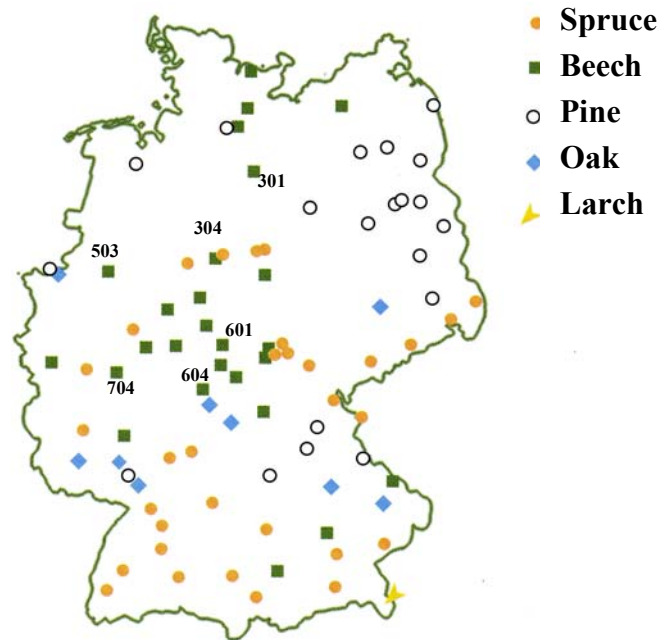
Figure 1-5: Intensive monitoring Level II sites in Germany (BMELF, unkown date; modified). The Level II site numeration is given for beech stands investigated in the present thesis (301: Luess, 304: Solling, 503: Haard, 601: Huenfeld, 604: Biebergemuend, 704: Neuhaeusel).

Adjacent to the Level II sites at the **Solling**, a **roof project** was started in a 60 year-old Norway spruce forest in September 1991 (Bredemeier et al., 1995; Bredemeier et al., 1998). The aim of the project was to investigate the ecosystem response to reduced acidity and N depositions as well as to drought stress. The Solling roof project was part of the large-scale European NITREX (NITRogen saturation EXperiment) and EXMAN (EXperimental MANipulation of forest ecosystems) projects (Beier et al., 1998; Bredemeier et al., 1995; Rasmussen and Wright, 1998). In the framework of the NITREX project, a series of studies have been conducted where N was added to N-poor sites by N fertilisation and N was removed from N-rich sites by under-canopy roofs (Tietema et al., 1998). Three transparent roofs (each 300 m^2) were installed below the canopy and 3.5 m above the surface organic layer of the spruce forest (Fig. 1-6) for manipulation of atmospheric nutrient and water inputs (Bredemeier et al., 1995; Bredemeier et al., 1998). In the "clean rain" roof plot ambient throughfall water was replaced by artificial throughfall water whose NH_4^+-N deposition was reduced by 85% and NO_3^--N deposition by 50% (pre-industrial deposition level) compared to the "control" roof plot receiving ambient N deposition of approx. 33 kg N ha^{-1} yr^{-1} (Corre and

Lamersdorf, 2004). Under the "clean rain" roof, sulphate and proton inputs were reduced as well. In 2001, a ^{15}N tracer experiment was additionally started under the "clean rain" and "control" plots to trace the fate of N depositions (Feng et al., 2008). The third roof plot was used for drought stress experiments. The main hitherto findings of the Solling roof project are presented in chapter 3.1.
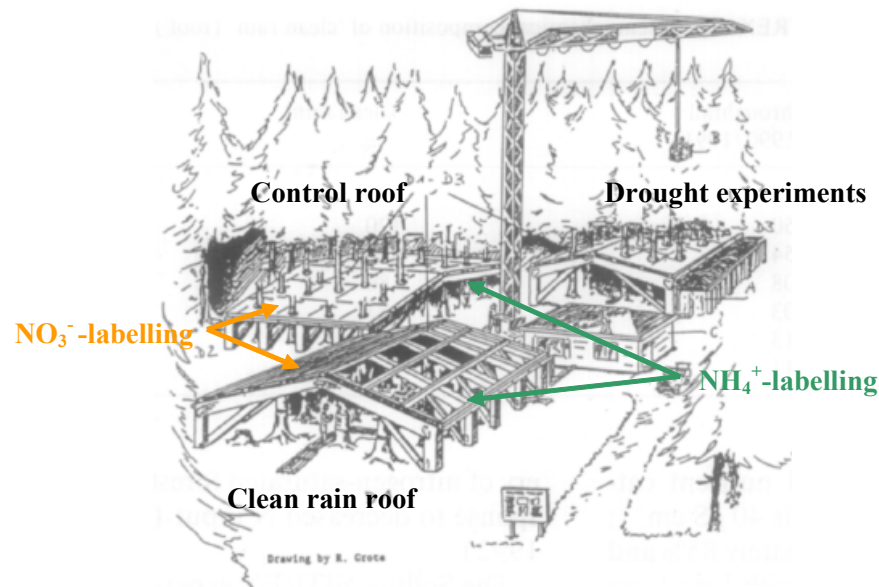
Figure 1-6: Drawing of the roof constructions at the Solling spruce stand, Germany (Bredemeier et al., 1995; modified). The roof project included three roofs: a "clean rain" roof with pre-industrial depositions, a roof for drought experiments, and a "control" roof with ambient depositions. The "clean rain" and "control" roofs were divided into two subplots for ^{15}N-labelling of NH_4^+ and NO_3^- depositions, respectively.

1.8. Objectives and outline

Up to the present chapter, a **general introduction** was given that imparted recent knowledge and facts about the alteration of the N cycle of forests ecosystems by increased N depositions, consequences of N deposition for harmful N_2O and NO emissions from forests soils, and the proposed mechanisms of N_2O and NO flux regulations in forests. Although the mechanisms of N oxide regulation are well known at the process level, the complexity of ecosystems makes understanding difficult at the ecosystem level. Despite high research efforts in the last two decades, the impact of atmospheric N depositions on N_2O and NO fluxes from temperate forest soils is still highly unclear. Regarding N oxide fluxes, forest ecosystems do not all respond in a similar way to N depositions most probably due to forest

type and humus form effects. Therefore, research undertaken in the present thesis attempted to answer the following overall **questions**:

> (1) What is the **origin of N$_2$O** emissions from temperate forest soils exposed to chronic high N depositions? How does **N deposition** contribute to **N$_2$O and NO$_x$** emissions? Is **turnover of leaf litter N** involved in N$_2$O emissions?
>
> (2) What **factors regulate N$_2$O and NO$_x$** emissions from temperate forest soils? Does soil **gas diffusivity** of the surface organic layer play a key role for seasonal N$_2$O emission patterns? How does **forest type** influence N oxide emissions?

The corresponding **hypotheses** were:

(1) Nitrogen depositions are mostly responsible for N$_2$O and NO$_x$ emissions whereas turnover of leaf litter N represents a minor source for N oxide emissions.

(2) German forest soils are believed to have generally high N turnover rates and N availability due to chronic high N depositions. However, releases of N$_2$O and NO$_x$ from soils strongly vary due to differences in gas diffusivity of the surface organic layer. Deciduous forests with moder type humus generally exhibit seasonal emission patterns of N$_2$O whereas deciduous forests with mull type humus and coniferous forests belong to the background emission type. In contrast, forests having BEP, and thus higher soil gas diffusivity, emit more NO$_x$ than forests having SEP.

The focus of the research was on German forest ecosystems receiving chronic high N deposition rates. Research activities took place at six Level II beech stands (*Fagus sylvatica*) and at two roofed and an unroofed Norway spruce stand (*Picea abies* (L.) Karst.) at the Solling. Beech and spruce represent the main German tree species. Since acid soil conditions dominate German forest soils, stands with low pH values were chosen.

To answer the posed questions, **five studies** were conducted. The manuscript of each study is presented as an individual chapter. **Study 1 to 3** aimed at answering the **first question** whereas **study 4 and 5** aimed at answering the **second question**.

1. Study (**chapter 2**): The objective was to examine (i) the direct **contribution of NH$_4^+$ and NO$_3^-$ depositions** to N$_2$O emissions, (ii) the involved **processes**, and (iii) the one-year effect including the remineralisation of deposited and immobilised N. The study was conducted at the Solling **spruce and beech** stands. In this study, a ^{15}N **isotope labelling technique** was used for the first time to determine N$_2$O **emission factors** for forest ecosystems. Additionally, different methods to calculate emission factors commonly used in literature are discussed.

2. Study (**chapter 3**): Due to the decline in atmospheric deposition rates since the 1980s, this study addressed the question how a N-enriched **spruce** stand **responds to declining N deposition** rates regarding N$_2$O and NO$_x$ emissions. The study was part of the **Solling roof project** where N deposition has been reduced to a pre-industrial level for 16–17 years.

3. Study (**chapter 4**): It was investigated whether **N of beech leaf litter** contributes measureably to N turnover, **N$_2$O emissions**, and N sequestration two and eleven years after litterfall. The study took place in the Solling beech stand where recently fallen beech leaf litter was replaced by ^{15}**N-labelled** beech leaf litter in 1997.

4. Study (**chapter 5**): The study dealt with the **regulation of N oxide fluxes** at ecosystem level. The aim was to ascertain the influence of **gas diffusivity and N turnover** on N oxide fluxes and to evaluate whether beech forests with moder type humus generally exhibit seasonal N$_2$O emission patterns but low NO$_x$ emissions. The study was carried out at **six Level II beech stands**.

5. Study (**chapter 6**): The purpose was to compare N oxide fluxes of the Solling spruce and beech stands and to examine the **impact of forest type** on short-term and long-term regulation of N oxide fluxes.

Subsequently to the studies, a **general discussion** of the results and **ideas for future research** are given. In addition, the successful application of the ^{15}N labelling technique regarding N$_2$O resulted in the start of the **development of a method** to determine ^{15}**N in NO**. Ideas, laboratory tests, and first results are presented in the **Appendix A**.

Direct Contribution of Nitrogen Deposition to Nitrous Oxide Emissions in a Temperate Beech and Spruce Forest – A ^{15}N Tracer Study

2.   Direct contribution of nitrogen deposition to nitrous oxide emissions in a temperate beech and spruce forest – A ^{15}N tracer study

Abstract

The impact of atmospheric nitrogen (N) deposition on nitrous oxide (N₂O) emissions in forest ecosystems is still unclear. Our study assessed the direct contribution of N deposition to N₂O emissions in temperate forests exposed to chronic high N depositions using a ^{15}N labelling technique. In a Norway spruce stand (*Picea abies*) and in a beech stand (*Fagus sylvatica*) at the Solling, Germany, we used a low concentrated ^{15}N-labelled ammonium-nitrate solution to simulate N deposition. Nitrous oxide fluxes and ^{15}N isotope abundances in N₂O were measured using the closed chamber method combined with ^{15}N isotope analyses. Emissions of N₂O were higher in the beech stand $(2.6 \pm 0.6 \text{ kg N ha}^{-1} \text{ yr}^{-1})$ than in the spruce stand $(0.3 \pm 0.1 \text{ kg N ha}^{-1} \text{ yr}^{-1})$. We observed a direct effect of N input on ^{15}N-N₂O emissions, which lasted for less than three weeks and was mainly caused by denitrification. No further increase in ^{15}N enrichment of N₂O occurred during a one-year experiment, which was probably due to immobilisation of deposited N. The annual emission factor for N₂O from deposited N was 0.1% for the spruce stand and 0.6% for the beech stand. Standard methods used in the literature applied to the same stands grossly overestimated emission factors with EF values of up to 25%. Only 6–13% of the total N₂O emissions were derived from direct N deposition. Whether the remaining emissions resulted from accumulated anthropogenic N deposition or native soil N, could not be distinguished with the applied methods. The ^{15}N tracer technique is a useful tool, which may improve estimates of the current contribution of N depositions to N₂O emissions.

Keywords: Nitrous oxide, ^{15}N isotope techniques, emission factor, N deposition, spruce forest, beech forest

2.1. Introduction

In Europe and in many other parts of the world, emissions of reactive nitrogen (N) have rapidly increased in the last decades mainly due to agricultural and industrial activities (e.g. Galloway et al., 2008; Vitousek et al., 1997). As a consequence, European forests have been exposed to high deposition rates of acidity and reactive N compounds (Berge et al., 1999). Pre-industrial European forests were supposed to be N-limited, however widespread N deposition has caused a shift in the forest's N status which under chronic N deposition may even lead to N-saturated conditions (Aber et al., 1998). Using N input-output balances, Brumme and Khanna (2008; 2009b) classified German forests into four groups with different N status that cover the complete N enrichment continuum. According to them 'steady state type forests' with mull type humus (STFa: forests where deposited N is accumulating in trees only and not in the soil) were probably widespread in Europe in pre-industrial times. They assumed that soil acidification caused these forests to lose organic matter and mineral N from

the soil changing them into 'degradation type forests' (DTF: forests where the mineral soil has become a source of N and C). With further acid and N depositions, these forests would change into 'accumulation type forests' (ATF: forests where deposited N and carbon is accumulating in the surface organic soil). At the end of the N enrichment continuum, forests could become another 'steady state type forests' with moder type humus (STFb: forests where deposited N is accumulating in trees and not in soil). Fluxes of nitrous oxide (N$_2$O), which is an important greenhouse gas (IPCC, 2007) and contributes to the chemical destruction of the stratospheric ozone layer (Crutzen, 1979), may increase if the soil becomes a source of N (transition from STFa → DTF) and deposited N exceeds the storage capacity of the ecosystem (transition from ATF → STFb). Consequently, European forests with chronic acid and N depositions frequently act as considerable source for N$_2$O (e.g. Brumme and Beese, 1992; Butterbach-Bahl et al., 1998; Papen and Butterbach-Bahl, 1999; Skiba et al., 1999).

Temperate forest soils are estimated to add between 0.1 and 2.0 Tg N$_2$O-N yr^{-1} to the atmosphere, which is between 0.6 and 11% of the total global N$_2$O emissions (Brumme et al., 2005; IPCC, 2001; Kroeze et al., 1999) indicating the high degree of uncertainty in the estimations. The role of atmospheric N depositions on N$_2$O emissions is highly unclear and is one of the reasons for this uncertainty (Pilegaard et al., 2006). The IPCC (2006) uses a default emission factor for N$_2$O of 0.01, which means that 1% of the N deposited in temperate forests contributes to N$_2$O emissions. The uncertainty of this emission factor is huge ranging from 0.002 to 0.05 where the higher fraction comes from deciduous forests and the lower fraction from coniferous forests (Brumme et al., 1999; Denier van der Gon and Bleeker, 2005). Tree species related differences in litter quality, litter structure, and soil moisture may play a role in the reported variation of emission factors, but still much uncertainty remains (Brumme et al. 1999, Pilegaard, 2006).

Three different approaches have been used to examine the impact of N depositions on N$_2$O fluxes: (1) regression analysis between N depositions and N$_2$O fluxes (e.g. Butterbach-Bahl et al., 1998), (2) comparison of similar forest ecosystems receiving different N loads (e.g. Skiba et al., 1999), and (3) N fertilisation experiments (e.g. Brumme and Beese, 1992). These approaches have the following limitations: (1) Regression analysis between N depositions and N$_2$O fluxes requires a strong correlation, which can only occur if direct N$_2$O emission of deposited N is significant. Moreover, N depositions are usually correlated with similar factors (e.g. precipitation) that also control N$_2$O fluxes (Butterbach-Bahl et al., 1998; Kitzler et al., 2006a). (2) Comparing sites receiving different N loads has the limitation that sites are usually not completely comparable in climate and soil properties. Finally, (3) studies have shown that

the application of mineral N to simulate N deposition does not commonly reflect the mode of chronic N depositions (Sitaula et al., 1995; Skiba and Smith, 2000).

The use of ^{15}N isotopes as a tracer is potentially a useful technique to investigate the impact of N depositions on N$_2$O fluxes in forests. The ^{15}N technique can be applied even if low direct N$_2$O emissions occur, it is independent of precipitation amount and does not cause artificially high mineral N concentrations in the soil. At present we are not aware of any study where the emission factor for N$_2$O from forest soils was estimated using ^{15}N tracer techniques. In forest ecosystems the ^{15}N tracing technique has only been used to distinguish between various sources of N$_2$O production (e.g. Ambus et al., 2006; Wolf and Brumme, 2002).

Our objectives were (i) to examine the direct contributions of ammonium (NH$_4^+$) and nitrate (NO$_3^-$) depositions to N$_2$O emissions and (ii) to follow the effect for one year including the remineralisation of deposited and subsequently immobilised N. We hypothesise that (1) N depositions contribute considerably to direct N$_2$O emissions which is caused by the availability of mineral N independent of mineralisation and nitrification rates and (2) N depositions contribute to significant amounts to N$_2$O emissions over one-year because of remineralisation of deposited N. To test our hypotheses, we conducted (i) a short-term as well as (ii) a one-year long lasting in-situ ^{15}N tracer experiment in a coniferous and in a deciduous forest stand which both have been exposed to high atmospheric N depositions for decades.

2.2. Materials and methods

2.2.1. Study area

The study was carried out at the Solling plateau in Central Germany (51° 46' N, 9° 34' E; 500 m a.s.l.; mean annual temperature: 6.9°C; annual precipitation: 1193 mm). The soil type is an acid Dystric Cambisol (silty loam), which has developed in a loess solifluction layer overlying Triassic sandstone bedrock. The humus type is a typical moder. A detailed site description is given by Bredemeier et al. (1995, 1998) and by Brumme and Khanna (2009a). Experiments took place in a 74-yr-old Norway spruce stand (*Picea abies* (L.) Karst.) and in an adjacent 160-yr-old beech stand (*Fagus sylvatica*). Soil characteristics for both sites are presented in Tab. 2-1.

Both stands have been exposed to high N depositions for decades. In 2007 and 2008, stand deposition amounted to 33 kg N ha^{-1} yr^{-1} in the spruce stand with NH$_4^+$-N:NO$_3^-$-N:N$_{org}$ in the ratios of 49:44:7 and to 20 kg N ha^{-1} yr^{-1} in the beech stand with a NH$_4^+$-N:NO$_3^-$-N:N$_{org}$ ratio of 47:38:16. Stand N deposition was usually measured in monthly intervals in both stands and represents the sum of throughfall deposition and stemflow. Stemflow was negligible in the

spruce stand. In the beech stand, data collection and chemical analyses were conducted by the Northwest German Forest Research Station (Meesenburg et al., 2009).

Table 2-1: Soil characteristics of the spruce and beech stands at the Solling site.

Soil depth and forest site	n	pH(H₂O)	n	Total organic carbon [mg C g^{-1}]	Total nitrogen [mg N g^{-1}]	C/N ratio	n	Bulk density [g cm^{-3}]	Humus mass [Mg ha^{-1}]
Organic layer									
Spruce	3	3.6 (0.1)[a]	13	447 (13)	16.5 (0.4)[a]	27.2 (0.6)[a]	6		67.1 (4.4)[a]
Beech	3	4.0 (0.0)[b]	13	443 (12)	18.6 (0.7)[b]	24.1 (0.7)[b]	6		35.6 (1.9)[b]
0–5 cm									
Spruce	6	3.3 (0.0)	14	112 (7)	4.6 (0.3)	24.3 (0.8)[a]	4	0.76 (0.03)	
Beech	6	3.4 (0.0)	14	98 (11)	4.4 (0.3)	21.7 (1.4)[b]	6	0.89 (0.02)	
5–10 cm									
Spruce	5	3.5 (0.0)	13	39 (2)[a]	1.7 (0.1)	22.9 (0.8)[a]	5	1.00 (0.02)	
Beech	6	3.6 (0.0)	13	32 (2)[b]	1.7 (0.1)	19.8 (1.1)[b]	6	1.08 (0.04)	

Note: At each layer, means (± SE) followed by different letters indicated differences among the spruce and beech stands (independent t-test or Mann-Whitney U-test at $P \leq 0.05$).

2.2.2. Experimental design

We conducted the short-term experiment in August and September 2009 in order to examine the direct contribution of NH₄$^+$ and NO₃$^-$ depositions to N₂O emissions. Ten chamber bases were randomly installed in each stand, four of which were labelled once with ^{15}NH₄NO₃ solution (named NH₄$^+$-labelled) whereas the other four were labelled once with NH₄^{15}NO₃ solution (named NO₃$^-$-labelled; both contained 98 atom% ^{15}N). Two chambers served as controls. We reduced the number of control chambers in favour of treated chambers because the temporal and spatial variability of emitted natural ^{15}N in our previous studies in the beech stand indicated low values (see Appendix B). We added 0.7 L labelling solution per chamber base. The N concentration of the solution was 1.65 mg L^{-1}, which was within the range of the N concentrations measured in the throughfall. The labelled N was distributed down to 10 cm mineral soil depth within few hours after the application. In addition to the fluxes of N₂O and ^{15}N-N₂O, we determined air and soil temperature, soil moisture, and extractable mineral N (N$_{min}$ = NH₄$^+$-N + NO₃$^-$-N) content. Soil samples were taken at randomly chosen locations between the chamber bases. Measurements were conducted 3.5 hours (spruce) and 5 hours (beech), 24 hours, 48 hours, 7 days, and 21 days after the application of labelled N.

The one-year experiment was performed to examine the impact of NH₄$^+$ and NO₃$^-$ deposition on N₂O emissions including the remineralisation of the deposited N within one

year. At both stands we randomly installed 17 chamber bases for gas flux measurements and soil sampling. Seven of the chamber bases received a ^{15}NH$_4$NO$_3$ solution (named NH$_4^+$-labelled; 98 atom% ^{15}N) and another seven received a ^{15}NH$_4$^{15}NO$_3$ solution (named NH$_4$NO$_3$-labelled; 95 atom% ^{15}N). Of the seven chamber bases, five were used for gas flux measurements whereas soil samples were taken in two chamber bases. Three chamber bases served as controls for N$_2$O and ^{15}N-N$_2$O fluxes. All measurements as described above in the short-term experiment were also carried out in this experiment which was conducted from May 2007 to June 2008. Measurements were done every second week in the summer months and monthly in the winter months. The ^{15}N solution was always applied one week before the gas flux measurements (18 applications over the year). For one N application in the one-year experiment, the amount of N and water added were equal to the amount added with one application in the short-term experiment. To each chamber receiving N we added the equivalent of 0.78 kg N ha^{-1} yr^{-1}, which was 2.4% of the annual deposition in the spruce stand and 3.8% in the beech stand. The amount of water added was equivalent to 47 mm rain, which was approx. 4.8% of the annual precipitation.

2.2.3. N$_2$O and ^{15}N-N$_2$O measurements

Fluxes of N$_2$O were measured using the closed chamber method (static, vented chambers made of PVC; area: 0.2665 m^2; vol.: 18.9–29.3 L (beech), 27.5–34.7 L (spruce)). Gas samples of 100 mL were removed at three (May 2007 to January 2008) or four (February to June 2008 and 2009) regular time intervals following chamber closure (42 minutes at most) using a portable gas sampler equipped with a pressure sensor (Loftfield et al., 1997). Analysis of N$_2$O was carried out with a gas chromatograph equipped with an EC detector (GC 14A, Shimadzu, Duisburg, Germany). Fluxes of N$_2$O were calculated from the linear change of N$_2$O concentrations in the chamber versus time, after fluxes were adjusted for air temperature and atmospheric pressure. We tested a quadratic model for some data, but no clear advantage of the non-linear model emerged from the statistical indicators for the goodness of fit. Additionally, N$_2$O fluxes calculated using the quadratic model were not significantly different from fluxes calculated using the linear model. We further think that scattering of N$_2$O concentrations due to random errors during sampling and measurement were much larger than the effect of the chamber on the gas exchange and possible biases due to linear regression. The cumulative mean annual flux was calculated using the trapezoid rule. Samples for ^{15}N-N$_2$O analysis were collected in glass bottles (100 mL) closed with a butyl-hallow stopper at time point zero and after 24 to 28 minutes. Analysis of ^{15}N in N$_2$O was done using an isotope

ratio mass spectrometer coupled with a preconcentration unit (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany).

2.2.4. Chemical analyses

Mineral N was extracted after shaking 25 to 30 g fresh mineral soil with 100 mL 0.5 M K$_2$SO$_4$ solution or 15 g of the organic layer with 50 mL K$_2$SO$_4$ solution, using pre-washed filter papers. Extracts of K$_2$SO$_4$ were frozen until analysis. Mineral N analysis was carried out using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, The Netherlands). The water content was determined gravimetrically. Soil bulk density was determined using undisturbed soil samples that were oven-dried at 105°C and sieved to 2 mm to remove stones and living roots. The mass of the organic layer was measured using a metal ring (594 cm^2). Organic layer samples were oven-dried at 60°C and living roots were removed. Total carbon and nitrogen measurements were done using a CNS Elemental Analyzer (Heraeus Elementar Vario EL, Hanau, Germany) and ^{15}N concentrations using a Delta C plus isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). The pH was determined in distilled water (1:2.5 (v/v)) using 10 mL sieved soil or shredded organic material.

2.2.5. Calculation of ^{15}N abundance

Isotope abundances were expressed in absolute proportion [atom%]:

$$^{15}\mathrm{N}\,[\mathrm{atom\%}] = \frac{^{15}\mathrm{N}}{^{15}\mathrm{N} + ^{14}\mathrm{N}} \cdot 100 \tag{2.1}$$

The ^{15}N-N$_2$O abundance of N$_2$O emitted from the soil was calculated using the Keeling plot approach (Pataki et al., 2003; Tilsner et al., 2003):

$$^{15}\mathrm{N}_{\mathrm{emitted}} = \frac{^{15}\mathrm{N}_{\mathrm{chamber\,air}} \cdot c(\mathrm{N_2O})_{\mathrm{chamber\,air}} - ^{15}\mathrm{N}_{\mathrm{ambient\,air}} \cdot c(\mathrm{N_2O})_{\mathrm{ambient\,air}}}{c(\mathrm{N_2O})_{\mathrm{chamber\,air}} - c(\mathrm{N_2O})_{\mathrm{ambient\,air}}} \tag{2.2}$$

where $c(\mathrm{N_2O})$ is the concentration of N$_2$O and ^{15}N is the abundance of ^{15}N in N$_2$O calculated as described in Eq. (1). The ^{15}N$_{\mathrm{emitted}}$ excess value was calculated by subtracting the ^{15}N$_{\mathrm{emitted}}$ value of the control chambers. The ^{15}N$_{\mathrm{emitted}}$ excess value was used to obtain the ^{15}N-N$_2$O excess flux. In case of negative N$_2$O fluxes the ^{15}N$_{\mathrm{emitted}}$ calculation was not applicable since ^{15}N-N$_2$O emissions occurred from labelled soil but could not be quantified because we only measured net N$_2$O fluxes. Hence negative N$_2$O fluxes were omitted for ^{15}N-N$_2$O flux calculations, which included 35% of all flux values in the spruce stand and 17% of those in the beech stand.

2.2.6. Recovery of ^{15}N in N_2O and N_t

The recovery of ^{15}N from the tracers in emitted N_2O (emission factor) and in soil N_t was calculated using atom% values for ^{15}N mass calculations:

$$^{15}\text{N recovery}[\%] = \frac{m^{15}\text{N}_{rec}}{m^{15}\text{N}_{Tracer}} \cdot 100 \tag{2.3}$$

where $m^{15}\text{N}_{rec}$ could be $m^{15}\text{N}_{N2O}$ or $m^{15}\text{N}_{Nt}$, respectively: $m^{15}\text{N}_{N2O}$ is mass of emitted ^{15}N-N_2O excess in mg ^{15}N m^{-2} per time interval considered; $m^{15}\text{N}_{Nt}$ is mass of ^{15}N-N_t excess in mg ^{15}N m^{-2} after 19 tracer applications. Furthermore, $m^{15}\text{N}_{Tracer}$ is mass of ^{15}N excess in the tracer in mg ^{15}N m^{-2} applied since the start of the corresponding experiment.

We used two approaches to estimate the mass of emitted ^{15}N-N_2O between two consecutive ^{15}N-N_2O flux measurements. In the first approach, we used the trapezoid rule. As indicated by the short-term experiment, this approach does not account for the peak emissions during the first week due to the fact that the emissions were measured one week after the application leading potentially to an underestimation of ^{15}N-N_2O fluxes whereas fluxes in the time interval between the measurement and the next tracer application were possibly overestimated. We therefore tested a second approach where we assumed that the ^{15}N-N_2O fluxes displayed the same temporal trend as observed in the short-term experiment. In this approach, the relative proportion of the tracer emission of one measuring day on the emissions of the time interval between consecutive tracer applications was estimated using the short-term experiment data (Eq. 2.4).

$$\text{relative proportion of Ms on Is}[-] = \frac{\text{mean } ^{15}\text{N-N}_2\text{O}_{Ms}}{\text{mean } ^{15}\text{N-N}_2\text{O}_{Is}} \tag{2.4}$$

where Ms is a measuring day of the short-term experiment (fluxes for days on which no measurement took place were interpolated from available measured data) and Is is the time interval between two consecutive tracer applications in which the measuring day lies. The mean ^{15}N-N_2O_{Ms} flux (ng ^{15}N m^{-2} d^{-1}) represents the mean excess ^{15}N-N_2O flux of all replicates of one labelling treatment of the measuring day of the short-term experiment. The mean ^{15}N-N_2O_{Is} flux (ng ^{15}N m^{-2} interval^{-1}) represents the cumulative mean excess ^{15}N-N_2O flux of all replicates of one labelling treatment of the time interval between two consecutive tracer applications of the short-term experiment. The tracer emission of a measuring day from the one-year experiment was then transformed into the tracer emission of the period between two consecutive measurements by dividing it by its relative proportion (Eq. 2.5).

$$\text{cumulative } {}^{15}\text{N-N}_2\text{O}_{\text{IL}} \text{ [ng m}^{-2} \text{ interval}^{-1}] = \frac{{}^{15}\text{N-N}_2\text{O}_{\text{ML}}}{\text{relative proportion of Ms on Is}} \qquad \textbf{(2.5)}$$

where cumulative ^{15}N-N$_2$O$_{\text{IL}}$ represents the cumulative excess ^{15}N-N$_2$O flux of a time interval between two consecutive tracer applications of the one-year experiment and ^{15}N-N$_2$O$_{\text{ML}}$ (ng ^{15}N m^{-2} d^{-1}) is the excess ^{15}N-N$_2$O flux of the measuring day in this interval. The relative proportion could only be estimated from the short-term experiment for the NH$_4^+$-labelled and NO$_3^-$-labelled treatments. Thus, for the NH$_4$NO$_3$-labelled treatment the mean of both proportions was used. Finally, the annual cumulative excess ^{15}N-N$_2$O flux was calculated by adding up the emissions of all measurement intervals.

Emission factors calculated by the two methods did not show any significant differences for a particular treatment and stand, indicating that the higher values of emissions during the first week of application were balanced by the lower emissions of the following week(s). Therefore, we only report results from the first approach.

Nitrous oxide derived from N deposition (N$_2$O-NDFD) was assessed by using the following equation:

$$\text{N}_2\text{O-NDFD [kg ha}^{-1} \text{ yr}^{-1}] = m\text{N}_{\text{dep}} \cdot \frac{\text{EF}}{100} \qquad \textbf{(2.6)}$$

where mN$_{\text{dep}}$ is the mass of inorganic N of the stand deposition in kg ha^{-1} yr^{-1}; EF is the emission factor (units in %).

2.2.7. Statistical analyses

Prior to analysis the assumptions of normality of residuals (Shapiro-Wilk or Cramer-von Mises test) and homogeneity of variances (Levene test) were tested. If necessary, we transformed (log and arcsine) data prior to analyses. Two independent sample means were tested for significant differences using the independent Student's t-test, the Welch test, or the non parametric Mann-Whitney U-test. We used analysis of variance (ANOVA) for comparison of more than two sample means. The weighted harmonic mean was utilised for proportion data with different bases. These proportions were weighted prior to statistical analyses. Temporal pseudoreplication occurred with time series data (N$_2$O, ^{15}N-N$_2$O) because measurements were repeatedly done using the same chamber bases. Therefore, we applied linear mixed effects models (Crawley, 2007; Gueorguieva and Krystal, 2004). We set up a basic model including the forest type (beech and spruce) or the different ^{15}N labelling (NH$_4^+$-labelled, NO$_3^-$-labelled, NH$_4$NO$_3$-labelled) as fixed effects and the spatial replication (individual chamber) nested in time as random effects. The model was extended by a variance

function and by a first-order temporal autoregressive function if the extension increased the goodness of the fit of the model. The Akaike Information Criterion was used to assess the relative goodness of the fit. Simple and multiple regressions were performed using ordinary linear regression models or, if residuals were autocorrelated, generalized least squares extended by an autoregressive moving average function. Autocorrelation was checked with the Durbin-Watson test and by plotting the autocorrelation function. A non-linear least-squares model was used to estimate parameters of the non-linear multiple regression. Effects were considered significant if $P \leq 0.05$. In the text results are reported as means ± 1 standard errors. Statistical analyses were carried out using R 2.10.1 (R Development Core Team, 2009).

2.3. Results

2.3.1. Annual N_2O fluxes

Fluxes of N_2O were significantly lower in the spruce stand than in the beech stand ($P < 0.0001$; Fig. 2-1a, 2-1b). Cumulative N_2O emissions were 0.3 ± 0.1 kg N ha^{-1} yr^{-1} in the spruce stand and 2.6 ± 0.6 kg N ha^{-1} yr^{-1} in the beech stand. The beech stand displayed a distinct seasonality of N_2O fluxes with high emissions during the summer months and low emissions during the winter months. Labelling did not increase N_2O fluxes since we observed no significant differences in N_2O fluxes between the NH_4^+-labelled, NH_4NO_3-labelled, and the control chambers (data not shown).

2.3.2. Short-term ^{15}N tracer experiment

In the spruce stand, the application of the $NH_4^{15}NO_3$ tracer caused an immediate and significant increase in ^{15}N-N_2O fluxes (Fig. 2-2a). Subsequently, ^{15}N-N_2O emissions decreased again and no significant differences in ^{15}N-N_2O fluxes were found three weeks after the $NH_4^{15}NO_3$ tracer application compared to the reference value (pre-measurement at zero hours). Application of the $^{15}NH_4NO_3$ tracer also caused a significant increase in ^{15}N-N_2O fluxes 3.5 hours after the addition, but the increase was smaller than in the NO_3^--labelled plots (Fig. 2-2a). After one week no significant differences in ^{15}N-N_2O fluxes occurred in the NH_4^+-labelled plots of the spruce stand compared to the reference.
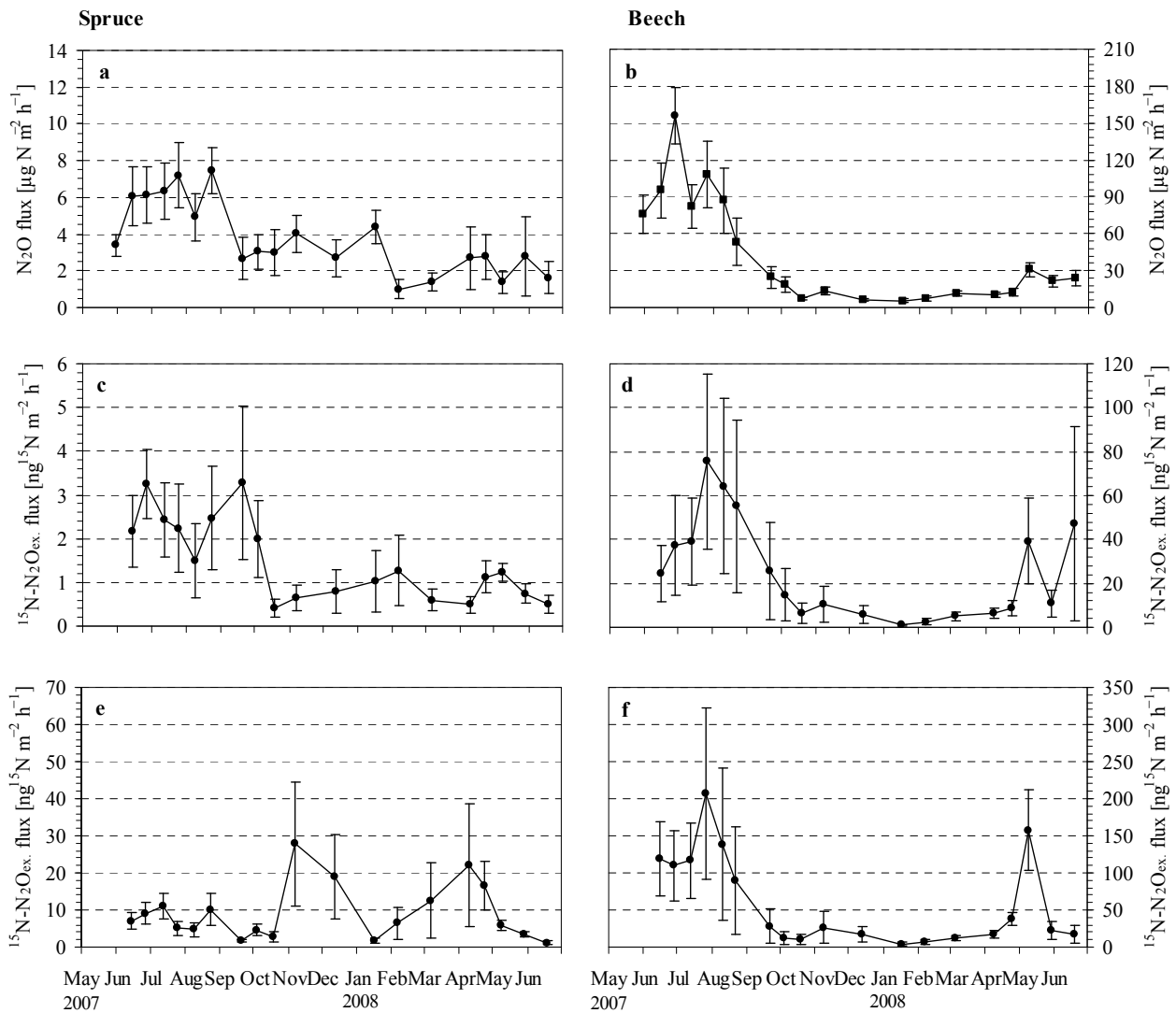
Figure 2-1: Mean (± SE, *n* = 13) N$_2$O fluxes (a, b) and mean (± SE, *n* = 5) ^{15}N-N$_2$O excess fluxes of the NH$_4^+$-labelled treatments (c, d) and NH$_4$NO$_3$ (double)-labelled treatments (e, f) of the spruce stand (a, c, e) and beech stand (b, d, f). Please note the different scales.

In the beech stand, a similar temporal pattern as in the spruce stand was found after tracer application. In the NO$_3^-$-labelled plots, we observed an immediate strong and significant increase in ^{15}N-N$_2$O fluxes (Fig. 2-2b). After one week, ^{15}N-N$_2$O fluxes had decreased and no differences compared to the reference measurement were observed. The application of the ^{15}NH$_4$NO$_3$ tracer only slightly but significantly increased ^{15}N-N$_2$O fluxes and again one week after the tracer addition no significant differences existed when compared to the reference value (Fig. 2-2b). In both spruce and beech stands, the NO$_3^-$-labelled plots displayed three times higher EFs during the first week compared to the NH$_4^+$-labelled plots (Tab. 2-2). For the first three weeks, the EFs calculated for the NO$_3^-$-labelled plots were approx. three times higher than the EFs calculated for the NH$_4^+$-labelled plots as well (Tab. 2-2). But in the beech stand, the difference between the EFs of the NO$_3^-$-labelled and NH$_4^+$-labelled plots decreased

and the EF values of the NH$_4^+$-labelled plots were 70% of the EF values of the NO$_3^-$-labelled plots (Tab. 2-2).
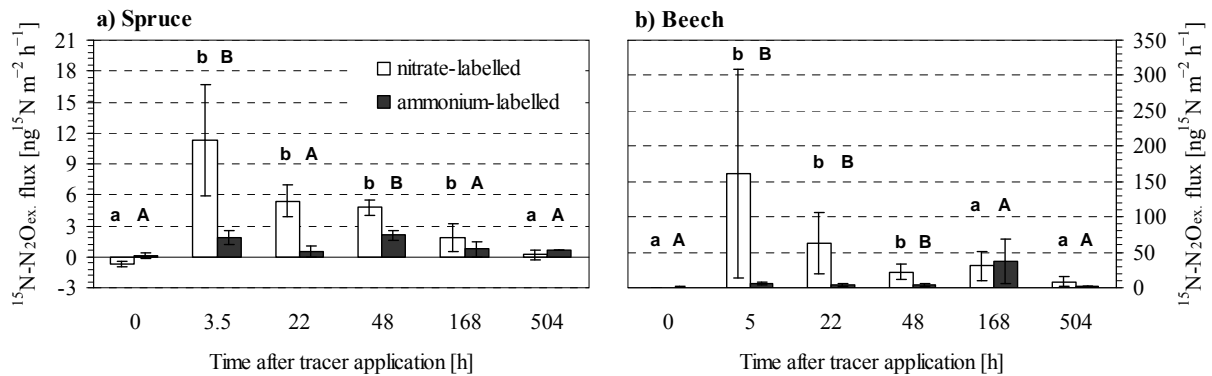
Figure 2-2: Mean (± SE, *n* = 4) ^{15}N-N$_2$O excess fluxes in the spruce stand (a) and beech stand (b) during the short-term experiment. Fluxes are given for the NO$_3^-$-labelled and NH$_4^+$-labelled treatments before (zero hours) and after the tracer application. Means followed by the same letter indicated no significant differences in ^{15}N-N$_2$O excess fluxes of one labelling treatment (NO$_3^-$ or NH$_4^+$-labelled) between zero hours and the time points after the tracer application (mixed effects models with linear contrasts at *P* ≤ 0.05). Please note the different scales.

However, for each stand differences in ^{15}N-N$_2$O emissions between NH$_4^+$-labelled chambers and NO$_3^-$-labelled chambers were not significant. Moreover, differences in ^{15}N-N$_2$O emissions of the NO$_3^-$-labelled chambers between both stands were not significant, but ^{15}N-N$_2$O fluxes of the NH$_4^+$-labelled chambers were significantly higher in the beech stand than in the spruce stand (*P* = 0.029).

Table 2-2: Emission factors (EFs) of N$_2$O during the first week and the first three weeks following tracer application in the spruce and beech stands. The emission factors were derived from the NH$_4^+$-labelled and NO$_3^-$-labelled treatments of the short-term experiment (*n* = 4).

	EF (1 week) [%]			EF (3 weeks) [%]		
Tracer	NH$_4^+$	NO$_3^-$	NH$_4$NO$_3$[*]	NH$_4^+$	NO$_3^-$	NH$_4$NO$_3$[*]
Spruce	0.01 (0.00)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.05 (0.02)	0.04 (0.01)
Beech	0.12 (0.09)	0.31 (0.18)	0.22 (0.10)	0.43 (0.33)	0.62 (0.33)	0.54 (0.22)

Note: Means (± SE); no significant differences were detected among stands, labellings, and EFs.
[*] The EF for the NH$_4$NO$_3$-labelled treatment was obtained by calculating the mean of the NH$_4^+$-labelled and NO$_3^-$-labelled treatments of the short-term experiment (*n* = 8).

Within one stand, fluxes of total N$_2$O were not significantly different in time during the short-term experiment and were 10-times higher in the beech stand (22.8 ± 3.6 µg N m^{-2} h^{-1})

than in the spruce stand (2.2 ± 0.5 µg N m^{-2} h^{-1}; $P = 0.009$). Since no changes were observed in fluxes we assumed that total N_2O fluxes were not changed by the tracer application. The same was true for the contents of NH_4^+, NO_3^- and soil moisture, which remained unchanged during the period of the short-term experiment.

2.3.3. One-year ^{15}N tracer experiment

In the spruce stand, fluxes of ^{15}N-N_2O were significantly higher in the NH_4NO_3-labelled treatment than in the NH_4^+-labelled treatment ($P = 0.0014$), though N_2O fluxes were not significantly different between the two labelled treatments (Fig. 2-1a, 2-1c, 2-1e, Tab. 2-3). Fluxes of ^{15}N-N_2O and N_2O were positively correlated for both labellings individually ($P \le 0.05$). Emissions of ^{15}N-N_2O of the NH_4NO_3-labelled treatment displayed high variability especially during the winter months when nitrate availability was low and any addition of nitrate with the NH_4NO_3 treatment may have contributed significantly to the ^{15}N-N_2O fluxes in the spruce stand (Fig. 2-1e). We did not observe continuous enrichment of ^{15}N in N_2O during the one-year experiment. The mean annual fraction of ^{15}N-N_2O on total N_2O-N was 0.04% (0.00–0.22%) for the NH_4^+-labelled treatment and 0.21% (0.01–0.71%) for the NH_4NO_3-labelled treatment. In the beech stand, we observed no significant differences in ^{15}N-N_2O fluxes between both treatments and beginning in July (1.5 months after the first labelling event), they followed a similar seasonal trend as the total N_2O flux in both treatments (Fig. 2-1b, 2-1d, 2-1f, Tab. 2-3). Fluxes of ^{15}N-N_2O and N_2O displayed strong correlations for both treatments ($P \le 0.0001$). The fraction of ^{15}N-N_2O on N_2O-N averages 0.06% (0.00–0.22%) for the NH_4^+-labelled treatment and 0.19% (0.04–0.49%) for the NH_4NO_3-labelled treatment. In both treatments ^{15}N-N_2O emissions were significantly higher in the deciduous stand than in the coniferous stand ($P \le 0.05$; Tab. 2-3).

Using the EF values approx. 0.12% (spruce) to 0.58% (beech) of the inorganic throughfall N deposition was lost as N_2O within one year, which corresponded to 12.5% (spruce) and 5.7% (beech) of the total annual N_2O emissions (N_2O-NDFD/N_2O-N, Tab. 2-3). For the spruce stand, the EFs of the first week and first three weeks were significantly lower than the EF of one year ($P = 0.03$) but no significant differences among EFs were found for the beech stand (Tab. 2-2, 2-3).

We recovered a large fraction of 59% (spruce) and 42% (beech) of the applied NH_4^+ in N_t of the organic layer and the surface 10 cm of the mineral soil (Tab. 2-3). In contrast, the recovery of NO_3^- tracer in N_t of the NH_4NO_3-treatment (obtained by subtracting the recovery

in the NH_4^+ treatment) was negligible in the beech stand and less than 10% in the spruce stand.

Table 2-3: Cumulative N_2O and ^{15}N-N_2O emissions, one-year emission factor (EF), N_2O derived from deposition (N_2O-NDFD), ratio of N_2O-NDFD to total N_2O emissions, and recovery of ^{15}N tracer in N_t of the organic layer and the top 10 cm of mineral soil. Results are derived from the NH_4^+-labelled and NH_4NO_3-labelled treatments of the one-year experiment.

	Labelling	N_2O [kg N ha^{-1} yr^{-1}]	^{15}N-N_2O [g ^{15}N ha^{-1} yr^{-1}]	EF [%]	N_2O-NDFD [g N ha^{-1} yr^{-1}]	N_2O-NDFD/ N_2O-N[*][%]	Recovery in N_t [%]
Spruce	NH_4^+	0.26 (0.06)[aA]	0.12 (0.04)[aA]	0.03 (0.01)[aA]	9.4 (3.0)[aA]	3.6 (1.1)[aA]	59.2 (11.6)[aA]
	NH_4NO_3	0.30 (0.13)[aA]	0.91 (0.47)[aB]	0.12 (0.06)[aA]	37.9 (19.3)[aA]	12.5 (6.4)[aA]	36.6 (6.6)[aA]
Beech	NH_4^+	3.15 (1.26)[bA]	1.79 (1.10)[bA]	0.46 (0.29)[aA]	79.6 (49.0)[aA]	2.5 (1.6)[aA]	41.7 (7.2)[aA]
	NH_4NO_3	1.75 (1.90)[bA]	4.28 (2.18)[bA]	0.58 (0.29)[aA]	99.3 (50.5)[aA]	5.7 (2.9)[aA]	20.7 (3.1)[aB]

Note: Means (± SE) (*n* = 5 for N_2O, ^{15}N-N_2O, EF, N_2O-NDFD and N_2O-NDFD/N_2O-N and *n* = 4 for recovery in N_t) followed by the same lower-case letters indicated no significant differences among both stands and the same labelling treatments. Means followed by the same capital letters indicated no significant differences among different labelling treatments within one stand (independent t-test at $P \leq 0.05$).
[*] The ratio N_2O-NDFD/N_2O-N represented the weighted harmonic mean with corresponding SE.

2.3.4. Relationship of N_2O flux rates to throughfall N depositions

In the beech stand, N_2O fluxes were positively correlated with total throughfall N depositions (P = 0.001; Fig. 2-3, Tab. 2-4) and with NH_4^+-N depositions (P = 0.001; Tab. 2-4). The slope of the regression equation, which described the modelled relationship between measured N_2O fluxes and measured N depositions, indicated the fraction of throughfall N deposition emitted as N_2O. The fraction was 25%. However, both N_2O fluxes ($P < 0.0001$; Fig. 2-4) and throughfall N deposition were also positively related with soil temperature (N deposition = temperature · 0.09 (±0.03) + 0.70 (± 0.27), r^2 = 0.37, P = 0.01, df = 14). When soil temperature was included in the regression equation the calculated EF was reduced from 25% to 8.6% (Fig. 2-4).

Table 2-4: Results of the regression analyses of N_2O-N fluxes and throughfall N depositions (total N, NH_4^+-N, NO_3^--N, and organic N depositions) and precipitation.

	N_t			NH_4^+-N			NO_3^--N			N_{org}			Precipitation		
	r^2	P	df	r^2	P	df	r^2	P	df	r^2	P	df	r^2	P	df
Spruce	-	ns	-	-	ns	-	-	ns	-	-	ns	-	0.30	0.042	12
Beech	0.53	0.001	14	0.53	0.001	14	-	ns	-	-	ns	-	-	ns	-

Note: ns is not significant

Throughfall N deposition was further positively correlated with precipitation ($P = 0.004$; not shown). For the spruce stand, we did not observe significant correlations between N$_2$O fluxes with N depositions. However N$_2$O fluxes were positively correlated with precipitation (Tab. 2-4).
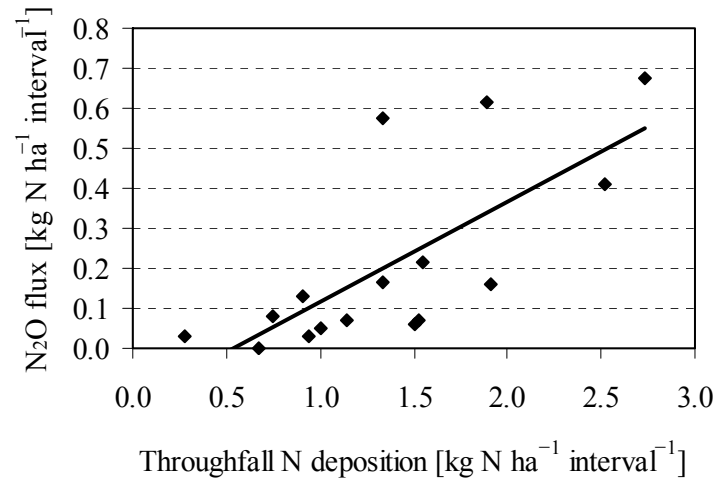
Figure 2-3: Relationship between N$_2$O fluxes (y) and total throughfall N depositions (x) in the beech stand from May 2007 to June 2008 ($y = x \cdot 0.25$ ($\pm$ 0.06) $-$ 0.14 ($\pm$ 0.09); $r^2 = 0.53$, $P = 0.001$, $df = 14$).
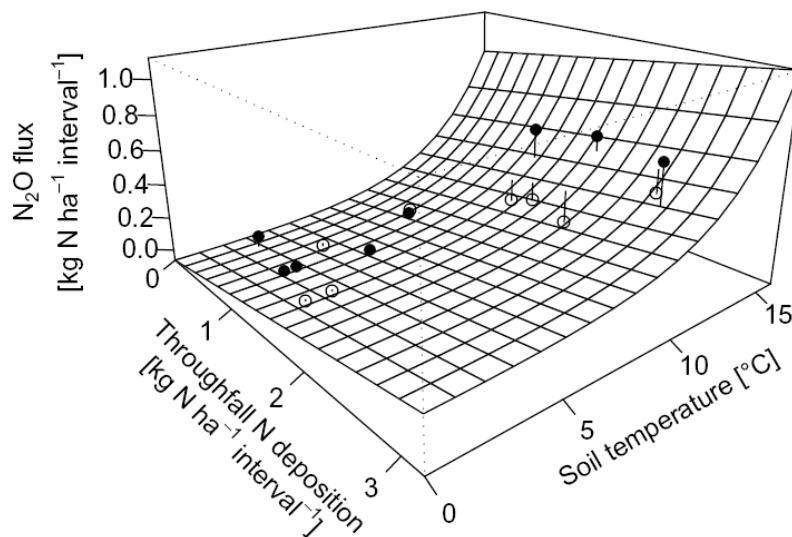
Figure 2-4: Relationship of N$_2$O fluxes (y) to throughfall N depositions ($x1$) and soil temperatures in 5 cm soil depth ($x2$) ($y = 0.086$ ($\pm$ 0.063) $\cdot x1 + 0.001$ ($\pm$ 0.000) $\cdot \exp(0.468$ ($\pm$ 0.242) $\cdot x2) - 0.042$ ($\pm$ 0.073); $r^2 = 0.78$, $P < 0.0001$, $df = 13$). The measured data are indicated as points, where black points are located above the surface area and white points are located below the surface area. The solid lines indicate the deviations of measured data from the model.

We also calculated EFs according to the fraction method, where EFs are calculated by relating measured total N_2O emissions to measured N depositions (for calculation see notes of Tab. 2-5). The EF obtained from this approach was 0.9% for the spruce stand and 13% for the beech stand.

2.4. Discussion

2.4.1. Annual N₂O fluxes

The relatively low N_2O fluxes from the spruce stand and the higher N_2O fluxes from the beech stand were within the range of N_2O emissions reported for other temperate coniferous and deciduous forests (Butterbach-Bahl et al., 2001; Macdonald et al. 1997; Oura et al., 2001; Zechmeister-Boltenstern, 2002; see also Tab. 2-5). The compiled values from literature (Tab. 2-5) further support the observation that deciduous forests generally tend to be a higher source for N_2O (> 20 µg N_2O-N m^{-2} h^{-1}) than coniferous forests (< 10 µg N_2O-N m^{-2} h^{-1}). We attributed the differences in N_2O fluxes between the investigated forest types to the differences in the structure and quality of the litter (e.g. pH, C/N ratio; s. Tab. 2-1). The laminar structure of the beech leaf litter layer of the moder type humus may have reduced gas diffusivity, whereas the needle litter of spruce may have created a well-aerated organic layer as was reported by Ball et al. (1997) for the Solling sites. As a result, the high N_2O emissions during the summer months were possibly produced by denitrification in anaerobic micro-sites in the soil due to high oxygen consumption by microorganisms and plants during high summer temperatures (Brumme et al., 1999). Wolf and Brumme (2002) identified denitrification as main process for N_2O production in the same beech stand which was investigated in the present study.

Net uptake of N_2O, as we observed in single chambers, may be ascribed to consumption of N_2O during denitrification to N_2 (Kroeze et al., 2007). The review by Kroeze et al. (2007) revealed that uptake of N_2O may occur at low oxygen and NO_3^- availability, even at fertilised sites, and that sites with small N_2O fluxes may change from sources to sinks depending on the soil conditions. At the beech stand, negative N_2O fluxes were almost exclusively measured during the winter months where soil moisture was high and soil NO_3^- concentrations were close to zero.

Table 2-5: Compilation of published studies that investigated the impact of N input on N$_2$O fluxes from deciduous and coniferous temperate forest soils. Studies are divided into fertilisation, deposition, and ^{15}N tracer experiments. The emission factor (EF) for N$_2$O is given for one year if not differently indicated.

Forest type	Type of N input	N$_2$O [μg N m^{-2} h^{-1}]	Emission factor [%]	Method used	Treatment	Reference
Deciduous	Fertilisation					
		0.68	0.03[‡]	EF$_{FB}$	NH$_4$NO$_3$-fertilised (120 (1st year) and 150 (2nd year) kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 2nd year; USA)	Bowden et al. (1991)
		0.57	0.06[‡]	EF$_{FB}$	NH$_4$NO$_3$-fertilised (37 (1st year) and 50 (2nd year) kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 2nd year; USA)	
		0.23			Unfertilised (USA)	
		89	1.6	EF$_{FB}$	(NH$_4$)$_2$SO$_4$-fertilised (140 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 6th and 7th year; Germany)	Brumme and Beese (1992)
		64	16[‡]	EF$_F$	Unfertilised (35 kg N ha^{-1} yr^{-1} deposition; Germany)	
		< 10	< 0.3	EF$_F$	NH$_4$NO$_3$-fertilised (150 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 13th year; USA)	Venterea et al. (2003)
		< 10	< 0.3	EF$_F$	NH$_4$NO$_3$-fertilised (50 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 13th year; USA)	
		< 10	< 0.3	EF$_F$	Unfertilised (8 kg N ha^{-1} yr^{-1} wet + dry deposition; USA)	
	Deposition					
		5.7	2[‡]	EF$_F$	25.6 kg N ha^{-1} yr^{-1} throughfall + stemflow dep. (Denmark)	Beier et al. (2001)
		83.3	22[‡]	EF$_F$	33 kg N ha^{-1} yr^{-1} wet deposition (Germany)	Brumme et al. (1999)
		9.1	2.4[‡]	EF$_F$	33 kg N ha^{-1} yr^{-1} wet deposition (Germany)	
		1.9	0.6[‡]	EF$_F$	28 kg N ha^{-1} yr^{-1} throughfall deposition (Germany)	
		7.2	2.4[‡]	EF$_F$	26 kg N ha^{-1} yr^{-1} throughfall deposition (Germany)	
		4.7	1.9[‡]	EF$_F$	21 kg N ha^{-1} yr^{-1} throughfall deposition (Germany)	
		7.3–9.0	~3.5[‡]	EF$_F$	20.2 kg N ha^{-1} yr^{-1} wet deposition (Austria)	Kitzler et al. (2006a)[a]
		5.9–7.4	~4.6[‡]	EF$_F$	12.6 kg N ha^{-1} yr^{-1} wet deposition (Austria)	
		2.3	1.27	EF$_F$	15.7 kg N ha^{-1} yr^{-1} throughfall deposition (Japan)	Oura et al. (2001)
		58.4	10	EF$_R$	20 kg N ha^{-1} yr^{-1} wet deposition (Germany)	Papen and Butterbach-Bahl (1999)
		29.7	25; 13	EF$_R$, EF$_F$	20 kg N ha^{-1} yr^{-1} throughfall + stemflow dep. (Germany)	This study
	^{15}N tracer					
		29.7	0.6	EF$_{15N}$	20 kg N ha^{-1} yr^{-1} throughfall + stemflow dep. (Germany)	This study

Table 2-5 continued.

Forest type	Type of N input	N_2O [μg N m^{-2} h^{-1}]	Emission factor [%]	Method used	Treatment	Reference
Coniferous	Fertilisation					
		4.0	0.1[‡]	EF$_{FB}$	NH_4NO_3-fertilised (120 (1st year) and 150 (2nd year) kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 2nd year; USA)	Bowden et al. (1991)
		3.0	0.5[‡]	EF$_{FB}$	NH_4NO_3-fertilised (37 (1st year) and 50 (2nd year) kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 2nd year; USA)	
		0.8			Unfertilised (USA)	
		3.11	0.35	EF$_F$	NH_4Cl-fertilised (31.4 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the beginning of the 3rd year (only growing season), estimated annual N_2O flux of 0.11 kg N ha^{-1} yr^{-1}); USA)	Castro et al. (1993)
		−1.12			Unfertilised (10 kg N ha^{-1} yr^{-1} wet + dry deposition; measurement only in the growing season; USA	
		1.1 (drained); 2.9 (wet)	0.1 (drained) 0.6 (wet)[‡]	EF$_{FB}$	NH_4NO_3-fertilised (35 kg N ha^{-1} yr^{-1} in small doses; chronic N addition exp.; measurement after 2 years; Sweden)	Klemedtsson et al. (1997)
		0.8 (drained); 1.3 (wet)			Unfertilised (12 kg N ha^{-1} yr^{-1} deposition; Sweden)	
		0.9	<0.1[‡]	EF$_F$	$(NH_4)_2SO_4$-fertilised (150 kg N ha^{-1}; single dose; measurement in the first 3 years; Germany)	Papen et al. (2001)
		−1.0			Unfertilised (Germany)	
		45.8	0.93 (1 month)	EF$_{FB}$	NH_4NO_3-fertilised (90 kg N ha^{-1}; single dose; measurement over 1 month; Norway)	Sitaula et al. (1995)[b]
		21.7	0.94 (1 month)	EF$_{FB}$	NH_4NO_3-fertilised (30 kg N ha^{-1}; single dose; measurement over 1 month; Norway)	
		8.3			Unfertilised (Norway)	
		5.7	0.6[‡]	EF$_{FB}$	Acid mist-fertilised (96 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement after 2 years; UK)	Skiba et al. (1998); Skiba et al. (1999)
		0.5	0.2[‡]	EF$_{FB}$	Acid mist-fertilised (48 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 3rd year, UK)	
		−0.3			Unfertilised (6.7 kg N ha^{-1} yr^{-1} wet + dry deposition; UK)	
		< 10	< 0.3	EF$_F$	NH_4NO_3-fertilised (150 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 13th year; USA)	Venterea et al. (2003)
		< 10	< 0.3	EF$_F$	NH_4NO_3-fertilised (50 kg N ha^{-1} yr^{-1}; chronic N addition exp.; measurement in the 13th year; USA)	
		< 10	< 0.3	EF$_F$	Unfertilised (8 kg N ha^{-1} yr^{-1} wet + dry deposition; USA)	

Table 2-5 continued.

Forest type	Type of N input	N_2O [$\mu g\ N\ m^{-2}\ h^{-1}$]	Emission factor [%]	Method used	Treatment	Reference
	Deposition					
		2.9	0.6[‡]	EF_F	41 kg N ha^{-1} yr^{-1} throughfall deposition (Germany)	Brumme et al. (1999)
		2.4	0.7[‡]	EF_F	31 kg N ha^{-1} yr^{-1} throughfall deposition (Germany)	
		14.8	6.5[‡]	EF_F	20 kg N ha^{-1} yr^{-1} throughfall deposition without N_{org} (Germany)	
		4–15	1.2–4.4[‡]	EF_F	30 kg N ha^{-1} yr^{-1} wet deposition (Germany)	Butterbach-Bahl et al. (1998)[a]
		−0.5–2.1	~1.3[‡]	EF_F	5–6 kg N ha^{-1} yr^{-1} wet deposition (Ireland)	
		16–32	~7–13[‡]	EF_F	20–22 kg N ha^{-1} yr^{-1} wet deposition (Germany)	Butterbach-Bahl et al. (2002)[a]
		5–10	~4.4[‡]	EF_F	~15 kg N ha^{-1} yr^{-1} wet deposition (Germany)	
		3.4–4.7	2.5–3.5	EF_F	10.6–11.9 kg N ha^{-1} yr^{-1} bulk deposition (Austria)	Kitzler et al. (2006b)[c]
		3.8	~1	EF_{FB}	~46.2 kg N ha^{-1} yr^{-1} total deposition (UK)	Macdonald et al. (1997)
		1.3			6.4 kg N ha^{-1} yr^{-1} total deposition (UK)	
		4.3	1.23	EF_F	30.6 kg N ha^{-1} yr^{-1} throughfall deposition (Japan)	Oura et al. (2001)
		16.4	0.5	EF_R	30 kg N ha^{-1} yr^{-1} wet deposition (Germany)	Papen and Butterbach-Bahl (1999)
		56	6	EF_{FB}	80.1 kg N ha^{-1} yr^{-1} NH_3 dep from poultry farm; 30 m downwind from farm (UK)	Skiba et al. (1998, 1999)[d]
		13			17.4 kg N ha^{-1} yr^{-1} NH_3 dep from poultry farm; 250 m down-wind from farm (UK)	
		3.4	0.9	EF_F	33 kg N ha^{-1} yr^{-1} throughfall deposition (Germany)	This study
	[15]N tracer					
		3.4	0.1	EF_{15N}	33 kg N ha^{-1} yr^{-1} throughfall deposition (Germany)	This study

Note: EF_F: Emission factor represented the fraction of N input that is re-emitted: EF_F = flux / N input · 100. EF_{FB}: Emission factor calculated for N fertilisation experiments or N deposition gradients, where N_2O background emissions are considered. Calculated for N fertilisation experiments: EF_{FB} = (flux from fertilised plot – flux from control plot) / N amount in fertiliser · 100. Calculated for N deposition gradients: EF_{FB} = ((flux at high N input – flux at background N input) / (high N input – background N input)) · 100. EF_R: Emission factor were derived from regression analysis between N_2O fluxes and N deposition rates. EF_{15N}: Emission factor were derived from ^{15}N tracer experiment; for calculations see this study.

[‡] Emission factor was calculated by the authors of this study.

[a] The compared sites were similar in climatic and edaphic conditions.

[b] Lysimeter study with re-established soil profiles from Scots pine forest.

[c] Spruce-fir-beech forest.

[d] Mixed woodland of pine, birch, oak, rowan, and elder.

2.4.2. Temporal dynamics of N_2O emissions derived from N depositions

The immediate increase in ^{15}N-N_2O fluxes that occurred within 3.5–5 hours in the short-term experiment after $NH_4{}^{15}NO_3$ and $^{15}NH_4NO_3$ tracer application indicates that deposited N contributed promptly to N_2O production. However, the contribution of N deposition to N_2O fluxes decreased within the following three weeks. In the spruce stand release of N_2O derived from the ^{15}N label occurred over a longer period than in the beech stand, where loss of deposited N as N_2O occurred entirely during the first three weeks. This short-lived increase in ^{15}N-N_2O emissions, which lasted approx. three weeks, was probably related to biotic and abiotic immobilisation of added N and to dilution processes. In contrast to our hypotheses, the repeated application of ^{15}N labelled N did not lead to any further increase in ^{15}N enrichment of N_2O emitted during the one-year experiment. This indicates that the main effect of N deposition on N_2O fluxes occurs when the mineral N enters the soil system ('short-lived effects'). The absence of 'medium-term' effect (up to one year) may be explained by continual immobilisation of N during the repeated additions and by the lack of any remobilisation of the immobilised N. Our recovery of NH_4NO_3 tracer in soil N_t also showed that a great proportion of N deposition was retained in the soil. Our finding was supported by output analysis by Brumme and Khanna (2009b) and Feng et al. (2008), which indicated that in both systems (spruce and beech) the deposited N was predominantly retained despite the amount of N deposition was in excess of that required for plant increment.

2.4.3. Processes of N_2O emissions derived from N depositions

The greater increase in ^{15}N-N_2O fluxes after $NO_3{}^-$ tracer application than after $NH_4{}^+$ tracer application suggests that denitrification was the dominant process for the input-derived emissions at both sites. Water addition probably created additional anaerobic micro-sites resulting in favourable conditions for denitrification, which mimics conditions when natural wet N deposition occurs. Denitrification was also found to be the dominant process at our beech stand at the Solling site by Wolf and Brumme (2002). The smaller ^{15}N-N_2O flux increase after $^{15}NH_4NO_3$ tracer application was probably caused by low activity of nitrifiers in these acid soils and the weak competition of nitrifiers for available $NH_4{}^+$ (Corre and Lamersdorf, 2004 (study conducted at Solling); Rennenberg et al., 1998). Fertilisation with $(NH_4)_2SO_4$ also delayed an increase in N_2O emissions by 14 days at our beech stand (Brumme and Beese, 1992), indicating low activity of nitrifiers and thus a delay of denitrification. In contrast, rapid increases in N_2O fluxes have been reported after fertilisation with $NO_3{}^-$-

containing fertilisers of coniferous forests (Klemedtsson et al., 1997; Macdonald et al., 1997; Sitaula et al., 1995).

For both stands, the fraction of ^{15}N-N$_2$O on N$_2$O-N of 0.05% for the NH$_4^+$-labelled treatment and of 0.20% for the NH$_4$NO$_3$-labelled treatment of the one-year experiment suggests that the contribution of NH$_4^+$ to N$_2$O emissions was 25% and of NO$_3^-$ 75%. Similar results were presented by Ambus et al. (2006) who showed that 62% of N$_2$O emissions in 11 European forests were derived from NO$_3^-$ and 34% were derived from NH$_4^+$.

2.4.4. Contribution of N depositions to N$_2$O emissions

The compilation of published studies on this subject showed a huge range of results for the impact of N input on N$_2$O fluxes in temperate forests similar as we found in our study (Tab. 2-5). Our study indicated that the emission factor (EF) depended on the method used for its calculation. The EF$_R$ value of 25% for our beech stand, which was calculated by using the regression method, was higher than the EF$_F$ values, which were obtained by the fraction method and ranged from 0.9% (spruce) to 13% (beech). The EF$_R$ and EF$_F$ were again both higher than the EF$_{15N}$ values derived from our ^{15}N tracer study (0.1% for spruce and 0.6% for beech stands). Further, a EF$_{FB}$ value (obtained from N fertilisation experiments; for calculations see notes of Tab. 2-5) of 1.6% was calculated for a long-term fertilisation experiment at our beech stand where 140 kg N ha^{-1} yr^{-1} were applied over a 5–6 years period (Tab. 2-5; Brumme and Beese, 1992).

In literature EF$_R$ values were among the highest reported. For example, a study in southern Germany reported an EF$_R$ of 10% for a beech stand (5.1 kg N$_2$O-N ha^{-1} yr^{-1}) and 0.5% for a spruce stand (1.4 kg N$_2$O-N ha^{-1} yr^{-1}) using the regression approach (Papen and Butterbach-Bahl, 1999; Tab. 2-5). Denier van der Gon and Bleeker (2005) combined results from several studies conducted in temperate forests and found an EF$_R$ value of 6.3% for deciduous forests and of 1.4% for coniferous forests. A positive correlation of N$_2$O + NO fluxes with N deposition was also reported for 15 European forests exposed to different rates of N deposition where between 2% and 32% of total N deposition were emitted as N$_2$O + NO (Pilegaard et al., 2006). Emission factors EF$_F$ show a wide range from <0.1% to 22% for both forest types (Tab. 2-5). Using the fraction method, Denier van der Gon and Bleeker (2005) found an average EF$_F$ of 2.4% for coniferous forests and of 6.5% for deciduous forests. The EF$_{FB}$ based on fertilised plots ranged from 0.1% to 0.9% for the coniferous forests and from 0.03% to 1.6% for the deciduous forests (Tab. 2-5). The results of EF estimations can be summarised as follows:

$$EF_{15N} \approx EF_{FB} << EF_F \approx EF_R$$

What factors cause the large differences in EFs when calculated employing different methods? The fraction of N$_2$O derived from inorganic N deposition was relatively small (13% on spruce and 6% on beech stands) indicating that sources other than direct N deposition were involved in N$_2$O emissions. The EF$_{FB}$ considers such background emissions e.g. from unfertilised plots in fertilisation experiments. In contrast to EF$_{FB}$, the EF$_F$ does not distinguish between possible background emissions and emissions directly derived from N deposition. Consequently values of EF$_F$ of the fraction method will overestimate direct N$_2$O emissions from deposition because it does not correct for such possible N$_2$O background emissions.

Emission factors based on fertilised plots (EF$_{FB}$) were in the same order of magnitude in our study as our EF$_{15N}$ values obtained by the ^{15}N tracer method. However, strong increases in N$_2$O fluxes from the fertilised plots compared to the unfertilised control plots have been reported (Tab. 2-5), which may result from the high mineral N concentrations following fertiliser application, which does not reflect conditions during atmospheric N deposition (Sitaula et al., 1995; Skiba and Smith, 2000) and may cause a positive priming effect (e.g. Fenn et al., 1998). Also the implicit assumption of the regression approach is that the N$_2$O emissions from N cycling are not affected by N depositions. However, this assumption may not be valid and may lead to artificially high estimates since both N deposition and N$_2$O fluxes are frequently positively related to a number of factors. Some of those factors also affect N$_2$O fluxes in a positive way causing systematic error of overestimating the EF$_R$ values. For an example, at times of high rainfall (and thus, high N deposition), high soil water content will stimulate denitrification and therefore N$_2$O fluxes, which originate mainly from N cycling in the soil and not from direct N deposition. The problem of complex relationships was further highlighted by the reduction of the calculated EF$_R$ from 25% to 8.6% in our beech stand when soil temperature was included in the regression equation. Our study demonstrated that EF$_{15N}$ values represent reliable and direct values of emissions by inorganic N depositions.

The low values of direct contribution of atmospheric depositions to N$_2$O emissions raise the question whether the background emissions are affected by decade long atmospheric N depositions, most of which may have been accumulated in the ecosystems. N cycling rates may have considerably changed due to long-term N depositions (Corre et al., 2007). With the methods employed here it was not possible to assess the effect of accumulated anthropogenic N on the background N$_2$O emissions. However, the literature compilation indicates that, in general, soils with a similar forest type but with higher N depositions had higher N$_2$O emissions than soils exposed to lower N depositions (Tab. 2-5). Assuming that N$_2$O emissions

were negligible before the onset of anthropogenic activity, the total contribution of N deposition to N₂O emission (direct and background emissions) is probably best described by the fraction of N₂O to current N deposition (EF_F). To obtain a representative EF_F value, mean values covering several years are necessary. This is illustrated by the mean EF_F value calculated for the beech stand from 1990 to 1998 ($8.2\% \pm 0.7\%$) compared to the higher EF_F calculated for 2007 to 2008 when N depositions were comparably low and N₂O emissions were comparably high.

2.5. Conclusion

The ^{15}N tracer method proved to be a precise approach for quantifying the direct contribution of atmospheric N deposition to the emission of N₂O. The technique allows the simulation of atmospheric throughfall N deposition without artificial fertilisation. The method provides the possibility to investigate the impact of N deposition on N₂O emissions in forests also when direct emissions are low. In contrast to the regression approach, this method does not include artefacts which may result from controlling rainfall or temperature conditions, as both would influence N₂O fluxes and N depositions. There are no uncertainties resulting from the comparison of different sites (as when regarding deposition gradients), which are usually not completely comparable in climate and soil conditions. Furthermore, EFs when calculated from fertilised plots may result in artificially high N₂O emissions because the pulse in mineral N concentrations does not reflect the true atmospheric N depositions and may also cause positive priming effects on the N cycling in soils. Whereas most of the other methods overestimate the direct emissions, the ^{15}N tracer method is a useful tool to measure direct emissions or background emissions.

Acknowledgments

Long-term reduction of N depositions alters the NO_x flux but not the N_2O flux in a N-enriched European spruce forest soil

3. Long-term reduction of N depositions alters the NO_x flux but not the N_2O flux in a N-enriched European spruce forest soil

Abstract

European temperate forest soils have been exposed to elevated nitrogen (N) and acid depositions for decades. High nitrous oxide (N_2O) and nitric oxide (NO) emissions have been reported from these forests. Since the 1980s, a decline in atmospheric deposition rates has been occurring. Our study addressed the question as to how in a N-enriched spruce stand (*Picea abies* (L.) Karst.) N oxide fluxes and N turnover processes have changed in response to the declining N depositions. Studies were conducted at the Solling roof site under a control-roof with ambient depositions and under a reduced-N-input-roof where N and acid depositions have been reduced to a pre-industrial level for 16–17 years. Open dynamic and closed chamber methods were used to determine NO_x and N_2O fluxes, respectively, and in situ incubation studies were conducted to measure net N mineralisation. Under long-term reduction of depositions, net nitrification and nitrate in soil solution were reduced to undetectable levels causing the soil to change from a net source for NO_x (0.62 ± 0.24 kg N ha^{-1} yr^{-1}) into a net sink (-0.33 ± 0.01 kg N ha^{-1} yr^{-1}). The uptake of NO_x was exclusively controlled by the NO_x concentrations of the forest air. Reversal of N enrichment did not affect annual N_2O fluxes (0.08 kg N ha^{-1} yr^{-1}) due to restricted denitrification in the well-aerated organic layer, but the origin of nitrate for denitrification changed from soil-borne N to deposited N. It was demonstrated that less than two decades of reduced N and acid depositions are sufficient to reduce the surplus N in this soil. Under these conditions, it was not the soil accumulated N but the rate of atmospheric N inputs which controlled N oxide fluxes.

Keywords: NO_x consumption, NO_x production, nitrous oxide, N deposition, nitrification, Solling roof project

3.1. Introduction

The global nitrogen (N) cycle has been changed due to the rapid increase in reactive N resulting from industrialisation and intensive agricultural activities during the last century (Berge et al., 1999; Galloway et al., 2008; Vitousek et al., 1997). Consequently, temperate forest ecosystems have been exposed to high atmospheric N and acid depositions, which have altered the geochemical cycles (Aber et al., 1998; Brumme and Khanna, 2009a). Increased N availability is held responsible for high nitrous oxide (N_2O) and nitric oxide (NO) emissions from European forest soils (e.g. Brumme and Beese, 1992; Gasche and Papen, 1999; Papen and Butterbach-Bahl, 1999; Skiba et al., 1999; van Dijk and Duyzer, 1999). Nitrous oxide represents a potent greenhouse gas and contributes to the catalytic decomposition of stratospheric ozone (Crutzen, 1979; IPCC, 2007) whereas the highly reactive NO is a secondary greenhouse gas (IPCC, 2007) and contributes to photochemical air pollution (Crutzen, 1979; Logan, 1983).

Since the end of the 1980s, political action has resulted in a decline in atmospheric N depositions and in particular in acid depositions in Europe (e.g. Meesenburg et al., 2009; Schöpp et al., 2003). Hence, the question arises of how forest ecosystems respond to and recover from declining N and acid depositions. Several research programmes have been initiated to study the effect of atmospheric depositions on forest ecosystems, such as the large-scale European NITREX (NITRogen saturation EXperiment) project (Bredemeier et al., 1995; Rasmussen and Wright, 1998). In the framework of this project, a reduction of throughfall deposition of 30 kg N ha^{-1} yr^{-1} in the control plot to 9.7 kg N ha^{-1} yr^{-1} in the clean rain plot has been conducted at the roof project at the Solling (Lamersdorf and Borken, 2004). Beside N, sulphur (−53%) and protons (−78%) were reduced. In this context, the major soil changes under the roof were:

(i)   Rapid reduction in nitrate (NO_3^-) leaching already one year after roof closure in the clean rain plot (Bredemeier et al., 1995, 1998; Xu et al., 1998).

(ii)   Progressive reduction in aluminium and sulphate concentrations of soil solution at 10 cm depths (Corre and Lamersdorf, 2004).

(iii)   No change in N_2O emissions and methane uptake two and nine years after reducing deposition inputs (Borken et al., 2002).

(iv)   A slight increase in gross N mineralisation rates, an increase in microbial ammonium (NH_4^+) immobilisation rates and a faster turnover of NH_4^+ and microbial N pools in the organic layer 10 years after roof closure (Corre and Lamersdorf, 2004).

(v)   No detection of nitrification and NO_3^- in soil solution 10 years after changing the input situation (Corre and Lamersdorf, 2004).

(vi)   Similar values of microbial biomass 10 and 14.5 years after reduction of N depositions, suggesting that lower N input probably was counterbalanced by the internal N turnover (Corre and Lamersdorf, 2004; Dörr et al., 2010).

(vii)   No response of soil pH up to 14.5 years after roof closure (Dörr et al., 2010; Lamersdorf and Borken, 2004).

(viii) No response in biomass of total nitrate reducer and denitrifier communities 15 years after reduction of N depositions (Kandeler et al., 2009).

Considering the decrease in nitrification and NO_3^- in soil solution little change in N_2O emissions were not expected. According to the "hole in the pipe" model by Firestone and Davidson (1989), the sum of N_2O and NO emissions is a function of N availability,

specifically of nitrification and denitrification, and should have declined due to reduced N input. Production of NO in soils mainly occurs via aerobic nitrification in contrast to N_2O, which is mainly released during anaerobic denitrification (Davidson et al., 2000; Conrad, 1996). Production of NO_x (NO + nitrogen dioxide (NO_2)) may further occur via the abiotic process of chemodenitrification in acid soils (van Cleemput and Baert, 1984). To date, no information about NO_x fluxes is available for the Solling roof project. However, high emissions of NO from other highly N-enriched coniferous forests have been reported (e.g Gasche and Papen, 1999; van Dijk and Duyzer, 1999). High NO but comparable low N_2O emissions from coniferous forests were ascribed to aerobic soil conditions (Pilegaard et al., 2006).

This study therefore addressed the question of whether long-term reduction of N depositions of 16–17 years below the critical load level of 14 kg N ha^{-1} yr^{-1} (de Vries et al., 1995) affected the exchange of NO_x and N_2O between soil and atmosphere, which might have a direct effect on the atmospheric chemistry.

3.2. Materials and methods

3.2.1. Study area

The study was conducted in a 74-yr-old Norway spruce stand (*Picea abies* (L.) Karst.) at the Solling plateau in Central Germany (51° 46' N, 9° 34' E; 500 m a.s.l.). The Solling is characterised by moderate temperature changes throughout the year with a mean annual temperature of 6.9°C, evenly distributed precipitation with annually 1193 mm (Meesenburg and Brumme, 2009), and chronic high N deposition rates. Triassic sandstone bedrock is covered by a loess solifluction layer, in which a Dystric Cambisol has developed. The humus type is a typical moder. The soil texture is a silt loam (23% sand, 62% silt, 15% clay). Further soil properties are shown in Tab. 3-1, for comparison a table including the same parameters can be found by Corre and Lamersdorf (2004) and a detailed site description is given by Bredemeier et al. (1995, 1998).

3.2.2. The Solling roof project

In September 1991, the Solling roof project was started, which was described in detail by e.g. Bredemeier et al. (1995) and Corre and Lamersdorf (2004). In brief, two transparent roofs (each of 300 m^2) were installed below the canopy of the spruce stand for manipulation of atmospheric nutrient inputs. The clean rain roof plot received pre-industrial depositions. Depositions of NH_4^+-N were reduced by 85% and depositions of NO_3^--N by 50% compared

to the control roof plot receiving ambient N depositions. The throughfall N depositions amounted to 12 kg N ha^{-1} yr^{-1} under the clean rain roof and to 35 kg N ha^{-1} yr^{-1} under the control roof in 2007–08. In 2007–08, the NH$_4^+$-N:NO$_3^-$-N:N$_{org}$ ratio was 26:54:20 under the clean rain roof and 37:54:12 under the control roof. The pH of the throughfall water was 5.0 under reduced depositions and 4.4 under ambient depositions. Throughfall water was continuously collected from the roof surface, pre-filtered (350 μm), and for the clean rain roof, de-ionised and chemically adjusted, before the water was almost simultaneously re-sprinkled under the roofs. In 2001, a ^{15}N tracer experiment was started to investigate the fate of N depositions. Therefore, each roof was divided into two subplots, one of which received a ^{15}NH$_4^+$-labelling and the other a ^{15}NO$_3^-$-labelling (see Fig. 1-6).

Table 3-1: Soil characteristics of the roof plots at the Solling spruce stand that received reduced and ambient N depositions.

Soil depth and forest site	pH(H$_2$O)	Total organic carbon [mg C g^{-1}]	Total nitrogen [mg N g^{-1}]	C/N ratio	Bulk density [g cm^{-3}]	Humus mass [Mg ha^{-1}]
Organic layer						
Reduced dep.	3.6 (0.1)	432 (9)	16.1 (0.7)	27.0 (1.0)		88 (7)
Ambient dep.[*]	3.6 (0.0)	427 (0)	18.3 (0.8)	23.4 (1.0)		89 (15)
0–5 cm						
Reduced dep.	3.4 (0.0)	94 (18)	4.2 (0.7)	22.2 (0.5)	0.78 (0.02)	
Ambient dep.	3.4 (0.0)	78 (8)	3.6 (0.3)	21.8 (0.8)	0.82 (0.08)	
5–10 cm						
Reduced dep.	3.6 (0.1)	37 (4)	1.9 (0.3)	19.8 (0.7)	1.15 (0.07)	
Ambient dep.	3.6 (0.0)	33 (5)	1.7 (0.3)	19.6 (0.9)	1.04 (0.18)	

Note: Means (± SE, *n* = 3, [*] *n* = 2).

3.2.3. Experimental design

The experiment was carried out under the NH$_4^+$-labelled subplots of the clean rain and control roof between June 2007 and August 2008. Additionally, the NO$_3^-$-labelled subplots were studied from May to August 2008. Five chamber bases, randomly installed for gas flux measurements, were used under each of the two NH$_4^+$-labelled subplots. Four chamber bases were randomly installed under each of the NO$_3^-$-labelled subplots in April 2008. We determined NO$_x$ and N$_2$O fluxes, air and soil temperature (at 2.5, 5, and 10 cm depth), soil moisture and extractable mineral N (N$_{min}$ = NH$_4^+$-N + NO$_3^-$-N) contents of the organic layer and of 0–5 and 5–10 cm depths of the mineral soil. For soil analysis, four fixed areas at each subplot were established, two of which were chosen randomly per sampling date to reduce the

amount of disturbance under the roofs. Measurements were conducted every second week in the summer months and monthly in the winter months.

3.2.4. NO$_x$ and N$_2$O flux measurements

We used the open dynamic chamber method for NO$_x$ measurements and the closed chamber method for N$_2$O measurements. All chamber bases under the roofs were permanently installed (area: 0.25 m^2). The insertion depth amounted to approx. 5 cm and the upper part of the bases consisted of a water trap for seal when the chamber lid was placed onto the base during the measurement. The enclosed air volume under the chambers ranged between 18.7 and 26.6 L. Chambers were made of PVC and were vented.

Flux determination of NO$_x$ took place on-site due to the high reactivity of NO$_x$. We used a mobile NO$_x$ analyser, which consisted of a calibration and a detection unit. Before analysis of NO$_2$ by a Scintrex LMA-3 chemiluminescence detector (Scintrex Unisearch, Ontario, Canada), NO was oxidised to NO$_2$ by a solid CrO$_3$ catalyst. A Teflon tube connected the analyser with a chamber, which we placed on the chamber base for five minutes. Concentrations of NO$_x$ were logged every five seconds (CR510 data logger, Campbell Scientific, Utah, USA). The total mean air flow through the chamber was 1 L min^{-1}. We calibrated before and after chamber measurements using a NO standard gas (3000 ppbv NO in N$_2$; Air Liquide GmbH, Germany).

For N$_2$O flux measurements, 100 mL gas samples were taken with a portable gas sampler equipped with a pressure sensor (Loftfield et al., 1997). The chambers were closed for 40 minutes at most and three to five gas samples were removed at regular intervals during closure. The samples were analysed with a gas chromatograph equipped with an EC detector (GC 14A, Shimadzu, Duisburg, Germany).

The linear change of gas concentration in the chamber versus time was calculated and multiplied by the ratio of chamber volume to soil surface area and by air density, which was adjusted for air temperature and atmospheric pressure. We estimated annual fluxes using the trapezoid rule.

3.2.5. Net N mineralisation rates

We determined net ammonification and nitrification rates at the NH$_4^+$-labelled subplots in summer 2008. Soil samples of the surface 10 cm of the soil (including the organic layer) were taken with a stainless steel core. We used four to five replicates. For each replicate, two samples were taken. One was removed from the core and divided into 2 cm sections of which

soil extracts for N_{min} analyses were prepared within few hours (t0 sample). The other soil core (t1 sample) was put into a PE bag and returned into the soil for undisturbed in-situ incubation, which lasted 23 days. After the incubation, soil cores were subject to the same treatment as the t0 samples.

3.2.6. Chemical analyses

Soil extracts were prepared adding 100 mL 0.5 M K_2SO_4 solution to 25 to 30 g fresh soil and 50 mL K_2SO_4 solution to 15 g of the organic layer. The samples were shaken for one hour before filtering. The extracts remained frozen until N_{min} analysis was performed using a continuous flow injection colorimeter (Cenco/Skalar Instruments, Breda, The Netherlands). The water content was determined gravimetrically. Bulk density was determined on undisturbed soil samples, which were oven-dried at 105°C. Stones and living roots were removed (sieving to 2 mm) and their volumes recorded. Samples of the organic layer were oven-dried at 60°C and living roots were removed for mass detection. We measured organic carbon and total nitrogen concentrations with a CNS Elemental Analyzer (Heraeus Elementar Vario EL, Hanau, Germany). Before measurements, mineral soil samples were dried at 60°C, sieved, and ground whereas organic material was shredded before grinding. We used sieved soil and shredded organic material (10 mL) for pH value determination in distilled water (1:2.5 (v/v)). For analysis of the particle size distribution, organic matter and Fe oxides were destructed using 30% H_2O_2 and 4% Na-dithionite-citrate before wet sieving of the sand and coarse silt fraction ($\geq$ 20 µm). Then, sedimentary fractionation according to the Atterberg method was conducted at 25°C for 21 hours (30 cm fall height; Schlichting et al., 1995).

3.2.7. Statistical analyses

The independent Student's t-test and the Welch-test were applied for comparison of two independent sample means. For testing more than two sample means, analysis of variance (ANOVA) or the Kruskal-Wallis H-test were used. Because of temporal pseudo-replication, we used linear mixed effects models for time series data (Crawley, 2007; Gueorguieva and Krystal, 2004). In these models, the spatial replication (individual chamber) nested in time was considered as random effect. A variance function and an autocorrelation function (first-order autoregressive function or autoregressive moving average function) were added if the extension increased the goodness of the model fit. The relative goodness of the fit was assessed by using the Akaike Information Criterion (AIC). Ordinary simple and multiple regression analyses were carried out. In case of autocorrelated residuals, generalized least

squares extended by an autocorrelation function were used for regression analyses. Autocorrelation was checked by the Durbin-Watson test and by plotting the autocorrelation function. The AIC was applied to obtain the minimal adequate model. We checked the assumptions of normality of residuals and homogeneity of variances prior to statistical analyses. Box-cox transformations were used where the assumptions were not met. Statistical analyses were performed on pseudo-replicates since the roofs were not replicated. Statistical significance was stated when $P \leq 0.05$. In the text results are reported as means ± 1 standard errors. All statistical analyses were conducted using R 2.12.1 (R Development Core Team, 2010).

3.3. Results

3.3.1. Effect of reduced N depositions on NO_x fluxes

The lower N depositions resulted in a reduction of the cumulative annual NO_x flux of 0.62 ± 0.24 kg N ha^{-1} yr^{-1} under ambient depositions to 0.33 ± 0.01 kg N ha^{-1} yr^{-1} under reduced depositions ($P \leq 0.001$; Fig. 3-1a). The marked seasonal dynamic with high positive emissions in summer month and zero emissions during the winter months under ambient depositions was not noticed on reduction in N depositions.

No significant differences in NO_x fluxes were observed between both subplots (NH_4^+ or NO_3^--labelled) of one roof (data not shown).

Including data of both roofs, fluxes of NO_x were explained best by the NO_3^- content of the organic layer and by soil temperature in 2.5 cm depth as was indicated by a multiple regression analysis (Fig. 3-2; $r^2 = 0.626$, $P < 0.001$, $df = 49$). Under reduced N depositions, NO_x fluxes negatively depended on the concentration of NO_x in the ambient forest air as shown by multiple regression analysis (Fig. 3-3a; $r^2 = 0.749$, $P < 0.001$; $df = 21$). Under ambient depositions, NO_x fluxes depended on the NH_4^+ content of the organic layer, on soil temperature in 2.5 cm depth, and on the concentration of NO_x in the ambient forest air ($r^2 = 0.639$, $P < 0.001$, $df = 17$). However, the NH_4^+ content was the only factor in the minimal adequate model that significantly influenced the NO_x fluxes under ambient depositions.
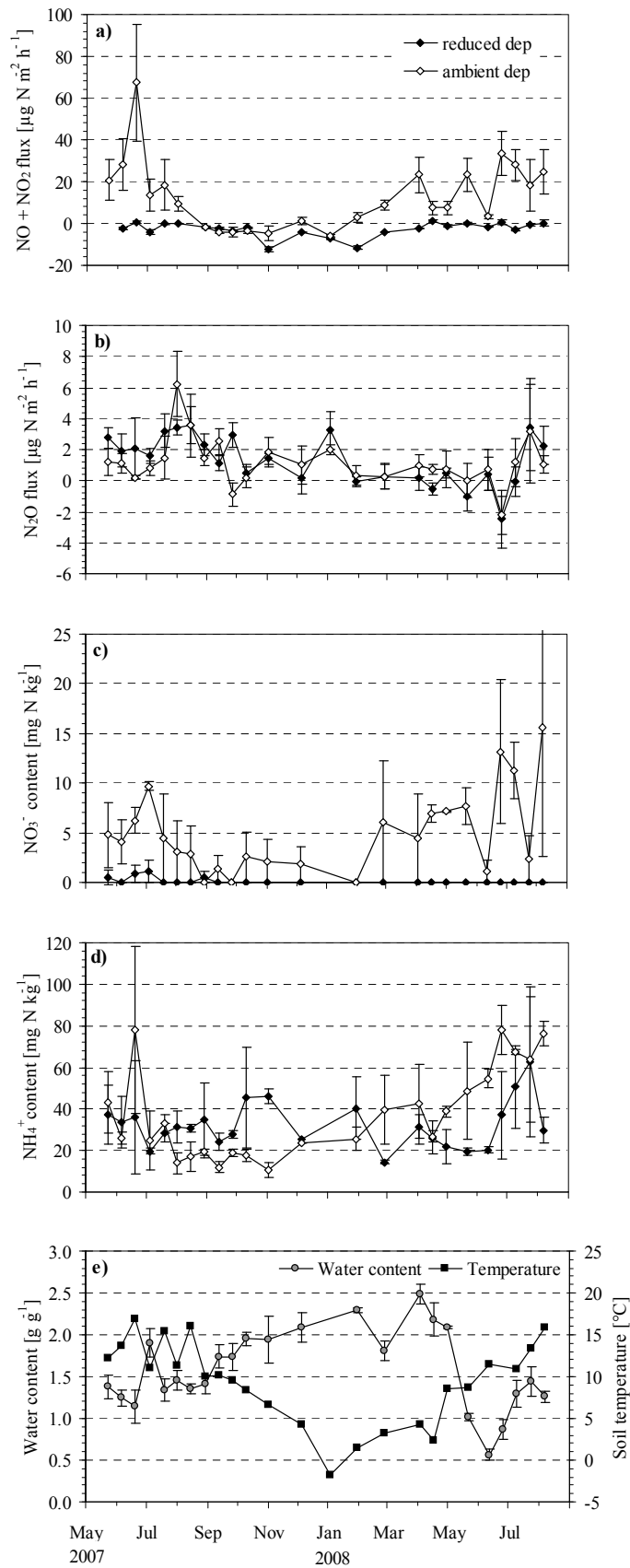
Figure 3-1: Mean (± SE, n = 5) NO + NO2 fluxes (a) and N2O fluxes (b) and mean (± SE, n = 2) NO3− contents (c) and NH4+ contents (d) of the organic layer under reduced and ambient N depositions at the Solling spruce from May 2007 to August 2008. The water content (n = 4) of the organic layer and soil temperature in 2.5 cm depth are mean values of both roofs (e). The fluxes and control parameters were measured at the NH4+-labelled subplots.
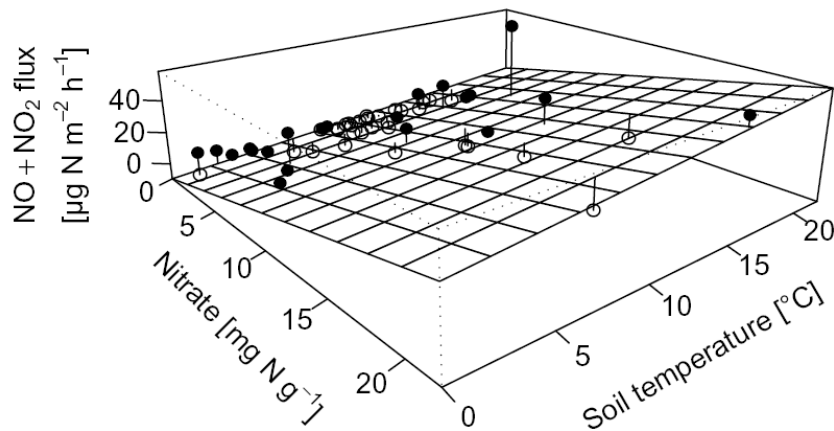
Figure 3-2: Relationship of (NO + NO₂)-N fluxes (y) to NO_3^--N contents ($x1$) and soil temperatures ($x2$) of the organic layer plotted as surface area. The regression equation was obtained including all data of both roof plots and both labellings (y = 2.10 (± 0.27) · $x1$ + 0.97 (± 0.34) · $x2$ − 9.67 (± 3.59); r^2 = 0.626, P < 0.001, df = 49). Measured data points are indicated as closed circles (above the surface) and open circles (below the surface) whereas solid lines indicate the deviation of measured data from the model surface.

3.3.2. Effect of reduced N depositions on N_2O fluxes

No significant differences in N_2O fluxes and seasonal pattern were observed between both roof plots (Fig. 3-1b) with similar values of fluxes under ambient depositions (0.08 ± 0.02 kg N ha^{-1} yr^{-1}) and under reduced depositions (0.08 ± 0.04 kg N ha^{-1} yr^{-1}). Fluxes of N_2O were significantly lower than NO_x fluxes under ambient depositions (Fig. 3-1; P = 0.04) in contrast to reduced depositions where N_2O fluxes were significantly higher than NO_x fluxes (Fig. 3-1; P < 0.001).

Between both subplots (NH_4^+ or NO_3^--labelled) of the roofs, no significant differences in N_2O fluxes were observed (data not shown).

Fluxes of N_2O depended on the water contents of the organic layer and soil temperatures in 2.5 cm depth when data from subplots under both roofs were included (Fig. 3-4; r^2 = 0.357, P < 0.001, df = 50).

Cumulative monthly fluxes of N_2O were positively related to monthly throughfall NO_3^- depositions under reduced depositions (Fig. 3-5; r^2 = 0.352, P = 0.025, df = 12) but not under ambient depositions.
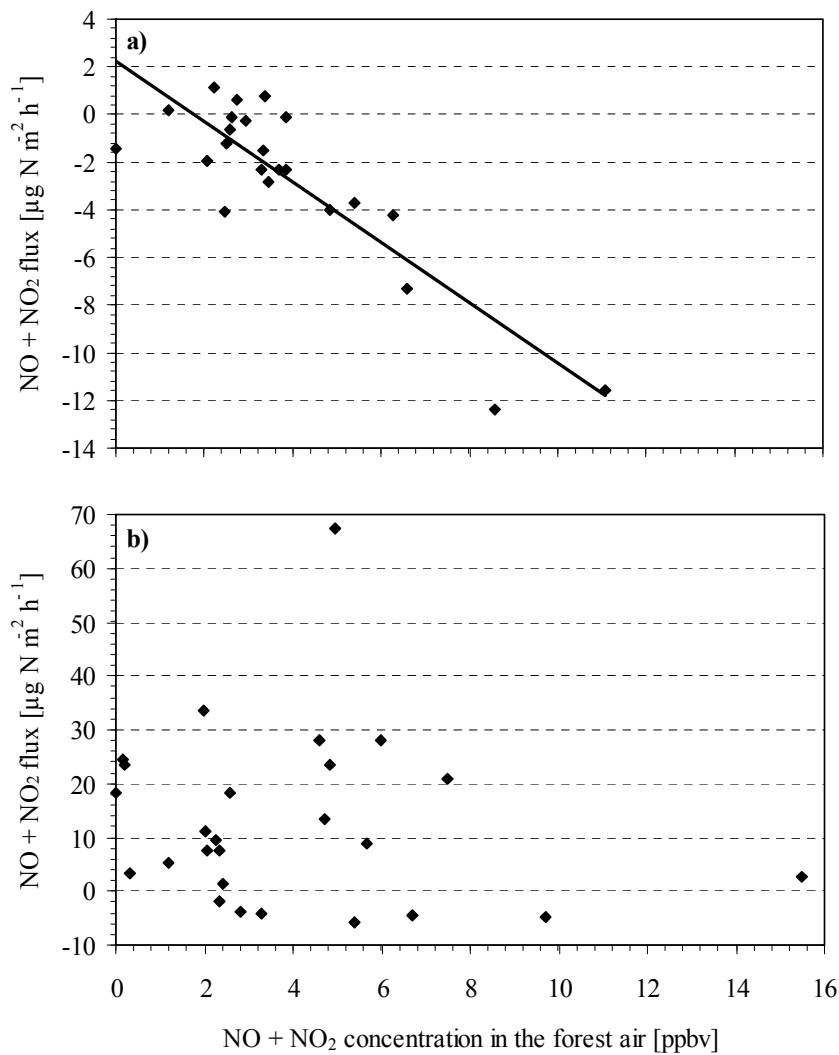
Figure 3-3: Fluxes of (NO + NO$_2$)-N (y) of the NH$_4^+$-labelled roof plot which received a) reduced N depositions and b) ambient N depositions plotted against the NO + NO$_2$ concentration in the forest air (x) from June 2007 to August 2008. There was a significant relationship between the fluxes and concentrations in the forest air under reduced N depositions ($y = -1.27$ (± 0.16)$\cdot x + 2.22$ (± 0.73); $r^2 = 0.749$, $P < 0.001$, $df = 21$).
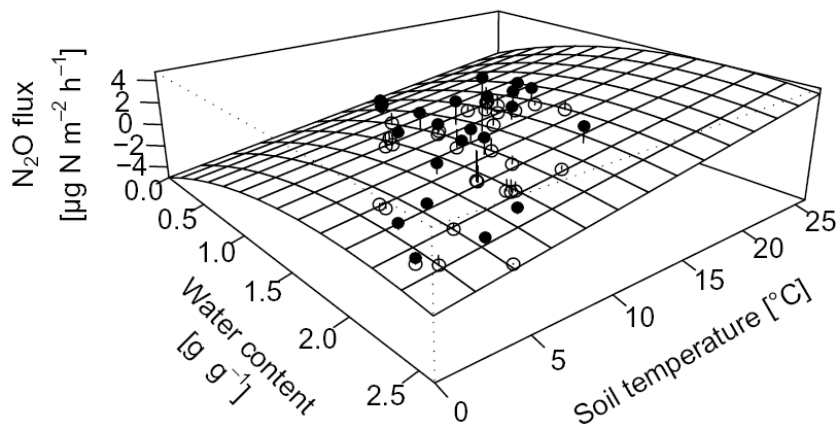
Figure 3-4: Relationship of N$_2$O-N fluxes (y) to water contents ($x1$) and soil temperatures ($x2$) of the organic layer plotted as surface area. The regression equation was obtained from all data of both roof plots and both labellings ($y = -1.24$ ($\pm$ 0.62) $\cdot$ $x1^2$ + 4.90 ($\pm$ 1.84) $\cdot$ $x1$ + 0.19 ($\pm$ 0.05) $\cdot$ $x2$ $-$ 5.03 ($\pm$ 1.38); r^2 = 0.357, P < 0.001, df = 50). Measured data points are indicated as closed circles (above the surface) and open circles (below the surface) whereas solid lines indicate the deviation of measured data from the model surface.
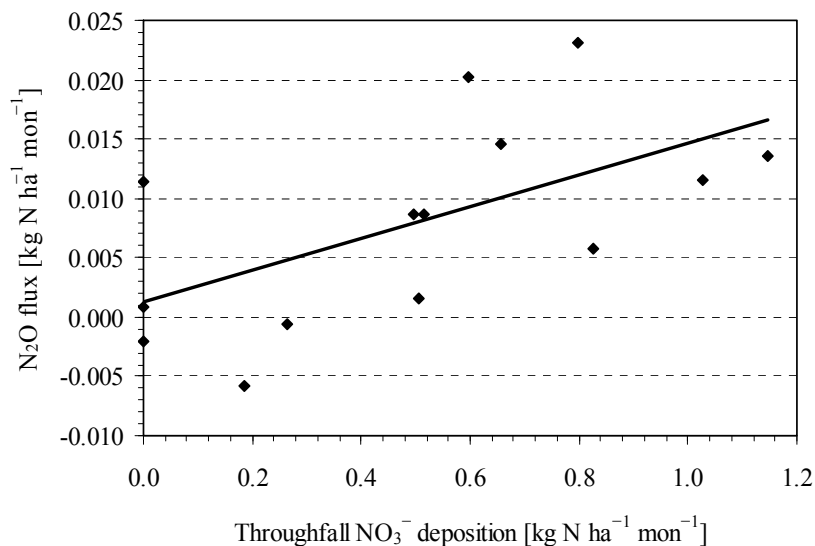
Figure 3-5: Relationship between cumulative monthly fluxes of N$_2$O and monthly throughfall NO$_3^-$ depositions from June 2007 to August 2008. The values derived from the NH$_4^+$-labelled subplot of the "clean rain" roof. The linear regression model was N$_2$O-N = 0.013 ($\pm$ 0.005) $\cdot$ NO$_3^-$-N + 0.001 ($\pm$ 0.003) (r^2 = 0.352, P = 0.025, df = 12).

3.3.3. Changing soil properties and N cycling under reduced N depositions

Net nitrification was not detectable in the surface 10 cm of the soil (including the organic layer) under reduced depositions (Fig. 3-6a). However under ambient depositions, net nitrification amounted to approx. 20% of total mineralised N and led to a higher NO_3^- content than under reduced depositions (Fig. 3-1c; Fig. 3-6b; $P = 0.002$). Net N mineralisation of the surface 10 cm of the soil was 25.7 ± 3.0 mg N m^{-2} d^{-1} under reduced depositions and 20.5 ± 4.0 mg N m^{-2} d^{-1} under ambient depositions. No significant differences occurred in net ammonification and nitrification rates among the five 2 cm intervals of one roof plot. Ammonium was the dominant inorganic N species in the soil under both roofs and no significant differences were observed in NH_4^+ contents in all three soil depths between both roofs (Fig. 3-1d). The water content as well as the soil temperature did not significantly differ between both roofs in the organic layer and the surface 10 cm of the mineral soil (Fig. 3-1e).

3.4. Discussion

3.4.1. Effect of reduced N depositions on NO_x fluxes

The long-term reduction (16–17 years) of N depositions to pre-industrial levels turned the spruce forest soil from a net source of NO_x into a net sink. Under ambient depositions, the seasonal NO_x emissions followed the dynamic of NH_4^+ and NO_3^- in the soil with high values in the summer months and low values in the winter months clearly indicating that the N turnover in the soil determined the NO_x fluxes. The high summer emissions disappeared under reduced N input conditions as also NO_3^-. Nitrification declined in the clean rain plot as was confirmed by our incubation experiment. Multiple regression analyses revealed that about 63% of the variation in NO_x fluxes could be explained by the NO_3^- content and the soil temperature in the surface organic layer when the data of both roof plots were included in the analysis. As the temperature was the same under both roofs, we propose that excess NO_3^- in soil solution is a good indicator for describing the magnitude of NO_x fluxes. Yet, the NO_x emissions under high N input conditions of the ambient roof plot were primarily controlled by the NH_4^+ content, the precursor of NO_3^-, which indicated nitrification as source for NO.

a) reduced deposition

$[mg\ N\ m^{-2}\ d^{-1}]$

b) ambient deposition

■ Ammonification ☐ Nitrification
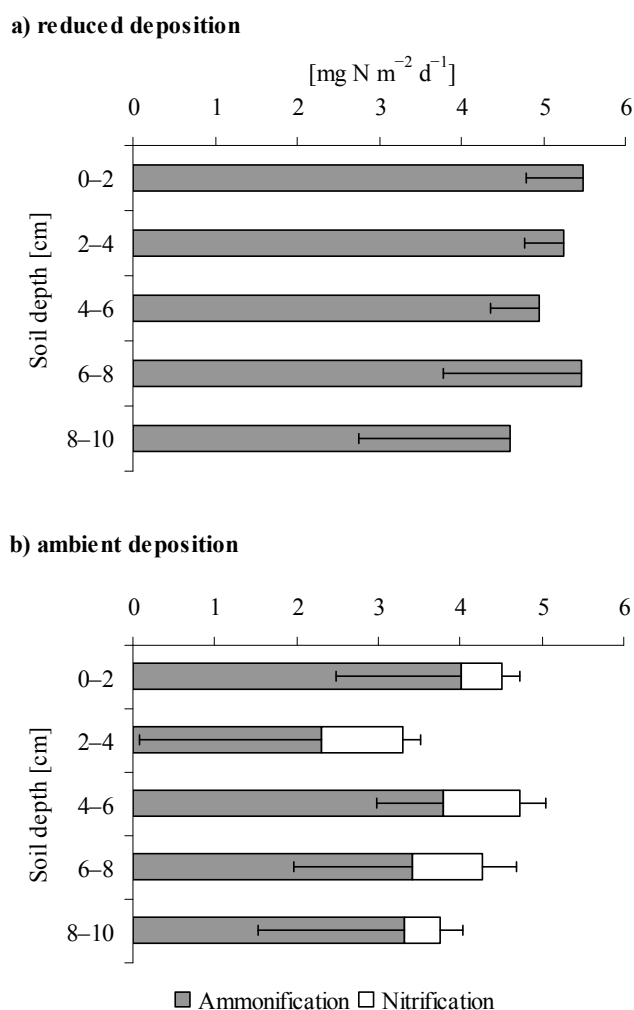
Figure 3-6: Mean (± SE) ammonification and nitrification rates of the Solling roof plots which received reduced (a; $n = 4$) and ambient N depositions (b; $n = 5$). The in-situ incubation was conducted with undisturbed soil columns in summer 2008 and lasted 23 days. No net nitrification was observed under reduced N depositions. Net ammonification and nitrification rates did not differ among the five 2 cm intervals of one roof plot. The depth 0–8 cm represents the surface organic layer, the depth 8–10 cm represents the surface mineral soil layer.

Several studies further supported the finding of the present study that mineral N availability in the soil and net nitrification rates were positively correlated to NO fluxes (e.g. Skiba et al., 1998, 1999; Venterea et al., 2003). Variation in net nitrification rates explained > 60% of the variation in NO fluxes in the highly N-enriched spruce forest in South Germany (Gasche and Papen, 1999). Venterea et al. (2003) reported an increase in NO emissions resulting from the increase in soil NO_3^- concentrations and net nitrification rates in a pine forest subject to long-term N fertilisation of 50 and 150 kg N ha^{-1} yr^{-1} (12–13 years). In addition, concentrations of NO_3^- and NH_4^+ linearly decreased, as also the NO fluxes, with increasing distance to a Scottish poultry farm (Skiba et al., 1999). Furthermore, NO_3^-

concentrations, which originally were below the detection limit, increased after one-time NO_3^- fertiliser application (~50 kg N ha^{-1}) in a Scots pine forest in Sweden, which resulted in an increase in NO emissions from < 3 up to 216 µg N m^{-2} h^{-1} (Johansson, 1984).

External N input often has been shown to stimulate the emission of NO_x by artificial N fertilisation (e.g. Johansson, 1984; Venterea et al., 2003) or by using gradients of N depositions (e.g. Butterbach-Bahl et al., 2002; Skiba et al., 1998, 1999). In our study, we took advantage of roofs in order to ensure similar edaphic and climatic conditions. However, the observed declines in nitrification rates, NO_3^- in soil solution, and NO_x emissions resulting from experimental long-term reduction of N depositions are conform to results of studies increasing the N inputs. There was a consistent pattern concerning the direction of the response, the absolute amount of NO_x emissions, however, was different. Highly N-affected forests display a wide range of NO_x emissions indicating that the relevance of N depositions for the emissions of NO_x is still not completely clear. It was generally reported that coniferous forests in areas marginally affected by N depositions emitted < 5 µg NO-N m^{-2} h^{-1} (Johansson et al., 1984; Pilegaard et al., 2006; Skiba et al., 1999; Venterea et al., 2003). Despite high throughfall N depositions of 30 kg N ha^{-1} yr^{-1}, the mean annual NO_x emissions amounted to only 0.62 ± 0.24 kg N ha^{-1} yr^{-1} in the present study. This is in contrast to the study by Gasche and Papen (1999) where mean annual NO_x fluxes of 5.4 kg N ha^{-1} yr^{-1} were reported under similar N input conditions. These authors found a positive correlation between weekly wet N depositions and NO fluxes at their spruce stand (Gasche and Papen, 1999). The correlation was stronger for the wet NH_4^+ deposition (r^2 = 0.60) than for the wet NO_3^- deposition (r^2 = 0.48). The reason for the observed differences between the stands might be ascribed to the N status of the forests and the capacity for nitrification. The incubation experiment of the present study indicated that only 20% of the mineralisation products were converted into NO_3^- under ambient depositions. Under reduced N input conditions nitrification did not occur although the NH_4^+ was still detectable. A tightly-coupled microbial NH_4^+ cycling could be responsible as was observed by Corre and Lamersdorf (2004) in a ^{15}N pool dilution study conducted prior to the establishment of ^{15}N labelling at the Solling roof project.

3.4.2. Uptake of atmospheric NO_x into N-limited forest soils

Net uptake of atmospheric NO_x was observed under reduced N depositions and during the winter months under ambient depositions. Uptake strongly increased with increasing concentration of atmospheric NO_x under reduced N input conditions whereas this relationship

was not found under ambient depositions. Effect of atmospheric NO concentrations on NO exchange between soil and atmosphere has previously been explained by the compensation point (e.g. Ludwig et al., 2001; Slemr and Seiler, 1991), which is defined as the atmospheric concentration at which the rate of NO production equals the rate of NO consumption. Compensation concentrations of less than 1 ppb_v NO to several hundred ppb_v NO have been reported with the highest values from N-enriched plots (Gasche and Papen, 1999; Ludwig et al., 2001). The NO consumption rate follows a first-order reaction with respect to the atmospheric NO concentration whereas the NO production rate is independent of the atmospheric NO concentration (Galbally and Johansson, 1989). Studies by Baumgärtner and Conrad (1992) further showed that NO consumption was not affected by N fertilisation indicating that NO production instead of NO consumption was presumably altered by long-term reduction of N depositions in the clean rain plot. We deduce from these observations that the high N depositions would cause the net NO_x flux into the atmosphere by increasing NO production during the summer months under ambient depositions. This effect on NO production obviously depended on the temperature indicated by low winter NO_x emissions or even NO_x uptake. Under low N input conditions and low nitrification rates, NO consumption became the prevailing process which is probably attributed to aerobic heterotrophic bacteria (Gödde and Conrad, 2000).

3.4.3. Effect of reduced N depositions on N_2O fluxes

In contrast to NO_x fluxes, the reduced N depositions did not affect the amount of N_2O emitted from the investigated spruce stand. Borken et al. (2002) came to the same conclusion two and nine years after the start of the roof experiment. In addition, Kandeler et al. (2009) reported no response in size of total nitrate reducer and denitrifier communities 15 years after roof closure. The emissions of N_2O were not correlated to the NO_3^- content of the soil. However, we noted a positive correlation of monthly NO_3^- depositions on N_2O emissions, which was only found under reduced N depositions indicating that NO_3^- from atmospheric deposition became the origin for N_2O. Since NO_3^- was not produced by nitrification under clean rain conditions, N_2O emissions may almost completely be derived from N depositions. At an adjacent unroofed spruce stand with ambient depositions, a ^{15}N tracer study revealed that 12.5% of the N_2O-N emissions were derived from inorganic N depositions (Eickenscheidt et al., 2011).

Under ambient depositions, NO_3^- in soil solution was detectable almost during the whole year and derived from NO_3^- depositions and from nitrification. The missing correlation

between N_2O emissions and NO_3^- depositions indicates that the available NO_3^- represented no limitation for denitrification. Thus, the question arises as to why N_2O emissions were low under ambient depositions despite sufficient amount of NO_3^- in the soil. In literature, low N_2O emissions have been reported from temperate spruce forests receiving both high and low N inputs (e.g. Bowden et al., 1991; Brumme et al., 1999; Butterbach-Bahl et al., 1998; Castro et al., 1993; Macdonald et al., 1997; Venterea et al., 2003).

The reason for this observation is the prevailing effect of oxygen on the production of N_2O, which was indicated by the dependence of N_2O fluxes on water contents and soil temperatures in both the ambient and the clean rain plots. The water content of the soil has a direct effect on oxygen availability due to its effect on oxygen diffusion whereas soil temperature indirectly controls the oxygen status through its effect on microbial activity and thus, oxygen consumption. Soil moisture and temperature were reported frequently to explain most of the variation in N_2O fluxes in European forests (e.g. Brumme 1995; Pilegaard et al., 2006; Schindlbacher et al., 2004) even in forests highly enriched in N (Brumme et al., 1999). Detailed studies of soil depth identified the organic layer, which frequently dries out, as the primary source for N_2O (Borken and Brumme, 1997). Ball et al. (1997) demonstrated that gas diffusivity of the organic layer was relatively high in a spruce stand adjacent to the roof plots, resulting in aerobic soil conditions. A well-aerated organic layer possibly restrained denitrification, which was assumed to be the main source of N_2O production. In addition, at exceptionally low soil moisture but high mineral N contents in soil solution in summer 2008, net uptake of N_2O into the soil was observed but at the same time high net NO_x emissions were noted under ambient N depositions.

3.5. Conclusions

The present study, which was conducted under controlled N-reduced conditions, pointed out that negative effects of N enrichment decline in less than two decades. We demonstrated that long-term reduction of N depositions turned the forest soil from a net source of NO_x into a net sink. The decline in nitrification rates was the primary reason for the reduction in NO_x emissions. Under the low N input conditions, the NO_x uptake was exclusively controlled by the NO_x concentration of the forest air. However, long-term reduction of N depositions and N availability in the soil played no role for N_2O fluxes at the investigated spruce stand since denitrification was limited by the factors influencing the oxygen status of the soil and not by those influencing the N availability.

Acknowledgments

Contribution of ^{15}N-labelled leaf litter to N turnover, nitrous oxide emissions and N sequestration in a beech forest during eleven years

4.   Contribution of ^{15}N-labelled leaf litter to N turnover, nitrous oxide emissions and N sequestration in a beech forest during eleven years

Abstract

Decomposition of leaf litterfall plays a major role for nitrogen (N) release and N sequestration in soils. However, little is known about to which extent beech leaf litter contributes to N turnover and nitrous oxide (N$_2$O) emissions within one decade after litterfall. In 1997, we exchanged recently fallen leaf litter by ^{15}N-labelled litter in a beech stand (*Fagus sylvatica*) at the Solling, Germany. Measurements were conducted 2–3 and 10–11 years after litter exchange. Two years after litter exchange, 92% of added ^{15}N was recovered in the surface 10 cm of the soil. The labelled N was primarily found in the upper part of the F layer of the moder type humus. Eleven years after litter exchange, 73% of the added ^{15}N was lost and the remaining 27% was mainly recovered in the lower part of the F layer indicating N sequestration. However, the remaining leaf litter was still subject to measurable N turnover and N$_2$O production and was not completely stabilised. Between 0.3% (eleventh year) and 0.6% (second year) of total annual N$_2$O emissions were attributed to beech leaf litter of a single year. Most of the annual N$_2$O emissions of 1.33 to 1.54 kg N ha^{-1} yr^{-1} were probably derived from older soil N pools.

Keywords: Nitrous oxide, beech leaf litter, ^{15}N isotope technique, N mineralisation, litter N dynamics

4.1. Introduction

Weathering of geological parent material contributes very marginally to mineral nitrogen (N) content of soils (Berg and Matzner, 1997; Parton et al., 2007). Thus, decomposition of litter and mineralisation inhere particular importance for N availability in forest ecosystems (Parton et al., 2007). Decomposition has been described as a three-stage process: (i) the early stage, (ii) the late stage, and (iii) the humus-near stage, latter is characterised by a decomposition rate close to zero (Berg and McClaugherty, 2003; Berg and Matzner, 1997).

During the early stage, initial litter N concentrations primarily drive net N release from litter (Parton et al., 2007). A rapid mass loss of native litter N but a simultaneous net increase in N and lignin contents of the decaying litter during the early stage was generally reported (Berg and McClaugherty, 2003; Laganière et al., 2010; Melillo et al., 1982; Zeller et al., 2000). Fahey et al. (2011) observed a continuous decrease in native litter ^{15}N of 20–30% in the first year after an addition of ^{15}N-labelled litter to a sugar maple stand. The loss, however, was balanced by a large N incorporation from external sources into the litter that resulted in an increase in N contents of 26–32%. External N incorporation balanced N loss over a 2-years period in three European beech forest soils and net release of N was measured in the third

year (Zeller et al., 2000; Zeller et al., 2001). Translocation of N from the soil to the decaying litter by fungi accounted for 25–35% of the exogenous N, whereas atmospheric N deposition accounted for 30–50% in a French beech forest (Zeller et al., 2000).

In the late stage of decomposition condensation of degradation products combined with import of nutrients produces recalcitrant humic compounds, which accumulate (Berg and McClaugherty, 2003). Berg and Matzner (1997) reported a rate-retarding effect of high initial litter N and soil N concentrations during the late stage of decomposition, which was ascribed to (i) the suppressing effect of N on the formation of ligninase in white-rot fungi, and (ii) condensation reactions in which lignin incorporates N, resulting in stable chemical structures. In forest ecosystems exposed to chronic high N depositions, leaching of organic N from the decaying litter but simultaneously reduced fungal N transport, due to sufficient supply of external N by depositions, may further promote N sequestration in soil organic matter (SOM; Fahey et al., 2011). An increase in organic layer and soil N has indeed been reported from forests exposed to chronic high N depositions (e.g. Meiwes et al., 2009).

Beech leaf litter is generally rich in N, water soluble compounds, tannin, and lignin (e.g. Berg and McClaugherty, 2003; Lorenz et al., 2004; Melillo et al., 1982) and European beech forests have often been exposed to chronic high N depositions. Thus, rapid decomposition in the early stage but slow decomposition in the late stage and temporary N sequestration may be expected (Berg and McClaugherty, 2003).

Mineral N, which is released from the decaying litter, may be utilised by micro-organisms for nitrification and denitrification processes during which nitrous oxide (N$_2$O) may be produced either as a by-product or intermediate (Davidson et al., 2000). Nitrous oxide is a potent greenhouse gas and contributes to the catalytic decomposition of stratospheric ozone (Crutzen, 1979; IPCC, 2007). High emissions of N$_2$O have been reported from European beech forest soils, which have been exposed to chronic high N depositions (Brumme and Beese, 1992; Papen and Butterbach-Bahl, 1999; Zechmeister-Boltenstern, 2002). However, one of our previous studies revealed that despite the high N$_2$O emissions of 2.6 kg N ha^{-1} yr^{-1} from an acid beech forest soil in Germany, only 6% of the total emissions were derived from N depositions of the recent year whereas the remaining part was derived from the soil N pool (Eickenscheidt et al., 2011). Therefore the question arises as to what extent the turnover of leaf litter N contributes to N$_2$O emissions.

Turnover of litter N is mostly studied through mass loss observations (e.g. Berg and McClaugherty, 2003) and only few studies have used stable isotope tracers (e.g. Fahey et al., 2011; Zeller and Dambrine, 2011), where the emphasis has been on the early-stage of

decomposition. Long-term ^{15}N tracer studies are required to improve our present knowledge about the processes of litter N dynamics and potential long-term N sequestration in the soil.

The focus of our study was to assess the contribution of beech leaf litter N to N mineralisation, N$_2$O emissions, and N sequestration in the soil 2–3 years and 10–11 years after litterfall application. We conducted a ^{15}N isotope litter exchange experiment in a beech stand exposed to chronic high N depositions at the Solling, Germany.

4.2. Materials and methods

4.2.1. Study area

The study was conducted in a 160-yr-old beech stand (*Fagus sylvatica*) at the Solling in Central Germany (51° 46' N, 9° 34' E, 500 m a.s.l., mean temperature: 6.9°C, annual precipitation: 1193 mm, stand deposition: 20 kg N ha^{-1} yr^{-1}). The soil type is a Dystric Cambisol (pH$_{H2O}$ ≤ 3.6; silty loam), which derived from Triassic sandstone covered by loess. The surface organic layer is a typical moder consisting of an L (litter) layer, F (fermentation) layer, and H (humus) layer with a thickness of 1–2 cm, 4–5 cm, and 2 cm, respectively. The organic layer in total stores 28 Mg C ha^{-1} and 1.3 Mg N ha^{-1} (Tab. 4-1). Leaf litter production amounted to 2.77 ± 0.25 Mg ha^{-1} yr^{-1} from 1991 to 2003, which corresponded to 34 ± 4 kg N ha^{-1} yr^{-1} (Khanna et al., 2009). A detailed site description can be found in Brumme and Khanna (2009a) and in Eickenscheidt et al. (2011; see chapter 2.2.1.).

Table 4-1: Characteristics of the surface organic layer and upper 2 cm of the A$_h$ horizon of the beech forest at Solling, Germany.

Horizon	Depth [cm]	C [%]	N [%]	C [Mg ha^{-1}]	N [Mg ha^{-1}]	C/N ratio
L	0–2	46.9 ± 0.2	1.67 ± 0.04	3.8 ± 0.3	0.14 ± 0.01	27.2
F	2–6	45.3 ± 0.6	2.01 ± 0.04	8.5 ± 0.8	0.38 ± 0.04	22.1
H	6–8	25.5 ± 4.1	1.24 ± 0.19	15.6 ± 2.0	0.77 ± 0.11	20.3
A$_h$	8–10	9.1 ± 1.7	0.47 ± 0.08	12.3 ± 1.4	0.64 ± 0.15	19.3

4.2.2. Experimental design

In December 1997, six chamber bases (area: 0.0559 m^2) were installed. We removed the freshly fallen beech leaf litter of the recent years and replaced it by 17 g ^{15}N-labelled beech litter (0.71 ± 0.01% N; 3.57 ± 0.07 atom% ^{15}N), which corresponded to the average annual leaf litter production. The ^{15}N-labelled litter was derived from beech trees that were grown in a greenhouse and irrigated by a ^{15}N solution. Three of the chamber bases were only used for soil sampling in 1999 and the other three bases were used for N_2O and ^{15}N-N_2O gas flux measurements in 1999 and 2008 as well as for soil sampling in 2008. Three additional chamber bases were installed as control chambers.

4.2.3. N_2O and ^{15}N-N_2O flux measurements

We measured N_2O and ^{15}N-N_2O fluxes from January 1999 to December 2000 and from May 2007 to July 2008 using the closed chamber method (chamber vol.: 5.4 L). Three N_2O gas samples were collected in evacuated glass flasks (100 mL) at three regular time intervals following chamber closure (36 minutes at most) using a portable gas sampler equipped with a pressure sensor (Loftfield et al., 1997). Analysis was done with a gas chromatograph equipped with an EC detector (GC 14A, Shimadzu, Duisburg, Germany). Samples for ^{15}N-N_2O measurements were removed at 0.5 and 36 minutes (1999–2000) and at 0.5 and 20 minutes (2007–08) and were stored in evacuated 100 mL glass bottles. For determination of the ^{15}N-N_2O natural abundance, we used three control chambers. An isotope ratio mass spectrometer coupled with a preconcentration unit was used for ^{15}N analysis (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany). Isotope abundances were expressed either in the δ notation [‰] or in absolute proportion [atom%]. We used the Keeling plot approach to calculate the ^{15}N-N_2O abundance of the N_2O emitted from the soil (Tilsner et al., 2003), which was described in detail in Eickenscheidt et al. (2011; see chapter 2.2.1.).

4.2.4. Soil sampling and in situ incubation

After gas flux measurements, soil sampling and an in-situ incubation of the soil was conducted within the chamber bases to determine net rates of ammonification, nitrification, and ^{15}N ammonification in summer 2008. For each chamber, two soil samples of the surface 10 cm of the soil were taken with a stainless steel core (diameter: 5.5 cm). The sample included the surface organic layer and the surface mineral soil (Tab. 4-1). Soil from one core was removed and divided into 2 cm sections, of which soil extracts for mineral N (N_{min} = NH_4^+-N + NO_3^--N) and $^{15}N_{min}$ analyses were prepared within few hours (t0 sample). The

other soil core (t1 sample) was put into a PE bag and was returned into the hole for undisturbed in situ incubation, which lasted 16 days. During the incubation, the mean temperature in 10 cm soil depth, which was logged every two hours, was 15.1 ± 1.2°C (standard deviation). After the incubation, soil cores were subject to the same treatment as the t0 samples. Mineral N extracts were obtained after shaking 30 g fresh mineral soil with 100 mL 0.5 M K$_2$SO$_4$ solution or 15 g of the organic layer with 50 mL K$_2$SO$_4$ solution. We analysed N$_{min}$ by using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, The Netherlands). A modified diffusion method (Corre et al. 2003; Stark and Hart, 1996) was applied for the determination of ^{15}N in the extracted NH$_4^+$ and nitrate (NO$_3^-$). However, net ^{15}N nitrification rates were not available due to failure of the isotope ratio mass spectrometer. In addition, total carbon (C) and nitrogen (N$_t$) contents (CNS Elemental Analyzer, Heraeus Elementar Vario EL, Hanau, Germany) and ^{15}N abundance in N$_t$ were determined in 1999 and 2008. Analyses of ^{15}N abundance (in N$_t$ and NH$_4^+$) were conducted with a Delta C plus IRMS (Finnigan MAT, Bremen, Germany).

4.2.5. Calculation of the mean residence time

The mean residence time (MRT) of N (and C) in the surface organic layer (0–8 cm) was assessed using the measured mass of N (C) in the surface organic layer divided by the mass of N (C) of the annual aboveground total litterfall (0.0675 Mg N ha^{-1} yr^{-1} and 2.581 Mg C ha^{-1} yr^{-1}; Khanna et al., 2009).

4.2.6. Statistical analyses

Statistical analyses were conducted with R 2.10.1 (R Development Core Team, 2009). We applied linear mixed effects models on time series data (Eickenscheidt et al., 2011; see chapter 2.2.1.). Results were given as mean ± 1 standard error and effects were considered significant where $P \leq 0.05$.

4.3. Results

4.3.1. Recovery and movement of ^{15}N in the soil

Two years after ^{15}N-labelled beech litter addition, 92% of the ^{15}N was recovered in the surface 10 cm of the soil, most of which (86%) was found in the upper part of the F layer of the surface organic layer at 2–4 cm soil depth (Fig.4-1a). Eleven years after the addition, 27% of the ^{15}N label was recovered with the highest recovery in 4–6 cm soil depth, the lower part of the F layer (Fig.4-1b). The changes of ^{15}N-N$_t$ contents in the soil profile from 1999 to 2008, with a decrease in 0–2 cm (P = 0.038) and 2–4 cm soil depths (P = 0.006) and an increase in 4–6 cm soil depth (P = 0.001), were significant. The ^{15}N-N$_t$/^{14}N-N$_t$ ratio within the surface organic layer (0–8 cm) decreased from 0.03% after two years to 0.01% after 11 years. The mean residence time (MRT) of N derived from the annual aboveground litterfall in the surface organic layer averaged to approx. 19 years and was higher than the MRT of C (10.5 years) in the surface organic layer.

4.3.2. Mineralisation of N and ^{15}N

Net ammonification and mineralisation, measured at about 15°C in the field, was low in 0–2 cm soil depth (L layer), highest in 2–4 cm soil depth (upper F layer; Fig. 4-2a), and decreased gradually below this depth. Net NH$_4^+$ immobilisation occurred in 8–10 cm soil depth (mineral soil). Net nitrification increased with soil depth within the organic layer but differences were not statistically significant (Fig. 4-2a). In total, net mineralisation amounted to 58 mg N m^{-2} d^{-1} within the surface 10 cm of the soil, half of which was nitrified.

Net ^{15}N ammonification in the surface 10 cm of the soil in general followed the trend of total net ammonification and in total amounted to 6 ± 1 µg N m^{-2} d^{-1} (Fig. 4-2b). Half of the total ^{15}N ammonification could be attributed to the 2–4 cm soil depth. About 0.6% of total net N ammonification could be ascribed to the exchanged leaf litter, assuming that the ^{14}N + natural ^{15}N contained in the ^{15}N-labelled litter participated equally in net ammonification as the ^{15}N label. Data for net ^{15}N nitrification were not available due to technical problems.

On average, the daily net ^{15}N ammonification of the surface 10 cm of the soil accounted for 0.031 ± 0.013% of the ^{15}N-N$_t$. The proportion was two times higher than the proportion of net native N (^{14}N + natural ^{15}N) ammonification per day of the native N$_t$ regarding the same depth interval, which was 0.015 ± 0.006%. However, the differences were not statistically significant. Also, no significant differences between these proportions occurred when the different depth intervals of the soil were considered separately (Fig. 4-3).

a)

$N_t \ [mg \ ^{15}N_{excess} \ m^{-2}]$

b)
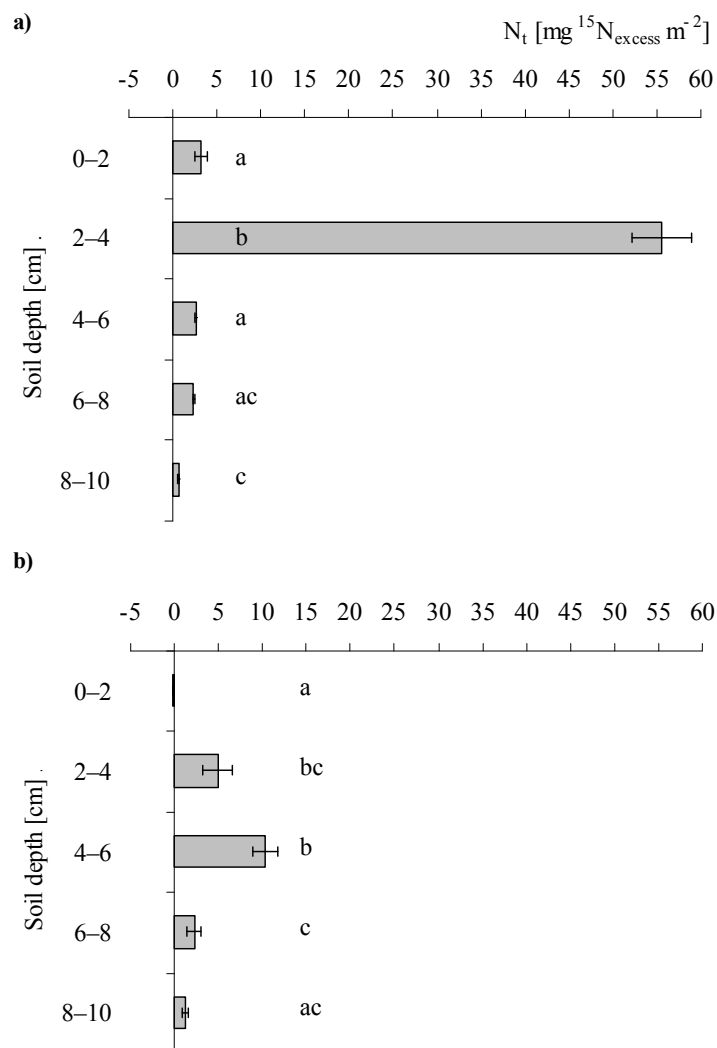
Figure 4-1: Distribution of soil ^{15}N in the surface 10 cm of the soil two years (a) and eleven years (b) after ^{15}N-labelled leaf litter addition in the beech stand. Among soil depths, means ($\pm$ SE; $n = 3$) followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at $P \leq 0.05$).

4.3.3. N₂O and ^{15}N-N₂O fluxes

Fluxes of N₂O displayed pronounced seasonality with high emissions during the summer months and low emissions during the winter months (Fig. 4-4). The cumulative N₂O emission amounted to 1.33 ± 0.26 kg N ha^{-1} yr^{-1} in 1999, 1.54 ± 0.88 kg N ha^{-1} yr^{-1} in 2000, and 1.35 ± 0.57 kg N ha^{-1} yr^{-1} in 2007–08.

In 1999–2000 ^{15}N-N₂O$_{emitted}$ abundances were significantly higher in the litter exchange chambers than in the control chambers (Tab. 4-2, Fig. 4-4; $P = 0.036$). Cumulative ^{15}N-N₂O emissions were 0.29 g ^{15}N ha^{-1} yr^{-1} in 2000 which was 0.04% of the added ^{15}N in leaf litter. Eleven years after the litter application, the recovery of ^{15}N in emitted N₂O decreased to 0.13

g ^{15}N ha^{-1} yr^{-1} in 2007–08 which was 0.02% of the added ^{15}N in leaf litter. However, the abundances of ^{15}N in N₂O emitted from the soil in 2007–08 did not significantly differ between the litter exchange chambers and the control chambers (Tab. 4-2, Fig. 4-5).
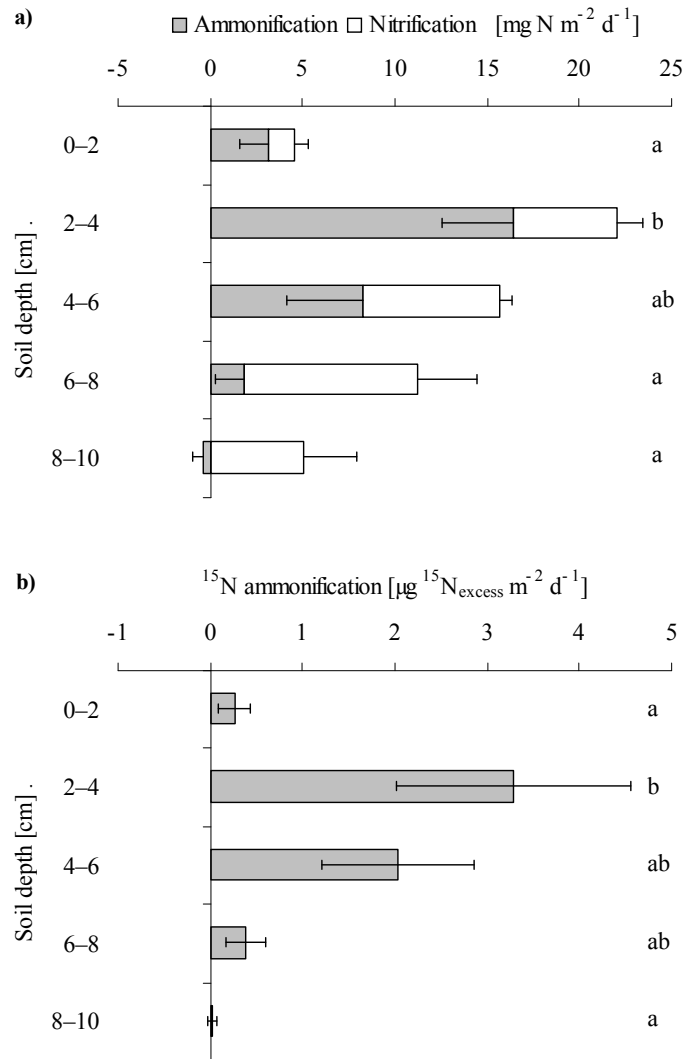
Figure 4-2: Net ammonification and nitrification rates (a) and net ^{15}N ammonification rates (b) of the surface 10 cm of the soil in the beech stand. The undisturbed in-situ incubation lasted 16 days and was conducted within the chamber bases of the litter exchange experiment in summer 2008. Among soil depths, means (± SE; n = 3) followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at P ≤ 0.05). Letters in figure a) belong to net ammonification rates and no significant differences occurred among net nitrification rates in different soil depths. Net ^{15}N nitrification rates were not available.

Assuming that ^{14}N + natural ^{15}N contained in the exchanged leaf litter participated equally in N$_2$O emissions as the ^{15}N label, then 0.3% (2007–08) and 0.6% (1999–00) of total annual N$_2$O emissions were derived from total N of a single litterfall year.

In 2007–08, the daily ^{15}N-N$_2$O emissions amounted to 0.0002% of ^{15}N-N$_t$ of the surface 10 cm of the soil, which was the same as the ratio of native N$_2$O emissions to native N$_t$ of the surface 10 cm of the soil assuming N$_2$O only derived from the top 10 cm of the soil.
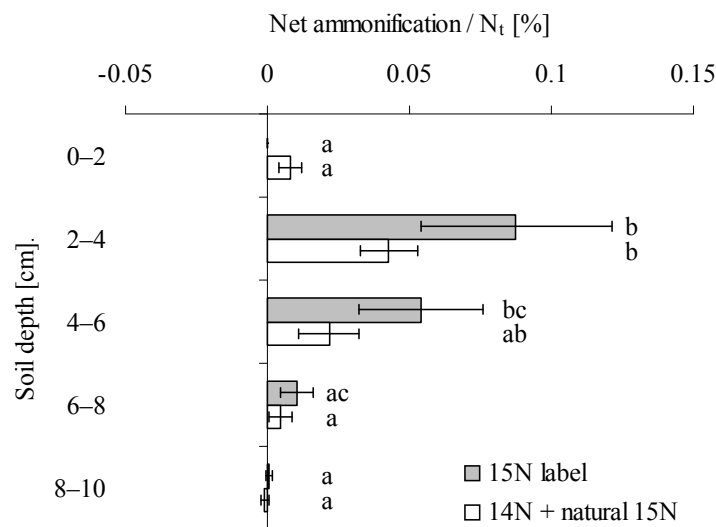
Figure 4-3: Ratio of daily net ammonification to Nt given for ^{15}N derived from labelled beech leaf litter and for native N (^{14}N + natural ^{15}N) for the five depth intervals. Among soil depths, harmonic means ($\pm$ SE; *n* = 3) followed by the same letter were not significantly different regarding ^{15}N or ^{14}N + natural ^{15}N, respectively (ANOVA, Tukey HSD-test at *P* $\leq$ 0.05). No significant differences occurred between ^{15}N and ^{14}N + natural ^{15}N regarding the different soil depths (Student's t-test at *P* $\leq$ 0.05).

Table 4-2: Abundances of ^{15}N in N$_2$O emitted from the soil of the beech stand during the measurements in 1999–2000 and 2007–08.

| | δ ^{15}N-N$_2$O$_{emitted}$ [‰] | |
	Control	Litter exchange
1999–2000	−24.3 (1.8)[a]	32.5 (24.5)[b]
2007–2008	−22.1 (3.3)[a]	−7.5 (6.5)[ab]

Note: Among treatments, means ($\pm$ SE; *n* = 3) followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at *P* $\leq$ 0.05).

4.4. Discussion

4.4.1. Relocation and sequestration of litter N in the soil

Recovery of 92% of the ^{15}N two years after the ^{15}N-labelled beech litter application was in line with other studies and pointed to low N losses during the first years of decomposition. Zeller et al. (2000) found between 80% and 85% of ^{15}N-labelled beech leaf litter three years after the litter application in the surface 30 cm of an acid soil that had a poorly developed moder type humus. Fahey et al. (2011) reported a recovery of over 90% for ^{15}N derived from sugar maple leaf litter application in the surface organic layer and surface 10 cm of the soil one year after litter application. However eleven years after litter application, 73% of the initially added ^{15}N had been mineralised, taken up by trees, leached, or emitted as gaseous N compounds in our study. The low turnover rate of the N pool of the surface organic layer when expressed by a high MRT of 19 years, in contrast to a MRT of 10.5 years for C, indicated high recovery of 27% of the ^{15}N of exchanged litter 11 years after litter addition.
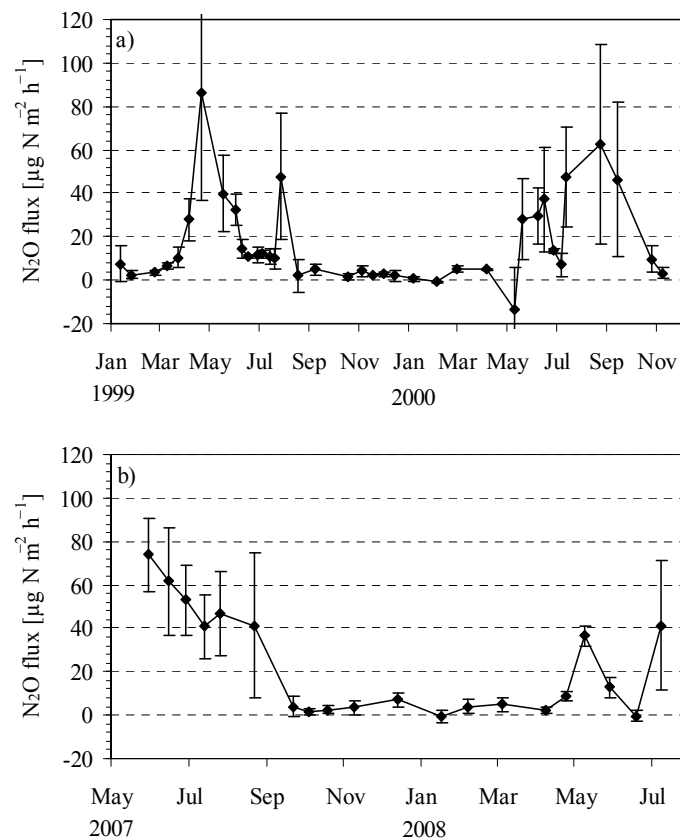
Figure 4-4: Mean (± SE; *n* = 3) N₂O fluxes in the litter exchange experiment of the Solling beech stand from January 1999 to December 2000 (a) and from May 2007 to July 2008 (b).

The annual amount of litter-N sequestration thus equals to about 9 kg N and significantly contributes to the mean total N sequestration of 21 kg N ha^{-1} yr^{-1} in the surface organic layer observed over a period of 35 years at the studied site (Meiwes et al., 2009). The site is characterized by high load of acid and nitrogen deposition (Meesenburg et al., 2009), which has led to an N-enriched but very acid soil with a low decomposition of the litter material and the absence of deep borrowing earthworms (Meiwes et al., 2009; Schaefer et al., 2009). Although the C/N ratio of the surface organic layer was constant over the last 35 years (Meiwes et al., 2009) the soil still is accumulating N as indicated by the low leaching losses and input-output balances (Brumme et al., 2009a). The most reasonable explanation is the stabilisation process which was induced by N depositions either directly by microbial N immobilisation or indirectly by plant uptake and the production of N-enriched litter including a big increase in fructification products (Khanna et al., 2009; Brumme and Khanna, 2009a).
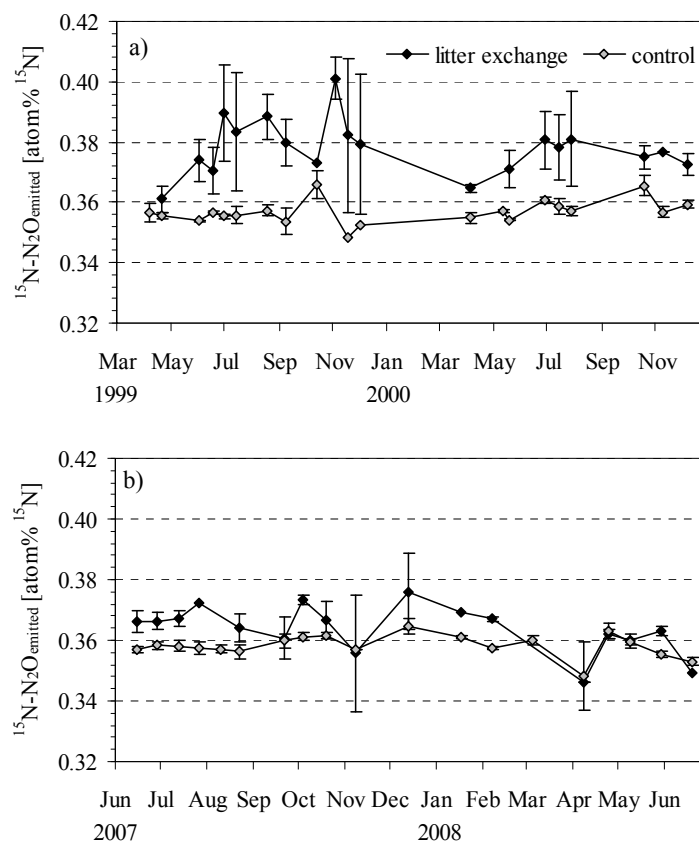
Figure 4-5: Mean (± SE; *n* = 3) ^{15}N abundances of N$_2$O emitted from the soil from the chambers where leaf litter was exchanged in 1997 and from the control chambers for the measurements in 1999–2000 (a) and in 2007–08 (b).

Only limited amount of the applied ^{15}N had been relocated to the lower part of the F layer (Fig.4-1), despite the huge amount of 28 Mg C ha^{-1} and 0.7 Mg N ha^{-1} which had been deposited through total aboveground litterfall since the beginning of the labelling experiment (Khanna et al., 2009). The surprisingly constant accumulation of ^{15}N in the H layer over the 11 years of observation revealed the lack of bioturbation though leaching of soluble organic and inorganic compounds between the F and H layer woulf have occurred. Labelled ^{15}N accumulated in the H layer was probably derived from initially high leaching of soluble organic N during the early-stage decomposition (first two years) as was also observed by Zeller et al. (2000). However, the downward movement of ^{15}N into the lower F layer might primarily be caused by litter deposition and decomposition. Limited relocation of litter ^{15}N from the organic horizons to the mineral soil within 4–5 years after litter application was also reported from three European beech forest soils which accumulated 7–13 Mg C on the mineral soil (Zeller and Dambrine, 2011; Zeller et al., 2001). The slow incorporation rate of litter N into the mineral-bound soil fraction was attributed to the absence of earthworms. Bird and Torn (2006) recovered very little ^{13}C and ^{15}N from pine needle litter below a 8 cm thick O-horizon in which the litter was placed two years after the beginning of the experiment. In contrast, Fahey et al. (2011) measured a comparable fast relocation of litter ^{15}N in the soil of a sugar maple forest with low earthworm activities. In their study, already one year after litter application the greatest proportion of recovered ^{15}N was found in 0–5 cm mineral soil depth. The mineral soil was covered by only 3.6 Mg C indicating a rapid incorporation of litter into the mineral soil.

Beside downward movement there is evidence that fungal hyphae may be responsible for an upward movement of N and C (Zeller et al., 2000). We observed that a significant proportion of applied ^{15}N was still present in the L layer two years after litter exchange. This may be due to fungal hyphae transporting N from decaying ^{15}N-labelled litter to fresh litter. Fungal transport of N amounted to 25–35% of exogenous N that was incorporated into decaying litter in an acid beech forest soil with low throughfall N depositions of 6–8 kg ha^{-1} yr^{-1} whereas 30–50% of the incorporated N might have come from deposited N (Zeller et al., 2000). In the sugar maple stand investigated by Fahey et al. (2011), 18% of external incorporated N in fresh litter was derived from the litter of the previous year. Bird and Torn (2006) suggested that as a result of fungal N translocation, organic horizons may provide a sink for leachable litter N. However, 11 years after litter exchange this process is of less importance in the present study as is indicated by a non-significant ^{15}N enrichment.

4.4.2. Mineralisation of leaf litter N

Nitrogen mineralisation amounted to 58 mg N m^{-2} d^{-1} in the surface 10 cm of the soil similar as was reported for this site in previous studies (Brumme et al., 2009a). The surface organic layer of the investigated stand was the primary horizon for N mineralisation and contributed to 77% to total net N mineralisation of the surface 20 cm of the soil (Brumme et al., 2009a). The present study showed an increased in the net ammonification and mineralisation rates within the soil profile from the L layer (0–2 cm) to the upper F layer (2–4 cm; Fig. 4-2), which was the most important layer for N turnover. The change in the N turnover could be attributed to the decrease in the C/N ratio from 28 to 22 (Tab. 4-1). Litter-bag studies with ^{15}N labelled beech litter by Zeller et al. (2000) showed that the shift from prevailing immobilisation to net mineralisation of N occurred after the first two years of decomposition when the C/N ratio had lowered to 25. In the present study ammonification and mineralisation continuously decreased with increasing soil depth below 2–4 cm depth (Fig. 4-2). Due to small changes in the C/N ratio other mechanisms might be responsible for this decrease. Respiration measurements conducted with surface organic matter of an adjacent beech stand showed a strong decrease in the release of CO_2 from 59 µg C ha^{-1} g^{-1} in the L layer to 12 and 3.9 µg C ha^{-1} g^{-1} in the F and H layer, respectively (Chodak et al., 2003). This clearly indicated a considerably decreased of mineralisation in the lower part of the surface organic layer probably caused by chemical stabilisation processes since physical stabilisation processes should be of minor importance in organic horizons. At the same beech stand, ^{15}N pool dilution studies conducted by Corre et al. (2003) revealed a gross mineralisation rate that was approx. four times higher than our net mineralisation rate indicating a high microbial recycling of N. Similar results were reported from other forest soils but their relevance for the N sequestration is still not clear (Venterea et al., 2004a).

About 0.6% of total net N ammonification could be ascribed to the exchanged leaf litter eleven years after litterfall, indicating that the labelled leaf litter N still participated in measureable amounts in N turnover. Compared to the native organic N twice as much labelled N was mineralised from the labelled N pool in the lower F layer and the H layer (Fig. 4-3). This demonstrated that the N from the comparably new ^{15}N-labelled beech litter was not preferentially stabilised compared to "older" native N, which was decomposed more slowly. Results from three European beech forests, where mineralisation of litter ^{15}N was 2–4-fold higher than mineralisation of native soil N 4–5 years after litter application, were in line with our findings (Zeller and Dambrine, 2011). The ^{15}N released from the decaying litter was mainly recovered in the coarse particulate organic matter fraction (> 200 µm; Zeller and

Dambrine, 2011). Bird et al. (2008) also reported that 58% of the ^{15}N of needle litter was recovered in the light fraction, which represents the SOM fraction with the highest turnover, in a Scots pine forest during the initial 1.5 years of their study.

4.4.3. N$_2$O emissions and contribution of leaf litter N

Emissions measured during our experiment were similar to the mean value of a 10-year measuring period at the Solling beech stand, which amounted to 1.92 kg N ha^{-1} yr^{-1} (Brumme and Borken, 2009). The beech stand belonged to the seasonal emission type where due to low oxygen availability in the soil, caused by high microbial and root respiration and low gas diffusivity in the surface organic layer, denitrification was promoted during the summer months (Brumme et al., 1999; Wolf and Brumme, 2002). Low gas diffusivity was the result of laminar undecomposed beech leaves in the L layer of the surface organic layer combined with high precipitation rate (Ball et al., 1997; Brumme and Borken, 2009). Leaching of NO$_3^-$ to the transitional zone between the H layer and the surface mineral soil presumably provided the substrate for denitrification. Brumme (1995) reported that high N$_2$O emissions occurred at temperatures of $> 10°C$ and water tensions < 200 hPa. Hence, years with high summer precipitation, as was the case for 2007 but not for 2008, exhibit high N$_2$O emissions (compare annual fluxes of Fig. 4-4).

The ^{15}N experiment indicated that the ^{15}N-labelled beech litter N measurably contributed to total N$_2$O fluxes. The contribution decreased from the 2–3 years measurement to the 10–11 years measurement after litter exchange due to the decrease in ^{15}N-labelled beech litter mass. The proportions of N$_2$O-N of total N were the same for labelled ^{15}N and native N in the surface 10 cm of the soil, despite the two times higher relative ammonification of labelled ^{15}N than that of native N. The surprising result may be the production of native N$_2$O in the mineral soil below 10 cm depth. However, we have no information about the proportion of ^{15}N nitrification of total ^{15}N but which probably could further clarify the observation.

Beech leaf litter N of a single year contributed between 0.3% (eleventh year) and 0.6% (second year) of the total annual N$_2$O emissions after litterfall. Thus, accumulated leaf litter N of the second to eleventh litterfall year only contributed approx. 5% to total annual N$_2$O emissions using an average emission of 0.46% (harmonic mean) for a single litterfall year.

The observation that neither leaf litter N during the 2–11 years period nor inorganic N depositions of the recent year (contribution of 6% to total N$_2$O emissions; Eickenscheidt et al., 2011) were primarily responsible for high N$_2$O emissions measured at the Solling beech stand, still raised the question of the origin of these emissions. The other sources of N$_2$O

emissions may include the first year litterfall N with possibly a rapid initial release of ^{15}N as also was reported by Zeller et al. (2000). However, we suppose that the older N pool of accumulated older litter material and accumulated deposited N may represents the major source for N$_2$O emissions from this soil.

4.5. Conclusions

Our study demonstrated that one quarter of the ^{15}N label of initially added beech leaf litter was still present in the surface 10 cm of the soil 11 years after litter addition. The high recovery of added ^{15}N, the constant accumulation of N in the H layer, and the possible fungal translocation of N from deeper horizons to fresh litter indicated some sequestration of added N in the organic layer. Movement of beech leaf litter N through the surface organic layer was very slow. However, the ^{15}N-labelled beech leaf litter still participated measurably in N turnover and N$_2$O production and obviously was not completely stabilised 11 years after litter exchange. The contribution of beech leaf litter of a single year to total annual N$_2$O emissions ranged between 0.3% (eleventh year) and 0.6% (second year). We suggest that the high N$_2$O emissions observed in the investigated beech stand resulted from accumulated N in older soil N pools, since neither leaf litterfall of the last 2 to 11 years nor N depositions of the recent year represented a significant source for N$_2$O.

Acknowledgment

Regulation of Soil N_2O and NO_x Fluxes in Temperate Beech Forests by Soil Gas Diffusivity, N Turnover, and Atmospheric NO_x Concentrations

5. Regulation of soil N_2O and NO_x fluxes in temperate beech forests by soil gas diffusivity, N turnover, and atmospheric NO_x concentrations

Abstract

High nitrous oxide (N_2O) and low nitric oxide (NO) emissions have been reported from acid temperate beech forest soils covered by an organic layer with moder type humus. Low soil gas diffusivity of the laminar litter layer is held responsible for the observation. The objectives of our study were (i) to evaluate whether acid beech forest soils generally exhibit high and seasonal N_2O emissions and (ii) to detect the influence of gas diffusivity on N oxide fluxes. We investigated six German beech stands that differed in soil texture and humus type. In two campaigns, we used chamber methods for N_2O and NO_x ($NO + NO_2 = NO_x$) measurements. Furthermore, we determined net N turnover rates and gas diffusivity on soil samples taken at each chamber. At one beech stand, we observed high N_2O emissions of up to $113\ \mu g\,N\,m^{-2}\,h^{-1}$, whereas at the other stands fluxes ranged from -2.4 to $7.4\ \mu g\,N\,m^{-2}\,h^{-1}$. Net nitrification of the organic layer together with soil diffusivity explained 77% of the variation in N_2O fluxes ($P = 0.001$). Fluxes of NO_x were low (-6.3 to $12.3\ \mu g\,N\ m^{-2}\,h^{-1}$) at all sites and appeared to be controlled by NO_x concentrations in the forest air. In most beech forest soils, air-filled porosity and diffusivity was high. Parameter estimates for the relationship between the apparent diffusion coefficient and ε are presented using well-established models such as the Penman-Millington-Quirk model. We could not confirm that in general acid beech forest soils with moder type humus exhibit high seasonal N_2O emissions. Our results suggest that at the site with high N_2O fluxes low gas diffusivity and high respiration rates caused anaerobic conditions that promoted denitrification. A combination of moder type humus on a loamy soil with high annual precipitation and high N turnover rates is required for high N_2O emissions. We concluded that high seasonal emissions are probably less common in German beech forests than previously assumed.

Keywords: Soil gas diffusivity, beech forest, nitrous oxide, nitric oxide, N mineralisation, nitrification, seasonal emission patterns, "hole in the pipe" model, Penman-Millington-Quirk model

5.1. Introduction

Temperate forest soils have been identified as a considerable source for the trace gases N_2O and NO in particular when forests are exposed to high N depositions (e.g. Brumme and Beese, 1992; Papen and Butterbach-Bahl, 1999; Skiba et al., 1999; van Dijk and Duyzer, 1999). Estimates of N_2O and NO emissions from temperate forest soils range from 0.1 to 2.0 Tg N_2O-N yr^{-1} (Brumme et al., 2005; IPCC, 2001; Kroeze et al., 1999) and from 0.2 to 0.4 Tg NO-N yr^{-1} (Davidson and Kingerlee, 1997; Gasche and Papen, 1999). Nitrous oxide is a potent greenhouse gas and contributes to the catalytic decomposition of stratospheric ozone (Crutzen, 1979; IPCC 2007). Nitric oxide and nitrogen dioxide (NO_2) are indirectly involved

in global warming through the production of radiative tropospheric ozone and contribute to the formation of acid rain (Crutzen, 1979; Logan, 1983).

Several studies have reported that in general N_2O emissions from deciduous forests were higher than those from coniferous forests (e.g. Brumme et al., 1999; Papen and Butterbach-Bahl, 1999) whereas this seems to be opposite for NO emissions (e.g. Pilegaard et al., 2006; van Dijk and Duyzer, 1999). Differences in litter quality and quantity are held responsible for these observations (e.g. Papen and Butterbach-Bahl, 1999). Soil N turnover rates are typically higher in deciduous forests than in coniferous forests due to higher litter quality (e.g. Ambus et al., 2006). Furthermore, the horizontal laminar structure of the litter layer of beech forests on acid soils has been found to function as a gas diffusion barrier (Ball et al., 1997; Brumme and Borken, 2009). Low gas diffusivity was responsible for high N_2O emissions during times of high soil respiration in a beech forest with moder type humus in contrast to a beech forest with mull type humus and a coniferous forest with moder type humus (Brumme et al., 1999). A literature review revealed that deciduous forests with mull type humus and acid coniferous forests with moder type humus, whose litter layer is thick but well-aerated, showed low non-seasonal emissions whereas deciduous forest with moder type humus showed a seasonal emission pattern with high summer emissions (Ball et al., 1997; Brumme and Borken, 2009; Brumme et al., 1999). Brumme et al. (1999) defined three types of N_2O emission patterns in temperate forests: (i) the seasonal emission pattern (SEP) with high N_2O emissions in wet summer, (ii) the background emission pattern (BEP) with low N_2O emissions during the whole year, and (iii) the event emission pattern (EEP). The authors hypothesised that soil gas diffusivity of the organic layer was mainly responsible for these differences whereas N was not a limiting factor under the high N load in most parts of the temperate zone.

Firestone and Davidson (1989) proposed the conceptual "hole-in-the-pipe" (HIP) model which postulates two levels of regulation of N oxide emissions from soils: (i) the sum of total N oxide production is a function of N availability, specifically of nitrification and denitrification rates (rate of N moving 'through the pipe') and (ii) the ratio of released gases (NO, N_2O, N_2) is a function of environmental factors such as O_2 availability and pH, which define the size of 'holes in the pipe' (Davidson et al., 2000). Whether aerobic nitrification or anaerobic denitrification prevails, depends on O_2 availability, which is controlled by gas diffusivity of the soil and O_2 consumption by micro-organisms and roots (Bollmann and Conrad, 1998; Brumme et al., 1999; Smith et al., 2003). In contrast to N_2O emissions, which are mainly derived from denitrification, NO emissions are probably the result of nitrification or chemodenitrification (Ambus et al., 2006; Wolf and Brumme, 2002; van Cleemput and

Samater, 1996). The oxygen and N mineralisation dynamics might thus be the most important drivers for high N_2O emissions and have been assumed to be responsible for seasonal emission patterns in the rainy seasons of the tropics and moist summer periods in the temperate regions (Brumme et al., 2005). However, to date N oxide fluxes, mineralisation and nitrification rates, and soil gas diffusivity have not been measured simultaneously at forest sites.

The objective of our study was to determine the gas fluxes of N_2O and NO_x along a gradient from F-mull to moder/mor type humus with different soil texture under high N deposition loads. At two field campaigns in six beech forests, in-situ fluxes of N_2O and NO and lab studies for N mineralisation, nitrification, and gas diffusivity were conducted. In our study we addressed the following questions: (i) Do all acid temperate beech forest soils with organic layers consisting of moder type humus exhibit high seasonal N_2O emissions and low NO_x emissions? (ii) Is gas diffusivity the key driver that controls the release of N oxides? Additionally, the present study focussed on the description of the relationship between the apparent diffusion coefficient and air-filled porosity in order to provide parameters for modelling.

5.2. Materials and methods

5.2.1. Study sites

We conducted our study at six beech stands (*Fagus sylvatica*), which were part of the German Level-II monitoring programme (Forest Intensive Monitoring Programme of the UNECE; Tab. 5-1, Fig. 1-5). The beech stands were >100 years old and were characterised by acid soil conditions. Soil types all were Dystric Cambisols, but sites differed in particle size distributions, humus type, and further climatic conditions. The surface organic layer type ranged from F-mull to moder/mor type humus. The stands have been exposed to different atmospheric N loads. The site characteristics and soil properties are presented in Tab. 5-1 and Tab. 5-2. Huenfeld had probably been limed in the past (H. Meesenburg, pers. communication, 2009).

5.2.2. Soil properties

At Solling, soil samples for soil characterisation were taken in December 2007. At the other five sites, soil samples were taken in October 2009. The mass of the organic layer was determined using a metal ring (594 cm^2). Organic layer samples were oven-dried at 60°C, living roots were removed, and organic material was shredded and ground for further

analyses. Soil bulk density was determined on undisturbed soil samples (0–5 and 5–10 cm depths; 250 cm^3), which were oven-dried at 105°C and corrected for the volume of roots and stones > 2 mm. Mineral soil samples were oven-dried at 60°C, sieved, and ground. Organic carbon and total nitrogen concentrations were determined using a CNS Elemental Analyzer (Heraeus Elementar Vario EL, Hanau, Germany). Sieved mineral soil or shredded organic material (10 mL) were used for pH measurements conducted in distilled water (1:2.5 (v/v)).

Table 5-1: Site characteristics of the six Level II monitoring beech stands in Germany.

	Haard	Neuhaeusel	Bieber-gemuend	Huenfeld	Luess	Solling
Level II site	503	704	604	601	301	304
Elevation [m]	70	390	450	410	101–150	500
Precipitation [mm yr^{-1}]	880	956	1037	706	784	1193
Mean temperature [°C]	9.5	8.1	7.6	7.2	8.0	6.9
Parent material	Cover sand over Haltener Sand	Pumic or loess solifluction layer over quartzite	Loess solifluction layer over Triassic sandstone	Loess solifluction layer over Triassic sandstone	Pleistocenic sands	Loess solifluction layer over Triassic sandstone
Humus type	Moder/Mor	F-Mull	Moder	F-Mull	Moder	Moder
Height org. layer [cm]	9.6	4.2	4.3	3.8	6.0	5.2
NO$_3$ deposition [kg N ha^{-1} yr^{-1}]	8.3	9.9	11.5	11.4	6.4	9.5
NH$_4$ deposition [kg N ha^{-1} yr^{-1}]	15.3	8.8	8.3	7.4	8.7	12.1
Throughfall N$_{min}$ deposition [kg N ha^{-1} yr^{-1}]	23.6	18	19.9	18.8	15.2	21.6
N leaching [kg N ha^{-1} yr^{-1}]	16	6	2	0	0	1
N uptake [kg N ha^{-1} yr^{-1}]	13	6	17	14	6	11

Note: Data derived from Haussmann and Lux (1997), Brumme and Khanna (2008, 2009a), Gehrmann (pers. communication, 2009), Meesenburg (pers. communication, 2009), and from 'www.wald-rlp.de' (accessed 2010).

The effective cation exchange capacity (CEC) was determined according to Koenig and Fortmann (1996). In short, soil samples were percolated with 100 mL 1 M NH$_4$Cl solution for four hours and the cations were quantified using an ICP-AES (Spectro Analytical Instruments, Kleve, Germany). Exchangeable protons were calculated from the pH of the NH$_4$Cl solution before and after percolation. Cation exchange capacity was calculated as the sum of the exchangeable Na, K, Ca, Mg, Mn, Fe, Al, and H ions whereas the base saturation (BS) was calculated as the sum of base cations (Na, K, Ca, and Mg) as percent of CEC. The particle size distribution was determined using the sieving and pipette method described by

Schlichting et al., 1995. Briefly, organic matter and Fe oxides were destructed using 30% H_2O_2 and 4% Na-dithionite-citrate before wet sieving of the sand and coarse silt fraction ($\geq 20\ \mu m$). Afterwards, sedimentary fractionation according to the Atterberg method was conducted at 25°C for 21 hours (30 cm fall height).

Table 5-2: Soil properties of the six German beech stands.

	Haard	Neuhaeusel	Bieber-gemuend	Huenfeld	Luess	Solling
Sand [%]	63.4 (1.0)[a]	23.6 (2.3)[b]	57.7 (0.7)[c]	54.4 (1.3)[c]	78.8 (0.8)[d]	22.2 (0.6)[b]
Silt [%]	31.0 (0.9)[a]	52.7 (2.0)[b]	29.8 (0.6)[a]	36.2 (1.5)[c]	17.4 (0.7)[d]	52.9 (0.5)[b]
Clay [%]	5.6 (0.2)[a]	23.7 (1.5)[b]	12.5 (0.4)[c]	9.4 (0.3)[d]	3.8 (0.4)[e]	24.8 (0.4)[b]
Humus mass [Mg ha^{-1}]	309 (38)[a]	50 (10)[bd]	56 (12)[b]	40 (6)[bd]	184 (7)[c]	36 (7)[d]
BD 0–5 cm [g cm^{-3}]	1.36 (0.13)[a]	0.83 (0.08)[c]	0.93 (0.09)[bc]	1.18 (0.17)[ab]	1.35 (0.28)[a]	0.89 (0.06)[c]
BD 5–10 cm [g cm^{-3}]	1.55 (0.07)[a]	1.07 (0.11)[b]	0.92 (0.09)[b]	1.50 (0.04)[a]	1.57 (0.11)[a]	1.08 (0.13)[b]
C content org. layer [%]	39.9 (0.9)[a]	30.8 (2.9)[b]	32.5 (1.0)[b]	35.6 (2.4)[ab]	33.5 (4.3)[ab]	45.5 (0.4)[c]
N content org. layer [%]	1.86 (0.06)[ac]	1.36 (0.10)[b]	1.54 (0.07)[ab]	1.23 (0.05)[b]	1.33 (0.16)[b]	2.07 (0.07)[c]
C/N ratio organic layer	21.5 (0.5)[a]	22.6 (0.9)[a]	21.2 (0.6)[a]	28.9 (1.8)[b]	25.1 (0.8)[ab]	22.1 (0.7)[a]
C content 0–5 cm [%]	14.17 (1.42)[a]	6.92 (1.19)[bc]	5.87 (0.62)[c]	3.25 (0.59)[d]	5.61 (0.91)[cd]	9.70 (0.84)[abc]
N content 0–5 cm [%]	0.57 (0.07)[a]	0.38 (0.07)[bc]	0.26 (0.03)[cd]	0.15 (0.02)[d]	0.23 (0.03)[d]	0.47 (0.04)[abc]
C/N ratio 0–5 cm	25.3 (1.0)[a]	18.2 (0.5)[c]	22.3 (0.5)[ad]	21.7 (0.9)[bde]	24.2 (0.3)[ae]	20.7 (0.3)[bcd]
pH (H$_2$O) 0–5 cm	3.6 (0.0)[a]	4.1 (0.1)[b]	3.7 (0.0)[c]	4.3 (0.1)[b]	3.7 (0.0)[c]	3.5 (0.0)[d]
CEC 0–5 cm [mmol$_c$ kg^{-1}]	108 (10)[a]	101 (8)[ac]	77 (3)[bc]	56 (5)[b]	49 (7)[b]	139 (6)[d]
BS 0–5 cm [%]	6.7 (0.6)[a]	8.1 (1.2)[a]	7.0 (0.8)[a]	45.1 (8.5)[b]	10.8 (0.9)[a]	10.0 (1.4)[a]

Note: Among the stands, means ($\pm$ SE, n=5) followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at $P \leq 0.05$). For the Solling, six replicates were taken for bulk density and humus mass and at all other stands four replicates were taken for humus mass. BD stands for bulk density of the mineral soil; CEC for effective cation exchange capacity, and BS for base saturation.

5.2.3. N$_2$O and NO$_x$ flux rates

Gas flux measurements were conducted during two field campaigns in May and July 2009. Five chamber bases (PVC; area: 0.07 m^2; mean volume of enclosed air: 17.6 L) were inserted approx. 3 cm into the soil at each site immediately before measurements. For N$_2$O flux measurements, we used the closed chamber method. Gas samples (100 mL) were taken at 0,

15, 30, and 45 minutes after chamber closure, using a portable gas sampler equipped with a pressure sensor (Loftfield et al., 1997). The samples were analysed with a gas chromatograph equipped with an EC detector (GC 14A, Shimadzu, Duisburg, Germany). For NO_x flux measurements, we used an open dynamic chamber method combined with a portable battery operated NO_x analyser (Scintrex Unisearch, Ontario, Canada). Calibration was conducted in the field before and after chamber measurements using a NO standard gas (3000 ppbv NO in N_2; Air Liquide GmbH, Germany). Nitric oxide was oxidised to NO_2 by a solid CrO_3 catalyst before analysis of NO_2 by a Scintrex LMA-3 chemiluminescence detector. A Teflon tube connected the analyser with a chamber, which we placed on the chamber base for five minutes. The total mean air flow through the chamber was 0.9 L min^{-1}. Concentrations of NO_x were logged every five seconds using a CR510 data logger (Campbell Scientific, Utah, USA).

In July 2009, we additionally measured N_2O fluxes and heterotrophic CO_2 respiration rates in the laboratory on undisturbed soil sample (0–9 cm and 0–19 cm), which were later used for determination of N_2 diffusion coefficients. The bottom of the PVC column, which contained the soil sample, was sealed and a chamber (PVC, area: 0.018 m^2; enclosed air volume: 2.8 L) was air-tightly fixed to the top of the column. The first sample (time point zero) was taken above the soil surface immediately before chamber closure. Further samples were removed after 5, 10, and 15 minutes after chamber closure. Samples were removed using a syringe and the sample (3 mL) was directly inserted into the gas chromatograph.

The linear change of gas concentration in the chamber versus time was calculated and multiplied by the ratio of chamber volume to soil surface area and by air density, which was adjusted for air temperature and atmospheric pressure.

5.2.4. Net N mineralisation rates

In May and July 2009, five PVC columns (height: approx. 0–19 cm, diameter: 15.1 cm) were inserted into the organic layer and upper mineral soil next to the chambers after gas flux measurements to determine net nitrification and mineralisation rates. The PVC columns were excavated and stored in PE bags. The soil samples were cooled during transport. In the laboratory, the organic layer and the upper 5 cm mineral soil of each column were separately mixed and a part of the soil (t0 samples) was removed for preparation of soil mineral N (N_{min}) extracts and gravimetrical soil water determination. The remaining part (t1 sample) was incubated in PE bags in the laboratory at the soil temperature (at 5 cm soil depth) during soil

sampling, which was approx. 9°C in May and 13.5°C in July. After 14 days, the incubated, disturbed soil samples were extracted using the same procedure as for the t0 samples.

For soil extraction, we added 100 mL 0.5 M K_2SO_4 solution to 30 g fresh soil or 50 mL K_2SO_4 solution to 15 g of the organic layer. The excess was filtered after shaking the sample solution for one hour. The extracts remained frozen until N_{min} analysis was done. The concentrations of extractable ammonium (NH_4^+) and nitrate (NO_3^-) were determined using a continuous flow injection colorimeter (Cenco/Skalar Instruments, Breda, The Netherlands). The NO_3^--N and N_{min} contents at the beginning and at the end of incubation were used to calculate the net nitrification and mineralisation rates.

5.2.5. Gas diffusion coefficients

Although we were interested in the gas diffusion coefficient of O_2, we chose N_2 as experimental gas, since its consumption and production during the experiment could be neglected, its concentration in air is comparatively constant and its molecular mass is similar to that of O_2. To determine the apparent diffusion coefficient (Ds) of N_2 in the soil, we sampled five undisturbed soil columns (height: approx. 0–19 cm, diameter: 15.1 cm) per site within the chambers in May and July 2009 after conducting the gas flux measurements. In July 2009, an additional column (height: approx. 0–9 cm, diameter: 15.1 cm) per chamber was taken next to each chamber for measurement of Ds. In May 2009, we determined the diffusion coefficient for the whole column and, after cutting off the lower part, for the upper 0–9 cm of the column. The undisturbed soil samples were stored in PE bags at 9°C in May and at 13.5°C in July. Before determination of the diffusion coefficient, soil columns had been acclimatised to room temperature for one night. We used the one-chamber method (e.g. Fluehler, 1972; Teepe, 1999) where soil columns were placed on a chamber (height: 15.6 cm; diameter: 15.1 cm) and the upper surface of the soil sample was exposed to the atmosphere (Fig. 5-1). The soil column and chamber were air-tightly linked to each other. The gas leakage rate was examined for each chamber before soil samples were analysed. A mesh prevented soil loss into the chamber. A magnetic stirrer at the bottom of the chamber provided a homogenisation of the gas mixture in the chamber. The chamber was equipped with two valves to flush the chamber and a septum to take gas samples with a syringe. The chamber was flushed with argon (Ar) to decrease the N_2 concentration in the chamber to 2–6%. Thus, we created a N_2 and Ar concentration gradient between the chamber and the ambient air. Gas samples (2 mL) were removed at several time points, depending on the rate of N_2 increase in the chamber. Usually we sampled six gas samples over a period up to 4 hours (for the 0–

19 cm soil samples) and up to 1.5 hours (for the 0–9 cm soil samples). Analyses of N_2 were conducted with a gas chromatograph coupled to a thermal conductivity detector (Delsi Instruments ENICA 21).
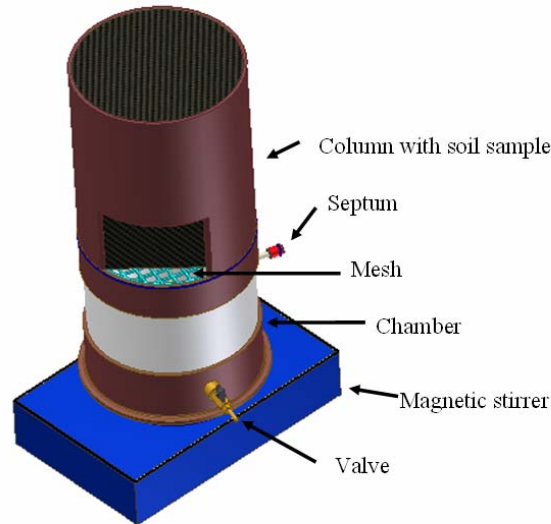
Figure 5-1: Design of the one-chamber method used for determination of soil gas diffusivity (drawn by H. Eickenscheidt).

Assuming stationary conditions, Fick's first law describes the rate of gas diffusion as (e.g. Fluehler, 1972; Teepe, 1999):

$$q = -Ds \cdot \frac{dc}{z} \tag{5.1}$$

where

q: rate of gas diffusion [$cm^3\ cm^{-2}\ s^{-1}$]

Ds: apparent diffusion coefficient of N_2 in the soil [$cm^2\ s^{-1}$]

dc: local difference in gas concentration [$cm^3\ cm^{-3}$]

z: length of the soil sample [cm], varied

The term dc/z indicates the mean concentration gradient in the soil sample at a specific time. If we assume that the mean gradient is consistent with the local gradient (Fluehler, 1972), we have a linear concentration gradient (Eq. 5.2).

$$\frac{dc}{dz} = \frac{c_C(t) - c_A}{z} \tag{5.2}$$

where

$c_C(t)$:   gas concentration in the chamber at time point t

c_A: gas concentration in ambient air, constant

In case of stationarity, the gradient and diffusion rate are constant in time. However, in our experiment the difference in N_2 concentration decreased with time (Eq. 5.3).

$$q = \frac{d(c_C(t) - c_A)}{dt} \cdot \frac{V_C}{A}$$
(5.3)

where

V_C: chamber volume [cm^3], constant

A: cross section area of the soil sample and chamber [cm^2], constant

Assuming quasi steady state conditions, i.e. that the gradient in N_2 changes with time but is locally constant at a specific time, and the gas diffusion rate changes with time, we can combine Fick's first law with Eq. 5.2 and 5.3 (Eq. 5.4).

$$\frac{d(c_C(t) - c_A)}{dt} \cdot \frac{V_C}{A} = -Ds \cdot \frac{c_C(t) - c_A}{z}$$
(5.4)

Rearrangement of Eq. 5.4 to solve for Ds and integration in the limits of integration $t=0, c_C(0)$ and $t=t', c_C(t')$ gives:

$$Ds = \frac{2.3 \cdot V_C \cdot z}{A} \cdot \frac{1}{t'} \cdot \log\left(\frac{c_C(0) - c_A}{c_C(t') - c_A}\right)$$
(5.5)

For comparison of samples, independent of the gases used, the apparent diffusion coefficient was expressed relative to that in free air (Ds/Da; $Da = 0.199$ cm^2 s^{-1}; Teepe, 1999). Since gas diffusion in soils is in particular dependent on air-filled porosity ε, we described Ds/Da as function of ε. We calculated total soil porosity (Φ) and ε of each soil sample. Particle density of minerals was assumed to be 2.65 g cm^{-3} and density of organic matter was assumed to be 1.5 g cm^{-3}.

5.2.6. Model fitting

Several empirically and mechanistically based linear or non-linear models have been developed to predict or describe the relative diffusion coefficient as a function of ε such as the Penman (1940) and Millington-Quirk (1961) models. We tested some of the well-established models. We presented three models with the best fit, namely an exponential model (Eq. 5.6), a power function model (Eq. 5.7), and the Penman-Millington-Quirk (PMQ) model (Eq. 5.8; Moldrup et al., 1997). Moldrup et al. (1997) transformed the Penman (1940) and Millington-Quirk (1961) models into a general form by introducing the tortuosity parameter m. The authors suggest $m = 3$ for undisturbed soils which exhibit high tortuosity. The model further

takes account of a soil type and water effect by considering Φ. We used the original PMQ model with $m = 3$, but we also fitted m (PMQ$_{mod}$).

$$\frac{Ds}{Da} = B \cdot e^{k \cdot \varepsilon} \tag{5.6}$$

$$\frac{Ds}{Da} = B \cdot \varepsilon^{k} \tag{5.7}$$

$$\frac{Ds}{Da} = 0.66 \cdot \Phi \cdot (\frac{\varepsilon}{\Phi})^{\frac{(12-m)}{3}} \tag{5.8}$$

where B and k are fitting parameters.

We used residual-based goodness of fit measures to evaluate and compare the models: sum of squared errors (SSQ), root mean square error (RMSE), and Akaike's Information Criterion (AIC; Akaike, 1974). The AIC was corrected for sample size according to Hurvich and Tsai (1989) and considers the number of fitted model parameters. A smaller AIC indicates better model performance. To evaluate model overestimation or underestimation, we calculated the bias (Moldrup et al., 1997).

5.2.7. Statistical analyses

Analysis of variance (ANOVA) and the Tukey HSD-test were used for comparisons of sites. We tested the assumptions of normality of residuals (KS test or Cramer-von Mises test) and homogeneity of variances (Levene test) prior to analysis. Where necessary, data were log or box-cox transformed. We used the Kruskal-Wallis H-test in case normality of residual errors was not obtained. Simple and multiple regression analyses were carried out. The minimal adequate model was obtained using the AIC. Due to non-normality of residual errors, regressions on field N_2O fluxes were performed using mean values of the replication chambers. Multiple regressions on NO_x fluxes were also performed using mean values since NO_x concentrations in the forest air were not replicated. Effects were considered significant if $P \leq 0.05$. Results are reported as means ± 1 standard errors. Statistical analyses were carried out using R 2.12.0 (R Development Core Team, 2010).

5.3. Results

5.3.1. N_2O fluxes

In May 2009, N_2O fluxes were significantly different among the six stands ($P = 0.001$; Fig. 5-2a). Solling displayed the highest N_2O emissions of 36 ± 11 µg N m^{-2} h^{-1} followed by Haard and Huenfeld with approx. 7 µg N m^{-2} h^{-1} whereas we measured net N_2O uptake at the remaining sites. Although N_2O emissions were high at Solling during the second campaign in July 2009 (113 ± 68 µg N m^{-2} h^{-1}), they were not significantly different among stands because of high variability (Fig. 5-2a; Tab. 5-3). The N_2O emissions measured at the laboratory at 27.5°C were differed among the stands ($P < 0.01$ for both the 0–9 cm and 0–19 cm columns). However, only the Solling showed significantly higher N_2O emissions in the laboratory than in the field measurement ($P = 0.001$; Tab. 5-3). Neuhaeusel displayed a potential for higher N_2O emissions and the other stands showed no differences (Tab. 5-3).
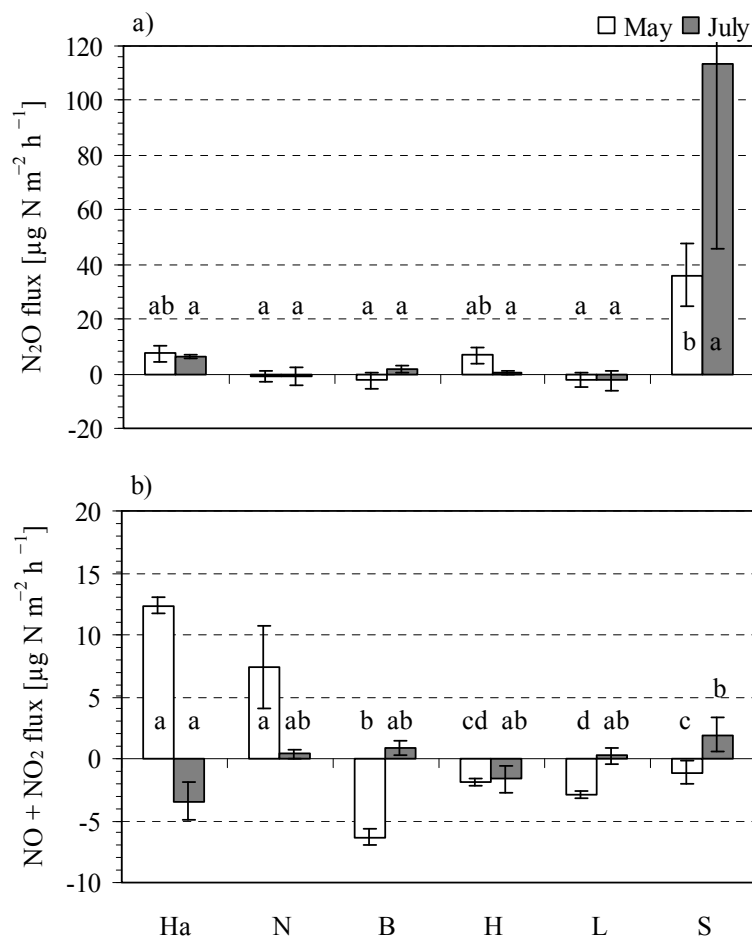
Figure 5-2: Mean (± SE, $n = 5$) N_2O fluxes (a) and NO + NO_2 fluxes (b) of the six beech stands in May and July 2009. Regarding one months, means followed by the same letter indicated no significant differences among stands (Kruskal-Wallis H-test or ANOVA, Tukey HSD-test at $P \leq 0.05$). Site abbreviations: Ha = Haard, N = Neuhaeusel, B = Biebergemuend, H = Huenfeld, L = Luess, and S = Solling.

5.3.2. NO + NO₂ fluxes

Fluxes of NO_x were low at all investigated stands and at both measuring dates and ranged from −6.3 to 12.3 µg N m^{-2} h^{-1}. The fluxes were significantly different among the stands in May ($P < 0.0001$) and July 2009 ($P = 0.017$; Fig 5-2b). In May, Haard and Neuhaeusel displayed NO_x emissions whereas NO_x was taken up at the other sites. In July, NO_x emissions were highest at Solling.

Table 5-3: Mean (± SE, *n* = 5) N₂O fluxes and heterotrophic CO₂ respiration rates measured in-situ at the six beech stands in July 2009 and in the laboratory at 0–9 cm soil columns (lab₉) and at 0–19 cm soil columns (lab₁₉), which both were taken at the six stands in July 2009.

	N$_2$O flux, field [µg N m^{-2} h^{-1}]	N$_2$O flux, lab$_9$ [µg N m^{-2} h^{-1}]	N$_2$O flux, lab$_{19}$ [µg N m^{-2} h^{-1}]	CO$_2$ flux, lab$_9$ [mg C m^{-2} h^{-1}]	CO$_2$ flux, lab$_{19}$ [mg C m^{-2} h^{-1}]
Haard	6.2 (0.5)[a]	45.2 (38.8)[a]	2.1 (6.4)[a]	196 (55)	249 (42)
Neuhaeusel	−1.2 (3.3)[a]	122.5 (122.2)[a]	131.9 (99.7)[a]	420 (102)	286 (55)
Biebergemuend	1.6 (1.6)[a]	−1.5 (3.5)[a]	18.9 (20.5)[a]	251 (18)	368 (94)
Huenfeld	0.2 (0.5)[a]	56.4 (38.8)[a]	29.6 (30.2)[a]	139 (17)	305 (83)
Luess	−2.4 (3.7)[a]	0.0 (2.9)[a]	4.1 (5.2)[a]	193 (72)	193 (30)
Solling	113.3 (67.4)[a]	2233.8 (447.7)[b]	943.0 (442.8)[b]	518 (74)	428 (127)

Note: For each stand, means followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at $P \le 0.05$). Gas flux measurements in the laboratory were conducted at 27.5°C at field moisture.

5.3.3. Control parameters

Net rates of N mineralisation and nitrification of the organic layer significantly differed among the six stands in May and in July 2009 ($P < 0.0001$, Fig. 5-3a, 5-3b). Both rates were highest at Solling. Neuhaeusel had a similarly high net nitrification rate as Solling in May 2009 and displayed the second highest net N mineralisation rates. We could not detected net nitrification at Luess and a negligible rate at Biebergemuend. Net rates of N mineralisation and nitrification of the surface 0–5 cm mineral soil also significantly differed among the six stands in May and in July 2009 ($P < 0.001$, data not shown). Neuhaeusel and Solling displayed similar net rates, which were significantly higher compared to the other sites.

Soil gas diffusivity in the 0–19 cm columns was significantly different among the stands in May ($P < 0.0001$) and July ($P = 0.022$; Fig. 5-4a). Gas diffusion coefficients Ds_{19} at Solling were the lowest at both measuring dates. Gas diffusion coefficients of the 0–9 cm columns also significantly differed among the stands in May ($P = 0.001$) and July ($P < 0.0001$; Fig. 5-4b). Again, Solling displayed the lowest Ds_9 at both dates as well as Huenfeld in July.

The water contents of the organic layer (Fig. 5-5d) and mineral soil (data not shown) were significantly different among the stands at both measuring dates ($P < 0.0001$). The water

content of the organic layer was highest at Solling at both dates and also at Haard in May (2.59 ± 0.14 g g^{-1}; Fig. 5-5d). The water content of the mineral soil was highest at Solling and Haard in both months and also at Luess in May. The water content of the mineral soil ranged from 0.34 g g^{-1} (Huenfeld) to 0.85 g g^{-1} (Solling) in May and from 0.24 g g^{-1} (Huenfeld) to 0.73 g g^{-1} (Solling) in July. The annual precipitation sum was also highest at Solling (Tab. 5-1).

Soil temperature in 5 cm depth ranged from 8.0°C to 9.8°C in May (mean 8.8°C) and from 12.5°C to 14.3°C (mean 13.5°C) in July at the six stands.
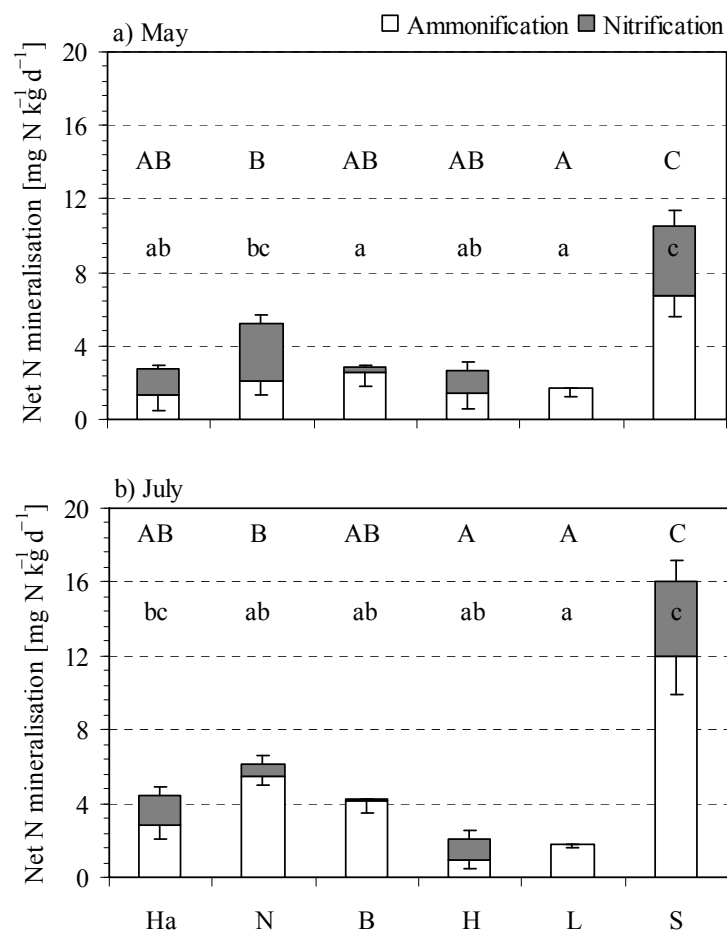
Figure 5-3: Mean (± SE, *n* = 5) net N mineralisation rates of the organic layer of the six beech stands measured on disturbed soil samples taken in (a) May and (b) July 2009. Upward error bars belong to net nitrification rates and downward error bars to net ammonification rates. Among stands, means followed by the same letter indicated no significant differences (ANOVA, Tukey HSD-test at *P* ≤ 0.05). Lower case letters belong to net nitrification rates and upper case letter to total net N mineralisation rates. Site abbreviations: Ha = Haard, N = Neuhaeusel, B = Biebergemuend, H = Huenfeld, L = Luess, and S = Solling.

5.3.4. Regulation of N$_2$O fluxes

Field N$_2$O fluxes were exponentially and positively related to the water content of the organic layer, net nitrification rates of the organic layer, net N mineralisation rates of the organic layer, and negatively to the N$_2$ diffusion coefficient Ds_{19} (Tab. 5-4; Fig. 5-5). Using multiple regressions, N$_2$O fluxes (y) could be best explained by net nitrification of the organic layer ($x1$) and the diffusion coefficient Ds_{19} ($x2$; Eq. 5.9; Tab. 5-4).

$$\log(y + 3.5) = 0.5\ (\pm 0.2) \cdot x1 - 130.9\ (\pm 55.9) \cdot x2 + 2.7\ (\pm 1.0) \qquad \textbf{(5.9)}$$

The model fit was almost similarly good using net N mineralisation rates of the organic layer (instead of nitrification rates) and Ds_{19} as the predictors (Tab. 5-4). In addition, using the water content of the organic layer instead of the diffusion coefficient resulted in similar good model fits (Tab. 5-4).

Table 5-4: *P*-values and coefficients of determination (r^2) for simple and multiple regression analyses between N$_2$O fluxes and control parameters.

Control factor	N$_2$O flux [µg N m^{-2} h^{-1}]	
	P-value	r^2
Simple regression		
Grav. water content O [g g^{-1}]	0.0154	0.46
Grav. water content M [g g^{-1}]	ns	
Temperature 5 cm depth [°C]	ns	
Net nitrification O [mg N kg^{-1} d^{-1}]	0.0020	0.63
Net mineralisation O [mg N kg^{-1} d^{-1}]	0.0016	0.65
Net nitrification M [mg N kg^{-1} d^{-1}]	ns	
Net mineralisation M [mg N kg^{-1} d^{-1}]	ns	
Ds_9 [cm^2 s^{-1}]	ns	
Ds_{19} [cm^2 s^{-1}]	0.0042	0.58
Air-filled porosity 0–9 cm [cm^3 cm^{-3}]	ns	
Air-filled porosity 0–19cm [cm^3 cm^{-3}]	ns	
Multiple regression		
Water content O × nitrification O	0.0026	0.73
Water content O × mineralisation O	0.0016	0.76
Ds_{19} × nitrification O	0.0013	0.77
Ds_{19}× mineralisation O	0.0015	0.76

Note: ns is not significant; O is organic layer; M is 0–5 cm mineral soil. Fluxes of N$_2$O were log-transformed for the analysis and $df = 10$ for simple regressions and $df = 9$ for multiple regressions.

Fluxes of N_2O measured in the laboratory were best explained by net N mineralisation rates of the organic layer and Ds of the corresponding column (for 0–9 cm columns: $P = 0.0001$, $r^2 = 0.52$, $df = 24$ and for 0–19 cm columns: $P = 0.0002$, $r^2 = 0.49$, $df = 26$). There was no significant relationship between these N_2O fluxes and heterotrophic respiration rates measured in the laboratory, but both fluxes were highest at Solling (Tab. 5-3)

Simple as well as multiple regressions were not significant when omitting the data of the Solling site.
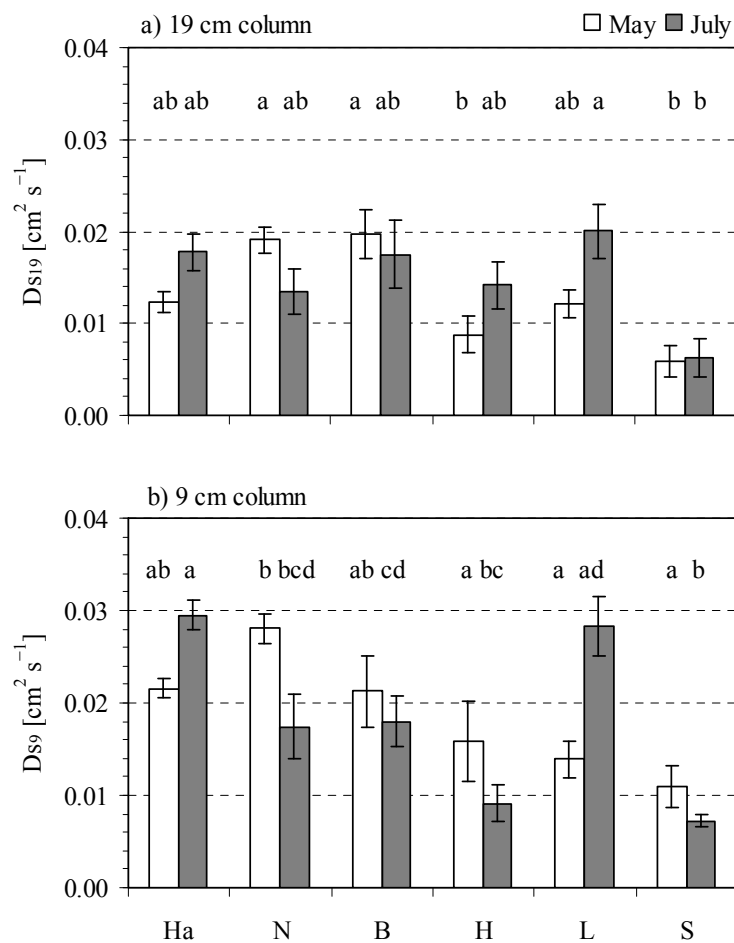
Figure 5-4: Mean (± SE, $n = 5$) apparent diffusion coefficients of N_2 given for the six beech stands, which were measured (a) on 0–19 cm soil samples (Ds_{19}) and (b) on 0–9 cm soil samples (Ds_9) taken in May and July 2009. Regarding one months, stand means followed by the same letter indicated no significant differences (ANOVA, Tukey HSD-test at $P \leq 0.05$). Site abbreviations: Ha = Haard, N = Neuhaeusel, B = Biebergemuend, H = Huenfeld, L = Luess, and S = Solling.
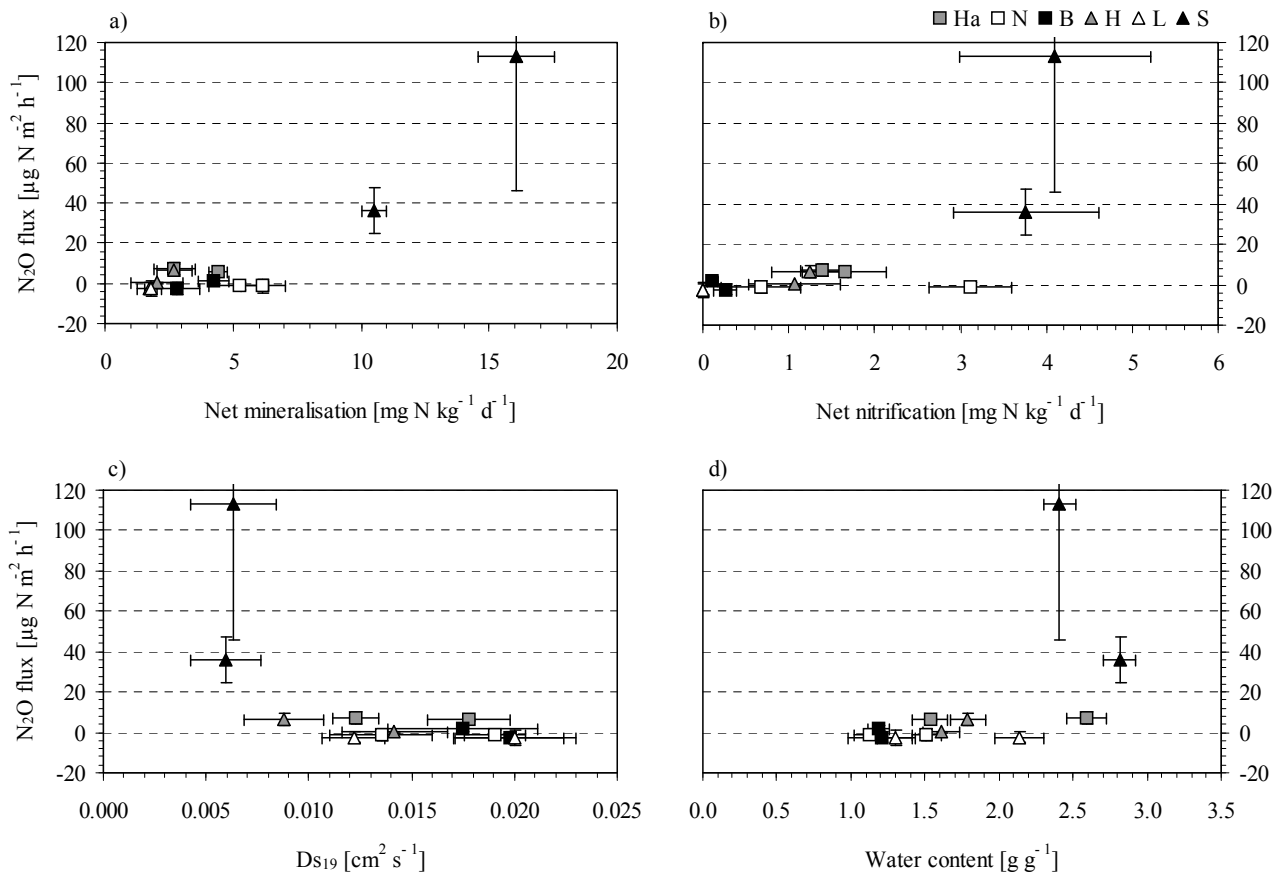
Figure 5-5: Fluxes of N$_2$O of the six beech stands plotted against (a) net N mineralisation rates of the organic layer, (b) net nitrification rates of the organic layer, (c) apparent diffusion coefficients of N$_2$ of the 0–19 cm soil samples (Ds_{19}), and (d) gravimetric water contents of the organic layer. The mean values (n = 5) from May and July 2009 are shown. Site abbreviations: Ha = Haard, N = Neuhaeusel, B = Biebergemuend, H = Huenfeld, L = Luess, and S = Solling.

5.3.5. Regulation of NO$_x$ fluxes

No significant simple and multiple regressions were observed among the NO$_x$ fluxes and the control parameters. However, fluxes of NO$_x$ could be explained best by NO$_x$ concentrations in the forest air (Fig. 5-6). Uptake of NO$_x$ into the soil increased with increasing atmospheric NO$_x$ concentrations. In Fig. 5-6, two linear slopes could be distinguished, first a linear slope that contained the data of Haard at both measuring dates and of Neuhaeusel in May and second, a linear slope that contained the remaining data. The minimal adequate regression model of all data (P = 0.11, r^2 = 0.52, df = 8) contained the NO$_x$ concentration in the forest air, which was the only significant control parameter (P = 0.04), the net nitrification rate of the organic layer (P = 0.09), and Ds_9 (P = 0.14).
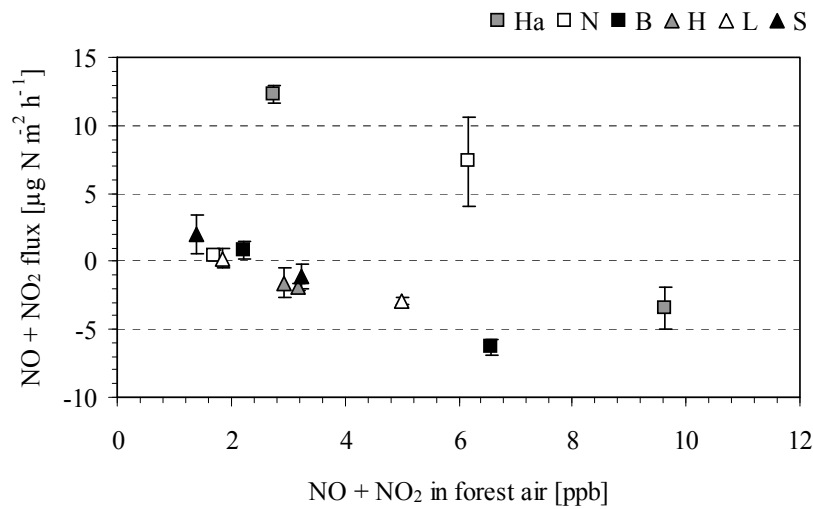
Figure 5-6: Mean (± SE, *n* = 5) NO + NO$_2$ fluxes plotted against NO + NO$_2$ concentrations in the forest air of the six beech stands. Mean fluxes were derived from measurements in May and July 2009. Site abbreviations: Ha = Haard, N = Neuhaeusel, B = Biebergemuend, H = Huenfeld, L = Luess, and S = Solling.

5.3.6. Regulation of the N$_2$O+NO$_x$ fluxes and the N$_2$O/NO$_x$ ratio

The total amount of N$_2$O and NO$_x$ released significantly and exponentially increased with increasing net nitrification rates (P = 0.002, r^2 = 0.64, df = 10) and net N mineralisation rates (P = 0.004, r^2 = 0.58, df = 10) of the organic layer. Due to occurrences of negative fluxes, calculation of the N$_2$O/NO$_x$ ratio was not applicable for most sites.

5.3.7. Dependence of Ds

We detected a linear positive relationship between Ds_{19} and sand content (P = 0.03, r^2 = 0.73) and a linear negative relationship between Ds_{19} and silt content (P = 0.03, r^2 = 0.72) and between Ds_{19} and clay content (P = 0.04, r^2 = 0.69). Soil gas diffusivity in the 0–19 cm columns also displayed a significant negative and linear relationship with the water content of the organic layer (P < 0.001, r^2 = 0.73).

The relative diffusion coefficient of N$_2$ further depended on air-filled porosity (Fig. 5-7). The exponential model, power function model, and PMQ model were similarly good in predicting Ds_9/Da (Tab. 5-5) and Ds_{19}/Da. For model fitting, we grouped the sites according to soil texture. The fitted parameters of the models are presented in Tab. 5-6. As an illustration, we fitted the power function model to the data of the 0–9 cm columns (Fig. 5-7).

Table 5-5: Goodness of fit according to statistical indicators when predicting the relative N_2 diffusivity Ds_9/Da of the 0–9 cm columns as a function of air-filled porosity ε using different models. Given are the sum of squared errors (SSQ), the root mean square error (RMSE), the bias, and Akaike's Information Criterion (AIC). Model fitting was conducted separately for Luess ($n = 10$) and the other sites (Neuhaeusel, Biebergemuend, Huenfeld, Solling; $n = 36$) due to the high sand content of approx. 80% atLuess. Haard was omitted since most 0–9 cm columns only consisted of organic material. The tortuosity parameter m was fitted in the modified Penman-Millington-Quirk (PMQ_{mod}) model whereas $m = 3$ in the original PMQ model.

	Model	SSQ	RMSE	Bias	AIC
Other sites					
	$Ds/Da = B \cdot \mathrm{e}^{(k \cdot \varepsilon)}$	0.0198	0.0235	−0.0001	−163.9
	$Ds/Da = B \cdot \varepsilon^{k}$	0.0197	0.0234	−0.0005	−164.2
	PMQ	0.1131	0.0561	0.0492	−105.3
	PMQ_{mod}	0.0198	0.0235	−0.0009	−166.0
Luess					
	$Ds/Da = B \cdot \mathrm{e}^{(k \cdot \varepsilon)}$	0.0045	0.0211	0.0007	−44.5
	$Ds/Da = B \cdot \varepsilon^{k}$	0.0038	0.0195	0.0006	−46.1
	PMQ	0.0054	0.0232	−0.0001	−46.9

Table 5-6: Values of fitted parameters for the exponential, power function, and PMQ_{mod} models, which predict the relative N_2 gas diffusivity Ds/Da of 0–9 cm soil columns and of 0–19 cm soil columns. Model fitting was conducted separately for Luess due to the high sand content of approx. 80%. Haard was omitted due to the thick organic layer. For parameter fitting, sites were further separated according to soil texture for the 0–19 cm columns (see Tab. 5-2).

	$Ds/Da = B \cdot \mathrm{e}^{(k \cdot \varepsilon)}$		$Ds/Da = B \cdot \varepsilon^{k}$		PMQ_{mod}
	B	k	B	k	m
0–9 cm column					
N,B,H,S-sites	0.002	7.4	1.22	3.9	−0.4
L-site	0.013	4.9	0.73	2.2	3.0
0–19 cm column					
N,S-sites	0.005	6.5	0.68	2.4	2.1
B,H-sites	0.011	4.6	0.39	1.8	0.2
L-site	0.036	2.5	0.22	0.9	3.3

Note: N = Neuhaeusel, B = Biebergemuend, H = Huenfeld, S = Solling, L = Luess.
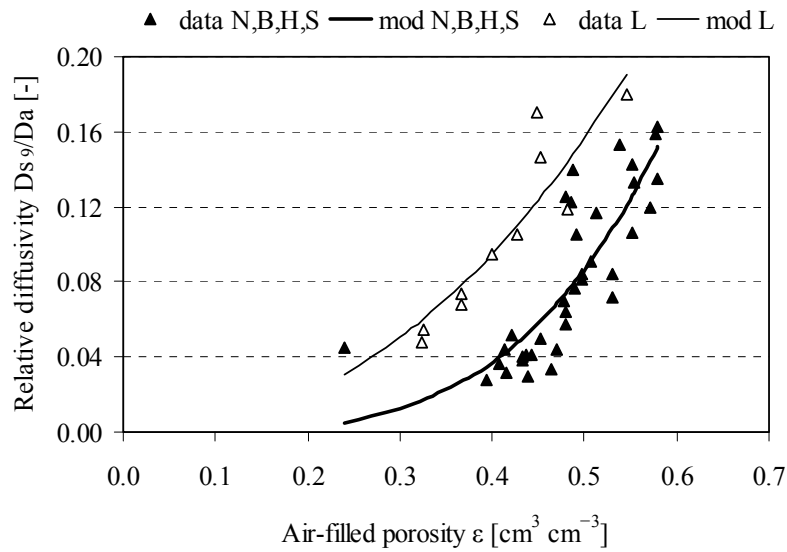
Figure 5-7: Dependence of the relative diffusivity of N$_2$ (*Ds/Da*) of the 0–9 cm soil columns on air-filled porosity. Data of Luess were separately indicated and modelled due to the high sand content of approx. 80%. A power function model (Eq. 5.7) was exemplarily fitted to the data. The corresponding fitting parameters are presented in Tab. 5-6. Site abbreviations: N = Neuhaeusel, B = Biebergemuend, H = Huenfeld, L = Luess, and S = Solling.

5.4. Discussion

5.4.1. Soil gas diffusivity

As was indicated by our results, soil texture is of importance for gas diffusivity, which is due to differences in pore size distribution. Total pore volume of fine-textured soils is higher but most pores are smaller than those in coarse-textured soils and molecular diffusion is slower through smaller pores (Currie, 1961). This phenomenon is shown in Fig. 5-7, where the sandy soil of Luess exhibit higher relative diffusivity compared to the more fine-textured soils at the same air-filled porosity. In fine-textured soils anoxic micro-sites are created at lower soil water contents than in coarse-textured soils (Bollmann and Conrad, 1998).

The relationship between the relative diffusivity of N$_2$ (*Ds/Da*) and air-filled porosity (ε) could be described by a power function. We found no evidence for dual-porosity (Fluehler, 1972; Kristensen et al., 2010). We attributed the observation to our high ε values. We suggest that our models are only valid for these ranges of ε: coarse-textured soils 0.3–0.6 cm^3 cm^{-3} and fine-textured soil 0.4–0.6 cm^3 cm^{-3} (the value at 0.25 cm^3 cm^{-3} possibly represents an outlier). We further expect a linear increase in *Ds/Da* with ε at low air-filled porosities for fine-textured soils (Kristensen et al., 2010). High air-filled porosity and relative diffusivity as

found in our beech stands can be ascribed to continuous macro-pores possibly resulting from roots.

The fitting parameters estimated for the exponential model fit well to the parameters proposed by Richter and Grossgebauer (1978). The authors used one model ($B = 0.0085$, $k = 6.8$) to describe the $Ds/Da - \varepsilon$ relationship for several soil textures (sand, silt, loam), thus the fitted values are ranged between our values (0–9 cm columns) for the sandy soil and fine-textured soils. The fitting parameters estimated for the power function model were also similar to values mentioned in Richter and Grossgebauer (1978). Moldrup et al. (1997) suggested a tortuosity parameter of $m = 3$ for undisturbed soils. This value also gave best model fits for the sandy Luess site. However, for the fine-textured soils the models performed better with lower values for m indicating even higher tortuosity of soils.

5.4.2. Regulation of N₂O fluxes

The Solling site displayed exceptionally high N_2O emissions compared to the other five beech stands and together with the lowest gas diffusivity we observed the highest net N mineralisation and nitrification rates at this site. However, mass of the moder surface layer and bulk density of the mineral soil (silty loam) were comparably low at Solling and were probably not solely responsible for low gas diffusivity. Soil texture and high clay contents were similar at Solling and Neuhaeusel, but gas diffusivity was high at Neuhaeusel compared to Solling. However, Neuhaeusel differed in humus type (F-mull) and in particular in annual precipitation and soil water contents, which were highest at Solling. Low diffusivity has already been reported for Solling by Ball et al. (1997). The determined relative diffusion coefficients of the beech stand at Solling were in good accordance with our results. Ball et al. (1997) showed that the diffusivity of the organic layer was similarly low than of the upper mineral soil at Solling whereas higher diffusivities of the organic layer were measured at a spruce stand and at a limed beech stand at Solling. Liming reduced N_2O emissions by 73% at Solling (Ball et al., 1997; Brumme and Beese, 1992). The observation was attributed to an alteration of the structure of the organic layer by earthworms (Borken and Brumme, 1997). In addition, a better nutrient status at the Solling compared to the other acid beech stands indicated by the highest cation exchange capacity, which resulted from high clay and soil organic matter contents, together with the second highest measured N deposition may have caused higher N turnover rates. High nitrification rates have previously been reported for this stand by Meiwes et al. (1998; see chapter 4 as well). Relationships between N_2O fluxes and

nitrification or mineralisation rates have further been found by Ambus et al. (2006), Gasche et al. (2002), Papen and Butterbach-Bahl (1999), and by Rosenkranz et al. (2006).

Nitrous oxide emissions did not follow a gradual change with any of the measured regulators. Instead, N_2O emissions abruptly increased at high mineralisation and nitrification rates and at low soil gas diffusivity (and high water content, respectively). No significant relationships were observed when omitting the Solling site. This pattern suggests that not only one but two or even more parameters have to be in a critical range for high emissions of N_2O.

Solling has been previously described as seasonal N_2O emission type with high N_2O emissions during the summer months, which derived from denitrification (Brumme and Borken, 2009; Eickenscheidt et al., 2011; Wolf and Brumme, 2002; see chapter 2). For a 10-year measuring period, mean annual N_2O emissions amounted to 1.9 kg N ha^{-1} yr^{-1} (Brumme and Borken, 2009). Low gas diffusivity probably results in anaerobic soil conditions in times of high soil respiration, which explains the observation of seasonal emission patterns. The significant increase in N_2O emissions at Solling at 27.5°C in the laboratory supports this hypothesis, since higher soil temperature increased soil respiration. Brumme (1995) found a correlation between N_2O emissions and soil respiration at Solling. According to the theory of anaerobic zone development, the increase in anaerobic soil volume fraction due to respiratory O_2 consumption causes an exponential increase in N_2O emissions (Smith et al. 2003).

The other five beech stands probably belonged to the background N_2O emission type. Likewise our observations, Brumme et al. (1999) found no correlations of N_2O fluxes with control parameters in forests with BEP. Fluxes of N_2O were similar to fluxes reported by Brumme and Borken (2009) for beech forests with mull type humus, by Butterbach-Bahl et al. (2002) for Scots pine forests, and by Papen and Butterbach-Bahl et al. (1999) for a spruce forest. These authors also observed uptake of atmospheric N_2O into the soil. Brumme and Borken (2009) hypothesised that background emissions are probably associated with heterotrophic nitrification. However, the increase in N_2O emissions during the laboratory study revealed a potential for higher N_2O emissions at least at Neuhaeusel. As mentioned before, this site only differed in precipitation and humus type and thus in gas diffusivity, but was similar to Solling in soil texture and net nitrification rates of the organic layer. Hence, high O_2 consumption resulting from high soil respiration rates at high soil temperatures was probably necessary to obtain anaerobicity and denitrification at Neuhaeusel (Tab. 5-3).

Fine-textured soils generally exhibit higher N_2O emissions than coarse-textured soils (e.g. Bollmann and Conrad, 1998; Skiba and Smith, 2000). Since molecular diffusion is slower in small pores, anaerobicity occurs more frequently and also consumption of NO via

denitrification due to longer residence times. Luess was characterised by properties which were unfavourable for denitrification such as a coarse soil texture (silty sand) and absent net nitrification due probably to comparably moderate N deposition rates and low biological activity in this poor sandy soil. Thus, N_2O fluxes were negligible during the field measurements and at high temperatures during laboratory measurements.

In addition, the results from Neuhaeusel indicated that texture is possibly more important for N_2O release from beech stands with mull type humus than humus type. High N_2O emissions of approx. 4 kg N ha^{-1} yr^{-1} have also been reported from an acid beech forest soil in Austria, which had a high nitrification activity, high precipitation, and a loamy silt texture but a mull type humus (Zechmeister-Boltenstern et al., 2002). Hence, soil conditions at the depth of N_2O production are most important and Brumme et al. (1999) reported that the contribution of the organic layer to N_2O emissions in deciduous forests was low at mull type humus sites (2.2–7.4%) compared to 53% at Solling with moder type humus.

We conclude that moder type humus of acid beech soils is not necessarily attended by SEP and that the thickness of the organic layer of a deciduous forest solely does not represent an indicator for the magnitude of N_2O emissions (Brumme and Borken, 2009; Schulte-Bisping et al., 2003). But high seasonal N_2O emissions from acid forest soils result from the combination of high N turnover rates and soil anaerobicity. We suggest that soil anaerobicity in the beech forests is most probably due to a tightly-packed laminar leaf litter layer of the moder type humus and loamy soil texture in combination with high summer precipitation during times of high soil respiration. Hence, measurement of both, N turnover rates and indicators for soil anaerobicity, are necessary for explanation of N_2O fluxes. The utilisation of the gravimetrical water content of the organic layer instead of the laboriously determined diffusion coefficient is sufficient for explaining N_2O fluxes in regression analyses indicating that the knowledge about the pore structure does not provide additional information.

5.4.3. Regulation of NO + NO$_2$ fluxes

Concentrations of NO_x in the ambient air have also been described as one of the main drivers for NO_x exchange between the atmosphere and soil by Gasche and Papen (1999) and by Ludwig et al. (2001; see chapter 3 as well). The linear slope that contained the Haard data obviously exhibited a higher compensation concentration of approx. 9 ppbv NO_x than the other slope of approx. 2 ppbv NO_x. Haard showed the highest N depositions of the investigated sites. Compensation concentrations of several hundred ppbv NO have been reported from fertilised plots (Ludwig et al., 2001). Gasche and Papen (1999) observed a high

NO compensation concentration of > 75 ppbv from a southern German spruce forest, which had been exposed to chronic high N depositions and exhibited high nitrification rates.

The observed uptake of NO_x could be ascribed to abiotic NO_2 uptake, oxidative consumption of NO via nitrification, reductive consumption via denitrification, or consumption of NO by aerobic heterotrophic bacteria, respectively (Gasche and Papen, 1999; Gödde and Conrad, 2000; Pilegaard et al., 2006). The uptake of NO_x at Haard in July despite high gas diffusivity could possibly be attributed to a heavy rainfall event during the measurement.

Other than N_2O fluxes, NO_x fluxes tend to increased with increasing diffusion coefficients (Ds_9), which suggested that NO probably derived from nitrification. A positive correlation between NO emissions and nitrification rates was reported by e.g. Gasche and Papen (1999) and Gasche et al. (2002). Nitrification rates were similar for the Neuhaeusel and Solling sites in May but Neuhaeusel had higher NO_x emissions most likely due to higher gas diffusivity compared to Solling where N_2O dominated.

Principally, fluxes of NO_x were low and comparable to fluxes measured in beech stands by Beier et al. (2001) and by Gasche and Papen (1999). The annual NO_x emissions amounted to 0.11 ± 0.05 kg N ha^{-1} yr^{-1} at Solling in 2007–08 (chapter 6). However, most studies reported NO fluxes. When regarding net NO_x fluxes, high NO production rates may be balanced by high NO_2 uptake rates (Gasche and Papen, 1999; Rosenkranz et al. 2006). Additionally, determination of net NO_x fluxes may be the reason for non-significant relationships with regulating factors.

5.5. Conclusions

We demonstrated that the "hole in the pipe" model, which was developed for the tropics, is also valid for explaining regulation of N oxide fluxes in European beech forest soils receiving chronic high N depositions. The sum of N_2O and NO_x fluxes depends on net N mineralisation and nitrification rates whereas the ratio of released gases depends on soil anaerobicity. The present study revealed an exponential relationship between N oxides and N turnover rates and between N oxides and soil gas diffusivity (water content), respectively. Furthermore, our study gave evidence that high N turnover rates in combination with soil anaerobicity result in high seasonal N_2O emissions from acid forest soils. However, moder type humus of acid beech soils is not necessarily attended by SEP. We believe that seasonal emission patterns are less common in German beech forests than previously assumed. However, to confirm our conclusion investigations at further forests exhibiting SEP are necessary.

Acknowledgments

Acknowledgments

Impact of Forest Type on Short-term and Long-term Regulation of N_2O and NO_x Fluxes from Temperate Forest Soils

6.   Impact of forest type on short-term and long-term regulation of N_2O and NO_x fluxes from temperate forest soils

Abstract

Temperate forest soils can act as a considerable source for nitrous oxide (N_2O) and nitric oxide (NO). However, the process understanding of frequently observed forest type effects on N oxide emissions is still insufficient. The objective of our study was (i) the comparison of N_2O and NO_x (NO + NO_2) fluxes of an acid temperate coniferous and deciduous forest soil exposed to chronic high N depositions and (ii) the determination of controlling factors, which regulate fluxes in the short and long term. We used closed and open dynamic chamber techniques to measured N oxide flux rates in a Norway spruce stand (*Picea abies* (L.) Karst.) and in an adjacent beech stand (*Fagus sylvatica*) for one year. Nitrous oxide fluxes were lower in the spruce stand (0.30 ± 0.06 kg N ha^{-1} yr^{-1}; background emission pattern) than in the beech stand (2.60 ± 0.59 kg N ha^{-1} yr^{-1}; seasonal emission pattern). The structure and quality of the litter probably determined N_2O fluxes in the long term by influencing soil gas diffusivity and soil N turnover rates. In the short term, N_2O emissions were regulated by soil nitrate content, temperature, and water content. Fluxes of NO_x were similar in both stands with 0.04 ± 0.08 kg N ha^{-1} yr^{-1} in the spruce stand and 0.11 ± 0.05 kg N ha^{-1} yr^{-1} in the beech stand. High water contents and NO_x consumption probably reduced NO_x emissions at our stands. Fluxes of NO_x could be best explained by the nitrate and water contents. We demonstrated for the first time that NO_2 emissions considerably contributed to total NO_x emissions with up to 100%. In conclusion, in the investigated stands forest type played a key role for N_2O emissions but presumably a minor role for NO_x emissions, which probably mainly resulted from chemodenitrification.

Keywords: Spruce forest, beech forest, nitrous oxide, nitric oxide, nitrogen dioxide, seasonal emission patterns, flux regulation

6.1. Introduction

European temperate forest soils may act as a considerable source for the trace gases nitrous oxide (N_2O) and nitric oxide (NO) (e.g. Brumme and Beese, 1992; Papen and Butterbach-Bahl, 1999; Skiba et al., 1999; van Dijk and Duyzer, 1999). Nitrous oxide is a potent greenhouse gas and contributes to the catalytic decomposition of stratospheric ozone (Crutzen, 1979; IPCC 2007). Nitric oxide and nitrogen dioxide (NO_2) are indirectly involved in global warming through the production of radiative tropospheric ozone and contribute to photochemical air pollution (Crutzen, 1979; Logan, 1983).

Temperate forest soils are estimated to emit 0.1–2.0 Tg N_2O-N yr^{-1} (Brumme et al., 2005; IPCC, 2001; Kroeze et al., 1999) and 0.2–0.4 Tg NO_x-N yr^{-1} (Davidson and Kingerlee, 1997; Gasche and Papen, 1999). The great uncertainties in regional and global N oxide budgets arises from the high spatial and temporal variability of N oxide fluxes (e.g. Brumme and

Borken, 2009; Groffman et al., 2000; Rosenkranz et al., 2006) and from insufficient process understanding of the importance of forest type (Ambus et al., 2006).

Aerobic nitrification and anaerobic denitrification are mainly responsible for N_2O and NO production in soils (Davidson et al., 2000). These biological processes are controlled by several abiotic factors such as temperature, soil moisture, and N availability (e.g. Davidson et al., 2000; Skiba and Smith, 2000). The conceptual "hole-in-the-pipe" (HIP) model (Firestone and Davidson, 1989) postulates that the rates of nitrification and denitrification (total amount of N moving through the pipe) determine total N trace gas emissions whereas environmental factors such as the water filled pore space (WFPS) control the ratio of released gases (NO, N_2O, N_2) (size of the hole in the pipe; Davidson et al., 2000). In acid soils, NO_x ($NO + NO_2$) can further be produced by chemodenitrification. During chemodenitrification, biologically produced nitrite is protonated to nitrous acid, which can spontaneously decompose or react with organic (e.g. amines, phenols) or inorganic (e.g. Fe^{2+}) compounds to NO and NO_2 (van Cleemput and Baert, 1984; van Cleemput and Samater, 1996).

Butterbach-Bahl et al. (1997) showed for the first time that the influence of tree species on soil properties control the ratio of NO to N_2O emitted. Subsequent studies further demonstrated that deciduous forests generally exhibit higher N_2O emissions compared to coniferous forests, whereas latter apparently exhibit higher NO emissions (Denier van der Gon and Bleeker, 2005; Papen and Butterbach-Bahl et al., 1999; Pilegaard, 2006; van Dijk and Duyzer, 1999). Brumme et al. (1999) classified forest types into three different temporal emission patterns of N_2O: the seasonal emission pattern (SEP), the background emission pattern (BEP), and the event emission pattern (EEP). The authors proposed that the state variables (e.g. amount and structure of the organic layer) determine the differences between SEP and BEP (long-term control) by reducing soil gas diffusivity. Thus, in addition to short-term control by e.g. weather conditions, long-term control seems to determine the 'size of the hole in the pipe' of SEP.

Markedly higher N_2O emissions from the beech stand than from the spruce stand were also reported from the intensely studied Solling forests in Germany (e.g. Borken et al., 2002; Brumme and Borken, 2009). However, NO fluxes have not yet been measured.

Thus, the objective of our study was (i) to quantify annual N_2O and NO_x fluxes from the acid temperate forest soils at Solling, and (ii) to determine the factors, which control N oxide fluxes in the short and in the long term.

6.2. Materials and methods

6.2.1. Study area

Our experiments took place in a 74-yr-old Norway spruce stand (*Picea abies* (L.) Karst.) and in an adjacent 160-yr-old beech stand (*Fagus sylvatica*) at the Solling plateau in Central Germany (51° 46' N, 9° 34' E, 500 m a.s.l.). The mean annual temperature was 6.9°C and the annual precipitation was 1193 mm. Triassic sandstone bedrock covered by a loess solifluction layer represented the parent material for soil development. The soil type was a Dystric Cambisol (FAO) and the humus type a typical moder. The sites were characterised by high soil acidity, low base saturation of < 10% down to 100 cm, and low bioturbation. Soil characteristics for both stands are shown in Tab. 6-1. In 2007 and 2008, stand N depositions was 33 kg ha^{-1} yr^{-1} in the spruce stand and 20 kg ha^{-1} yr^{-1} in the beech stand. A detailed site description is given in Bredemeier et al. (1995, 1998) and in Brumme and Khanna (2009a).

Table 6-1: Soil characteristics of the spruce and beech stands at the Solling, Germany.

Parameter	Soil depth	n	Spruce	n	Beech
pH (H$_2$O)					
	Organic layer	3	3.6 (0.1)[a]	3	4.0 (0.0)[b]
	0–5 cm	6	3.3 (0.0)	6	3.4 (0.0)
	5–10 cm	5	3.5 (0.0)	6	3.6 (0.0)
Organic carbon [mg C g^{-1}]					
	Organic layer	13	447 (13)	13	443 (12)
	0–5 cm	14	112 (7)	14	98 (11)
	5–10 cm	13	39 (2)[a]	13	32 (2)[b]
Total nitrogen [mg N g^{-1}]					
	Organic layer	13	16.5 (0.4)[a]	13	18.6 (0.7)[b]
	0–5 cm	14	4.6 (0.3)	14	4.4 (0.3)
	5–10 cm	13	1.7 (0.1)	13	1.7 (0.1)
C/N ratio					
	Organic layer	13	27.2 (0.6)[a]	13	24.1 (0.7)[b]
	0–5 cm	14	24.3 (0.8)[a]	14	21.7 (1.4)[b]
	5–10 cm	13	22.9 (0.8)[a]	13	19.8 (1.1)[b]
Bulk density [g cm^{-3}]					
	0–5 cm	4	0.76 (0.03)	6	0.89 (0.02)
	5–10 cm	5	1.00 (0.02)	6	1.08 (0.04)
Humus mass [Mg ha^{-1}]					
	Organic layer	6	67.1 (4.4)[a]	6	35.6 (1.9)[b]
Sand [%]					
	0–10 cm	8	22.8 (1.1)	5	22.2 (0.6)
Silt [%]					
	0–10 cm	8	62.2 (0.9)[a]	5	53.0 (0.5)[b]
Clay [%]					
	0–10 cm	8	15.0 (0.3)[a]	5	24.8 (0.4)[b]

Note: Means (± SE) with different letters indicated differences between the spruce and beech stands (independent t-test and Mann-Whitney U-test at $P \leq 0.05$).

6.2.2. Experimental design

At each stand, 17 permanently inserted chamber bases (area: 0.2665 m^2) were randomly installed in May 2007. We used 13 bases for N_2O and NO_x flux measurements and we took soil samples in the remaining four bases. Soil samples (organic layer, 0–5 cm, and 5–10 cm mineral soil) were used to determine soil moisture and extractable mineral N (N_{min} = NH_4^+-N + NO_3^--N) contents. We recorded soil temperature in 2.5, 5, and 10 cm depths. Measurements were conducted every second week in the summer months and monthly in the winter months from May 2007 to June 2008.

6.2.3. N_2O and NO_x flux measurements

We used the closed chamber method for N_2O measurements. The static, vented chambers had a volume of 27.5–34.7 L (spruce) and of 18.9–29.3 L (beech). We removed gas samples of 100 mL with a portable gas sampler equipped with a pressure sensor (Loftfield et al., 1997) at three to four regular time points during chamber closure, which lasted 42 minutes at most. Analysis was conducted with a gas chromatograph equipped with an EC detector (GC 14A, Shimadzu, Duisburg, Germany).

We used the open dynamic chamber method for NO_x flux measurements, which we carried out on-site using a mobile NO_x analyser. A chamber, which was connected to the analyser by a Teflon tube, was placed on the chamber bases for five minutes. The mean air flow through the chamber was 1 L min^{-1}. Nitric oxide was oxidised to NO_2 by a solid CrO_3 catalyst before analysis with a Scintrex LMA-3 chemiluminescence detector (Scintrex Unisearch, Ontario, Canada). Concentrations of NO_x were logged every five seconds using a CR510 data logger (Campbell Scientific, Utah, USA). Calibration was carried out before and after chamber measurements using a NO standard gas (3000 ppbv NO in N_2; Air Liquide GmbH, Germany).

The linear change of gas concentration in the chamber versus time was calculated and multiplied by the ratio of chamber volume to soil surface area and by air density, which was adjusted for air temperature and atmospheric pressure. We estimated annual fluxes using the trapezoid rule.

6.2.4. Chemical analyses

The water content was determined gravimetrically. Soil extracts for N_{min} analyses were prepared by adding 50 mL 0.5 M K_2SO_4 solution to 15 g of the organic layer and 100 mL K_2SO_4 solution to 25 to 30 g fresh soil. The sample-solution was shaken for one hour before we filtered the excess through pre-washed filter papers. The extracts were immediately

frozen. Continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, The Netherlands) was used for N_{min} analyses. We determined bulk density on undisturbed soil samples, which were oven-dried at 105°C and sieved to 2 mm. We used a metal ring (594 cm^2) to quantify the mass of the organic layer. Living roots were removed and the organic layer samples were oven-dried at 60°C. We measured total carbon and nitrogen contents with a CNS Elemental Analyzer (Heraeus Elementar Vario EL, Hanau, Germany). For pH value determination in distilled water (1:2.5 (v/v)), we used 10 mL sieved soil or shredded organic material. For analysis of the particle size distribution, we performed wet sieving of the sand and coarse silt fraction ($\geq$ 20 µm) following destruction of organic matter (30% H_2O_2) and Fe oxides (4% Na-dithionite-citrate). Subsequently, sedimentary fractionation according to the Atterberg method (25°C, 21 hours, 30 cm fall height) was conducted (Schlichting et al., 1995).

6.2.5. Statistical analyses

Two independent sample means were tested for significant differences using the independent Student's t-test, the Welch test, or the non parametric Mann-Whitney U-test. We used analysis of variance (ANOVA) for comparisons of more than two sample means. Prior to analysis the assumptions of normality of residuals (Shapiro-Wilks or Cramer-von Mises test) and homogeneity of variances (Levene test) were checked. We transformed data where necessary and possible. Linear mixed effects models (Crawley, 2007) were applied for time series data (N_2O, NO_x, N_{min}, H_2O) because of temporal pseudoreplication. The basic model included the forest type as fixed effect and the spatial replication (individual chamber) nested in time as random effect. If an extension increased the goodness of the fit, which was assessed using the Akaike Information Criterion (AIC), the model was extended by a variance function and by a first-order temporal autoregressive function. We conducted ordinary simple and multiple regression analyses. Generalized least squares extended by an autocorrelation function were used for simple regression analyses where residuals were autocorrelated. Autocorrelation was tested using the Durbin-Watson test and the plot of the autocorrelation function. Multicollinearity in explanatory variables of the multiple regression models was tested using the variance inflation factor. The minimal adequate model was received by using the AIC. We considered effects as significant if $P \leq 0.05$. Results are reported as means $\pm$ 1 standard errors. For statistical analyses, we used R 2.10.1 (R Development Core Team, 2009).

6.3. Results

6.3.1. Annual N_2O and NO_x fluxes

In the spruce stand, N_2O fluxes were significantly lower with cumulative annual emissions of 0.30 ± 0.06 kg N ha^{-1} yr^{-1} than in the beech stand with 2.60 ± 0.59 kg N ha^{-1} yr^{-1} ($P < 0.0001$; Fig. 6-1). We observed a pronounced seasonality in the beech stand with high emissions during the summer months and low emissions during the winter months. Fluxes of NO_x were not significantly different between both stands (Fig. 6-1) and were 0.04 ± 0.08 kg N ha^{-1} yr^{-1} in the spruce stand and 0.11 ± 0.05 kg N ha^{-1} yr^{-1} in the beech stand. During the summer months, NO_x fluxes were higher than during the winter months where mainly an uptake of NO_x into the soil occurred. In both stands, N_2O emissions were significantly higher than NO_x emissions ($P = 0.018$ (spruce) and $P = 0.007$ (beech)).
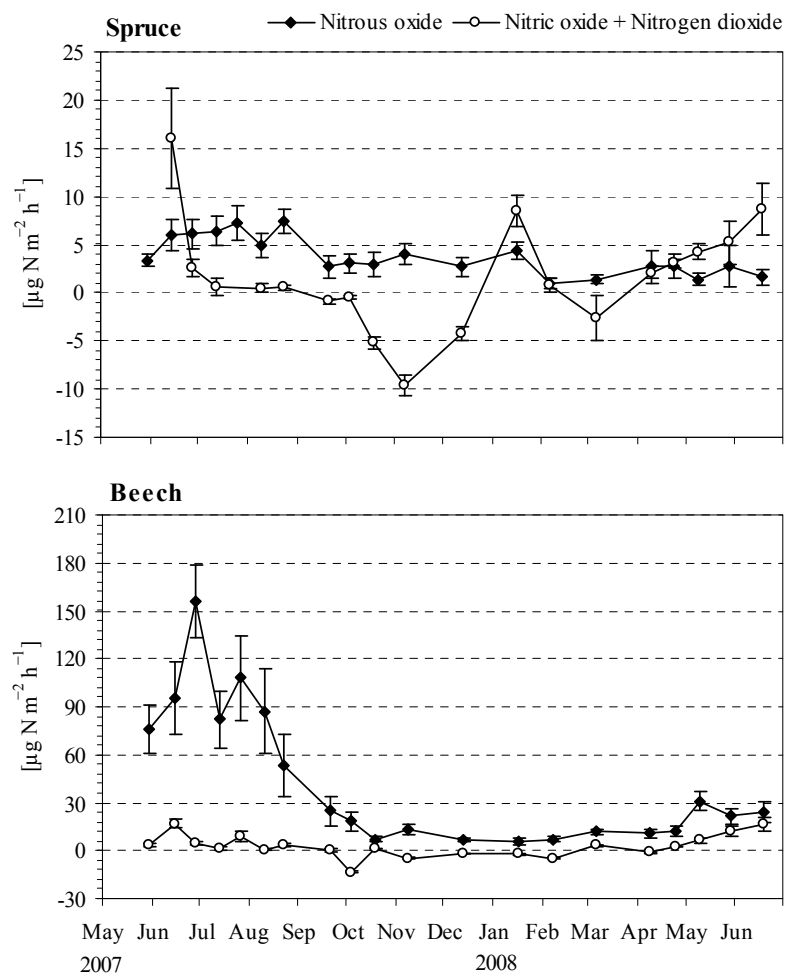
Figure 6-1: Mean ($\pm$ SE, n = 13) N_2O and NO + NO_2 fluxes of the spruce (top) and beech (bottom) stands from May 2007 to June 2008. Please note the different scales.

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The NO converter of the NO_x analyser was removed for some measurements. The measurements showed that not only NO was emitted from the soils but also NO_2 (Fig. 6-2). For the presented example, emissions of NO_2 accounted for approx. 88% of total NO + NO_2 emissions (Fig. 6-2). On 9 May 2008, total NO + NO_2 emissions of one chamber were 12.9 µg N m^{-2} h^{-1} and NO_2 emissions, which were measured 1.5 hours later, were 13.5 µg N m^{-2} h^{-1}. Thus, on that day approx. 100% of the total emissions were NO_2 emissions (data not shown).
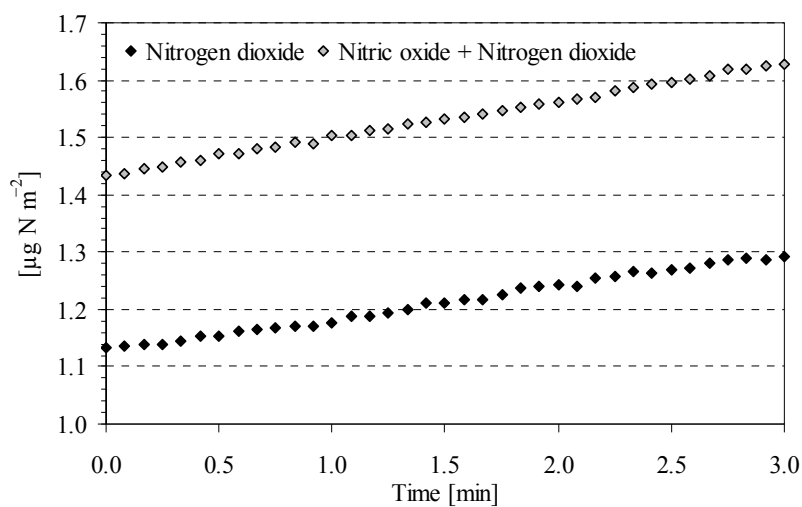
Figure 6-2: Example of the kinetic of NO_2 increase in the chamber's atmosphere during chamber closure compared to the kinetic of NO + NO_2 increase. The data were derived from the beech stand on 24 April, 2008. The observed increases corresponded to a NO_2 emission of 3.4 µg N m^{-2} h^{-1} and to a NO + NO_2 emission of 3.9 µg N m^{-2} h^{-1}. Please note that the _y_-axis does not start at zero.

6.3.2. Regulation of N_2O and NO_x fluxes

Ammonium contents in the mineral soil were significantly higher in the spruce stand than in the beech stand ($P = 0.007$ (0–5 cm) and $P = 0.016$ (5–10 cm); Fig. 6-3b). We found no significant differences between stands in NO_3^- contents (Fig. 6-3a). The gravimetrical water content was slightly higher in 5–10 cm mineral soil depth of the spruce stand compared to the beech stand ($P = 0.01$; Fig. 6-3c). The calculated water-filled pore space (WFPS) in 5–10 cm mineral soil depth ranged from 52% to 79% in the spruce stand and from 58% to 78% in the beech stand.
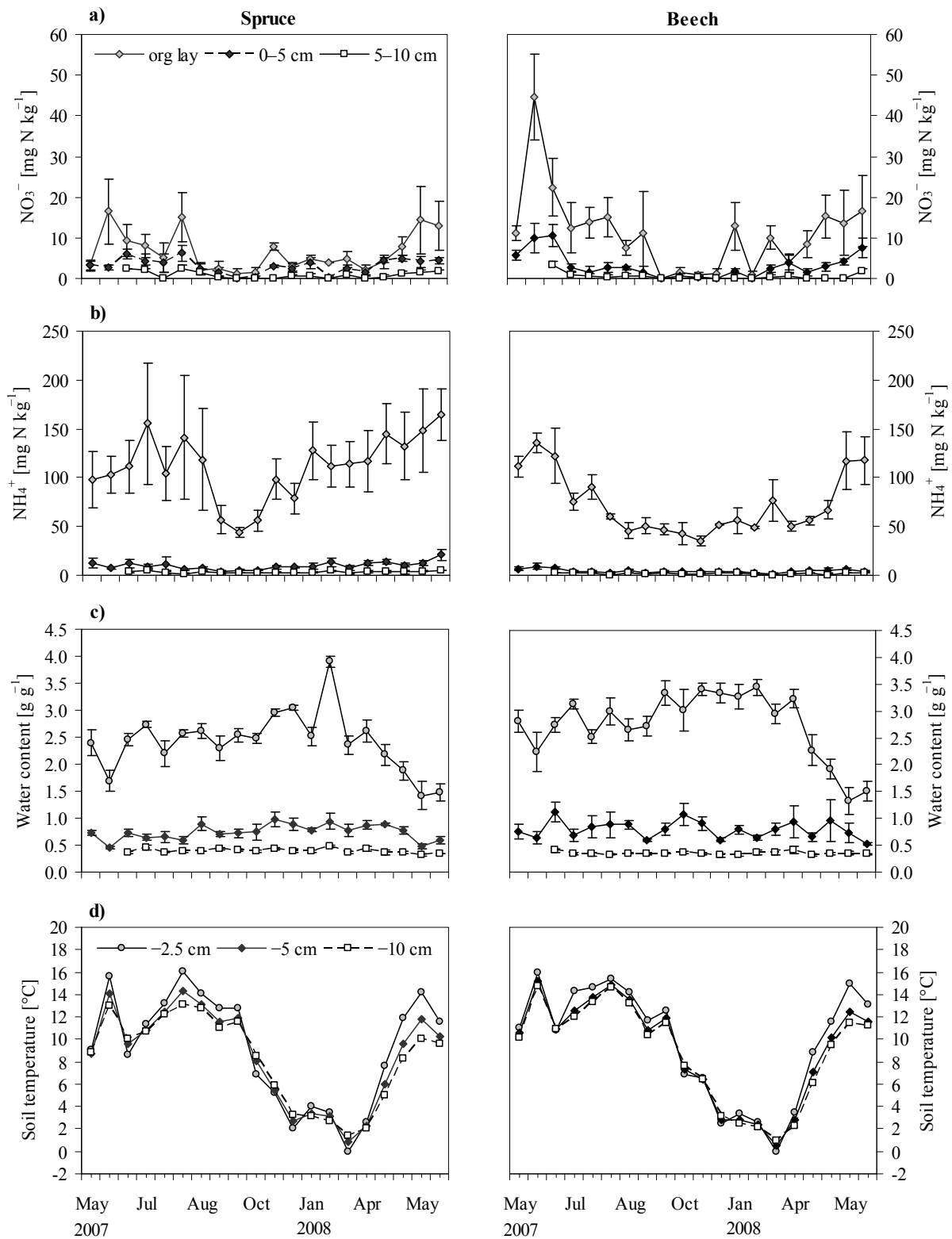
Figure 6-3: Mean (± SE, *n* = 4) NO$_3^-$ (a), NH$_4^+$ (b), and water contents (c; organic layer and 0–5 cm and 5–10 cm mineral soil) and soil temperatures (d) in three soil depths given for the spruce stand (left side) and beech stand (right side) from May 2007 to June 2008.

In the spruce stand, N_2O fluxes were controlled by soil temperature in 5 cm depth (ST_5; $r^2 = 0.30$, $P = 0.01$, $df = 18$) according to the following equation:

$$N_2O\text{-}N = 0.25 \, (\pm 0.09) \cdot ST_5 + 1.59 \, (\pm 0.87) \tag{6.1}$$

In the beech stand, N_2O fluxes exponentially depended on soil temperature in 10 cm depth (ST_{10}) and linearly on the NO_3^- and water contents of 0–5 cm mineral soil depth ($r^2 = 0.70$, $P < 0.001$, $df = 15$) according to the following equation:

$$N_2O\text{-}N = 4.26 \, (\pm 1.25) \cdot \exp(0.20 \, (\pm 0.17) \cdot ST_{10}) + 5.18 \, (\pm 2.23) \cdot NO_3\text{-}N + \\ 76.24 \, (\pm 38.16) \cdot H_2O - 67.64 \, (\pm 32.00) \tag{6.2}$$

Fluxes of NO_x of both stands could be best explained by the NO_3^- and water contents of the organic layer ($r^2 = 0.66$, $P < 0.0001$, $df = 35$; Fig. 6-4).
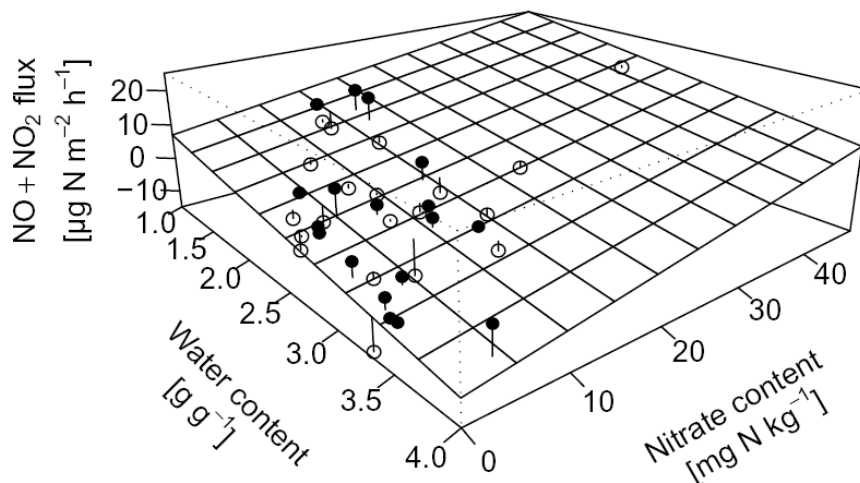
Figure 6-4: Relationship of ($NO + NO_2$)-N fluxes (y) to the water content ($x1$) and NO_3^--N content ($x2$) of the organic layer plotted as surface area. The regression equation was obtained from the data of both stands ($y = -5.18 \, (\pm 1.14) \cdot x1 + 0.38 \, (\pm 0.08) \cdot x2 + 12.20 \, (\pm 3.38)$; $r^2 = 0.66$, $P < 0.0001$, $df = 35$). Measured data points are indicated as closed circles (above the surface) and open circles (below the surface) while solid lines indicate the deviation of measured data from the model surface.

6.4. Discussion

6.4.1. Regulation of N_2O fluxes in the beech stand

Fluxes of N_2O in the beech stand fitted well to the mean flux of 1.92 kg N ha^{-1} yr^{-1} for a 10-year measuring period at this Solling site (Brumme and Borken, 2009) and were in the upper range of N_2O emissions published for other temperate deciduous forests (e.g. Butterbach-Bahl et al., 2001; Zechmeister-Boltenstern, 2002). The beech stand showed a

seasonal emission pattern, which is characterised by high N_2O emissions during the summer months and low emissions during the winter months (Brumme et al., 1999). Denitrification is responsible for the summer emissions observed in this acid beech forest soil (Wolf and Brumme, 2002). The reason for high emissions probably is the increased occurrence of anaerobic micro-sites in the soil due to high oxygen consumption by microorganisms, roots, and mycorrhiza at high summer temperatures but simultaneously hindered oxygen transport into the soil (Brumme et al., 1999; see chapter 5.4.2.). It was shown by Ball et al. (1997) that the laminar structure of the beech litter layer of the moder type humus reduced gas diffusivity at this beech stand (see chapter 5.4.2.).

Nitrate, which is the substrate of denitrification, soil temperature, and water content explained 70% of the variation in N_2O fluxes in the beech stand. Brumme et al. (1999) also reported that seasonal patterns of N_2O emissions in broad-leaved forests are produced by short-term control of these abiotic factors.

Microbial activity exponentially depends on soil temperature since enzymatic processes strongly increase with temperature as long as other factors are not limiting. However, Brumme (1995) reported Q_{10} values of up to 14 for a gap in the beech stand where N_2O fluxes were not restricted by soil moisture. A comparable high temperature response of Q_{10} values up to 19 were observed in our study. These values indicated that microbial activity was not solely responsible for the high N_2O fluxes during the summer months but that at least one additional process regulated the fluxes, which is believed to be gas diffusion due to temperature-induced positive feedbacks.

Soil water content is a further important control parameter because it determines gas and substrate transport and with it the anaerobicity of the soil and also microbial activity. The water contents of the mineral soils at the Solling sites were high and ranged within the reported optimal WFPS of 60–95% for maximal N_2O emissions (Davidson et al., 2000; Schindlbacher et al., 2004; Skiba and Smith, 2000). Soil temperature and soil moisture explained most of the variation in N_2O fluxes in several studies (e.g. Papen and Butterbach-Bahl et al., 1999; Pilegaard et al., 2006; Schindlbacher et al., 2004; Wu et al., 2010). In summer 2008, N_2O emissions were comparably small although soil temperature was high, but low precipitation caused lower water contents, which probably diminished microbial activity. Brumme (1995) demonstrated that high N_2O fluxes only occurred at temperatures $> 10°C$ and water tensions $< 200\,hPa$ at the Solling beech stand. Huge interannual variability in N_2O fluxes have also been reported e.g. by Butterbach-Bahl et al. (2002) and Macdonald et al. (1997).

6.4.2. Regulation of N₂O fluxes in the spruce stand

The spruce stand belonged to the background emission type (Brumme et al., 1999), which showed no pronounced seasonality in N_2O emissions. However, 30% of the variation in N_2O fluxes could be explained by soil temperature. Brumme et al. (1999) even found neither a short-term nor a long-term control of N_2O emissions in forests with BEP. Our fluxes fitted well to emissions of 0.30 and 0.41 kg N_2O-N ha^{-1} yr^{-1}, which were recorded at our investigated spruce stand in 1993–94 and 2000–01 (Borken et al., 2002). Similarly low annual N_2O emissions from coniferous forests have been reported by e.g. Lamers et al. (2007), Macdonald et al. (1997), and Oura et al. (2001). To date, high N_2O emissions as observed in deciduous forests have not been observed in temperate coniferous forests. The reason probably is higher gas diffusivity of the surface organic layer despite similar soil moisture contents of the organic layer and mineral soil compared to the beech stand. The needle litter built a loose and permeable organic layer, which was reflected in approx. 70% higher gas diffusivity at the surface of the organic layer observed in a spruce stand than in the beech stand at Solling (Ball et al., 1997). In the mentioned spruce stand, which is located adjacent to our spruce stand, the organic layer contributed with 73% to total N_2O emissions compared to 53% in the beech stand (Borken and Brumme, 1997). Brumme et al. (1999) exchanged the litterfall at the Solling sites. Emissions of N_2O increased in the spruce stand receiving beech litter whereas emissions decreased in the beech stand receiving spruce litter.

6.4.3. Impact of forest type on N₂O flux regulation

Higher N_2O emissions from deciduous forests than from coniferous forests have previously been reported in the literature (Ambus et al., 2006; Butterbach-Bahl et al., 2002; Pilegaard et al. 2006). We concluded that soil NO_3^- content, temperature, and water content played a key role for N_2O flux dynamics of soils within our forest sites by controlling denitrification and nitrification processes in the short term (e.g., Schindlbacher et al., 2004; Skiba and Smith, 2000; Szukics et al., 2009). However, on a regional scale factors such as forest and soil type have been identified to be the key drivers (Brumme et al., 2005; Groffman et al., 2000; Pilegaard et al., 2006; Schulte-Bisping et al., 2003). In the present study, we attributed the differences found in N_2O fluxes between the investigated beech (SEP) and spruce stands (BEP) to the different structure and quality (e.g. pH, C/N ratio, lignin content) of the litter. The pH value of the organic layer of the beech stand was higher and the C/N ratio of the organic layer and mineral soil was lower compared to the spruce stand. Investigations in several European forest sites showed that N_2O emissions were negatively correlated with the

C/N ratio (Ambus et al., 2006; Pilegaard et al., 2006), since net nitrification rates are enhanced at declining C/N ratios (Ambus et al., 2006). Thus the differences in litter quality and structure probably not only had an impact on gas diffusivity, as described above, but also on N turnover rates, which was also suggested by Groffman et al. (2000). Gross N mineralisation rates of the organic layer were moderately high and comparable for both stands whereas gross nitrification rates were markedly lower than total mineralisation rates and were lower in the spruce than in the beech stand (Corre and Lamersdorf, 2004; Corre et al., 2003; the spruce stand is referred to as ambient no-roof plot). In-situ incubation of undisturbed soil samples of the top 10 cm of the organic layer and mineral soil revealed that in the beech stand net mineralisation and nitrification rates were high with 58 and 29 $mg\,N\,m^{-2}\,d^{-1}$ (chapter 4.3.2.). The comparably high net nitrification rates in the beech stand provide substrate for denitrification. But in the spruce stand an accumulation of NH_4^+ in the soil solution was observed most likely due to lower nitrification rates and to higher N depositions than in the beech stand. Ammonium contents exceeded NO_3^- contents in both stands, indicating that NH_4^+ was not limiting nitrification.

6.4.4. Impact of forest type on NO_x flux regulation

A trend towards higher NO_x emissions from the beech stand than from the spruce stand is in contrast to the general finding that NO emissions from coniferous forest soils exceeds emissions from the deciduous forest soils (e.g. Butterbach-Bahl et al., 1997; Gasche and Papen, 1999; Pilegaard et al., 2006). However, Venterea et al. (2004b) also reported higher NO emissions from hardwood (4.4 $\mu g\,N\,m^{-2}\,h^{-1}$; primarily beech-maple) than from softwood plots (0.6 $\mu g\,N\,m^{-2}\,h^{-1}$; primarily spruce-fir). The authors proposed that temperate forests influenced by sugar maple have lower C/N ratios and possibly favour higher rates of nitrification compared to the softwood stands. Measurements of N oxides at 15 European forest sites revealed that nitrification was the main source for NO production whereas denitrification was the main source for N_2O production (Pilegaard et al., 2006). In the beech stand NO emissions were low despite high nitrification rates. Low diffusivity might have caused longer residence time of NO in the soil which enhanced the probability of NO consumption by denitrification (Gasche and Papen, 1999).

Furthermore, in our study net NO_x fluxes were determined, which can be low in spite of high production rates due to consumption rates of similar magnitude (Butterbach-Bahl et al., 1997; Conrad, 1996, Gasche and Papen, 1999). Fluxes of NO_x determined in our study were

comparable to fluxes from a Danish and a South German beech forest soil (Beier et al., 2001; Gasche and Papen, 1999).

6.4.5. Short-term regulation of NO_x fluxes

Regarding the short-term regulation, nitrate and water content of the organic layer explained 66% of the variation in NO_x fluxes of both stands. Skiba et al. (1994) also proposed soil NO_3^- contents as best predictor for NO fluxes. Fluxes of NO generally are correlated with soil moisture (e.g. Gasche and Papen, 1999; van Dijk and Duyzer, 1999). The relationship shows an optimum at low to medium soil water contents, since substrate diffusion and microbial activity is limited at low water contents and oxygen diffusion is limited at high water contents (Davidson et al., 2000; Pilegaard et al., 2006). However, the optimum can substantially vary between 15 and 85% WFPS depending on the soil type (Gasche and Papen, 1999; Ludwig et al., 2001; Schindlbacher et al., 2004; van Dijk and Duyzer, 1999). The negative relationship that we found in our study indicated that the water contents of the Solling soils were high and beyond the optimum for NO production. Several studies further found that NO fluxes exponentially depended on soil temperature because of its effect on microbial activity and soil gas diffusion rates (e.g. Beier et al., 2001; Gasche and Papen, 1999; Schindlbacher et al., 2004). In our study no such relationship was found, thus factors other than temperature obviously limited NO production. However in Mid-January 2008, a comparatively high emission rate of 8.5 μg NO_x-N m^{-2} h^{-1} was measured in the spruce stand, which we ascribed to a thawing event because soil temperature in the organic layer was zero degree until 1.5 days before measurement. In general, we assume that peak emissions of NO and N_2O (e.g. Goldberg et al., 2008, 2010; Teepe et al., 2000) were negligible in winter 2007–08. Wu et al. (2010) found a strong impact of freezing-thawing periods on N_2O emissions in 2005 and 2006 in South Germany but not in 2007 and 2008, where freezing-thawing marginally occurred.

A further factor that regulated NO_x fluxes in the short term was the NO_x concentrations in the ambient air. The high uptake rates of NO_x into the soil observed in the beech stand in October (13.3 μg N m^{-2} h^{-1}) and in the spruce stand in November (9.6 μg N m^{-2} h^{-1}) resulted from high NO_x concentrations in the ambient air, which were approx. 21 and 15 ppbv, compared to an average of < 3 ppbv during the residual investigation period. Consumption of NO_x depends on the NO_x concentration in the ambient air and NO_x is taken up by the soils when the apparent compensation concentration is exceeded (Conrad, 1996; Gasche and Papen, 1999). Published compensation concentrations range between < 1 ppbv and several

hundred ppbv (Ludwig et al., 2001). Ludwig et al. (2001) suggested that low compensation concentrations are associated with properties which are unfavourable for NO production processes. At the Solling sites, compensation concentrations were obviously low as well as NO_x emissions.

6.4.6. Uptake and emissions of NO_2

During the winter months soils mainly acted as net NO_x sink. In our study, abiotic NO_2 uptake was thought to be mainly responsible for net NO_x uptake, which we concluded from some measurements where both NO_x and NO_2 fluxes were analysed. Butterbach-Bahl et al. (1997) also observed that NO_2 consumption rates exceeded NO emission rates in a beech forest in South Germany.

Our study further brought forward that NO_2 was emitted from the Solling soils and accounted for up to 100% of total NO_x emissions. Chemodenitrification obviously was an important source of NO and NO_2 (van Cleemput and Baert, 1984; van Cleemput and Samater, 1996) in these acid soils having high soil organic matter and ferrous iron contents in soil solution. Most studies exclusively observed deposition of NO_2 (Butterbach-Bahl et al., 2002; Gasche and Papen, 1999; Rosenkranz et al., 2006). However, Butterbach-Bahl et al. (1997) found NO_2 emissions during short periods of low NO_2 concentrations in the ambient air of about 5 ppbv.

6.5. Conclusions

Our study demonstrated that N_2O emissions dominated total N oxide emissions in the spruce and beech stand, which both had high soil water contents. However, the beech stand displayed high and seasonal N_2O emissions whereas N_2O emissions were comparably low throughout the whole year in the spruce stand. Thus, forest type played a major role for N_2O emissions due to differences in litter quality and structure, which most likely resulted in higher soil N turnover rates and lower gas diffusivity of the organic layer in the beech stand compared to the spruce stand. Emissions of NO_x were similar and low in both stands. High water contents and NO_x consumption may have reduced NO_x emissions from nitrification. Furthermore, there is evidence that abiotic processes are mainly responsible for NO_x uptake and emissions in our acid forest soils.

Acknowledgment

Chapter 7

General Discussion
and Outlook

7.   General Discussion and Outlook

7.1. What is the origin of N$_2$O and NO$_x$ emissions from temperate forest soils exposed to chronic high N depositions?

7.1.1. Origin of N$_2$O emissions

The application of **^{15}N labelling techniques** in the present study **shed new light on the origin of N$_2$O emissions** from temperate forest soils exposed to chronic high N depositions. The first study (chapter 2) revealed that only **6% of total N$_2$O emissions** were derived **from N depositions of the last year** in the Solling **beech** stand, which exhibited seasonal N$_2$O emissions with high emissions during the summer months (2.6 kg N ha^{-1} yr^{-1}). Similarly, in the Solling **spruce** stand, which exhibited low background emissions throughout the whole year (0.3 kg N ha^{-1} yr^{-1}), only **13% of total N$_2$O emissions** were derived **from N depositions** of the last year. The first study further gave evidence that deposited N almost exclusively contributed to N$_2$O emissions in the first three weeks after depositions had occurred. In addition, turnover of total **leaf litter N** in the surface organic layer was involved in N$_2$O production but probably also provided a minor **contribution** to total annual **N$_2$O emissions** in the Solling **beech** stand as was indicated by the third study (chapter 4). Thus, the question arises as to where the remaining N$_2$O emissions originated from.

Studies that investigated the fate of N depositions in forests have reported that soils, in particular the surface organic layer, represent the largest sink for N inputs (Feng et al., 2008; Fenn et al., 1998; Nadelhoffer et al., 1999). Immobilisation of deposited N may occur via biotic or abiotic processes (e.g. Corre and Lamersdorf, 2004; Davidson et al., 2003; Fitzhugh et al., 2003). A change in the soil N pool accompanied by an increase in surface organic layer and an accumulation of SOM have been reported from European forests receiving chronic high N depositions (e.g. Berg and Matzner, 1997; Brumme and Khanna, 2008; Brumme et al., 2009b). At the Solling beech stand an increment in surface organic layer N pools of 21 kg N ha^{-1} yr^{-1} but no change in C/N ratios was observed over the last 30 years, which could probably ascribed to an accumulation of N depositions (Brumme and Khanna, 2008; Meiwes et al., 2002). Berg and Matzner (1997) proposed a retardation of the later stages of organic matter (OM) decomposition by increased N availability (chapter 4.1.). Changes in the chemical bond structure of OM due to incorporation of external N and suppression of humus-degrading enzymes by high N availability in soils are held responsible for the accumulation of N in OM (e.g. Berg and Matzner, 1997). Thus, immobilised N depositions may contribute to N oxide emissions following remobilisation years after N had been deposited. This

assumption could be confirmed in the present study using the **long-term ^{15}N tracer study** of the **Solling roof project** where NH_4^+ and NO_3^- depositions have been labelled since 2001 (chapter 1.7., 3.2.2.). In the framework of the second study (chapter 3), ^{15}N-N_2O fluxes were measured from May 2007 to August 2008 (data were not included in chapter 3). Assuming a similar contribution of N depositions to N_2O emissions following tracer application under the "control" roof than at the adjacent spruce stand, which was investigated in the first study (chapter 2), then NH_4^+ depositions of the last year contributed 2% and NO_3^- depositions contributed 17% to total N_2O emissions. However, **98%** (NH_4^+ depositions) **and 83%** (NO_3^- depositions) **of total labelled N_2O** emissions derived **from the years 2001–07**. Hence, **remobilised N depositions significantly contributed to N_2O emissions** later than one year after deposition of N.

The present study demonstrated that **in the largest part N_2O emissions of temperate forest soils probably are the result of chronic atmospheric N inputs** as was prior suggested by N fertilisation studies (e.g. Sitaula et al., 1995; Skiba et al., 1998, 1999) and comparisons of forest sites receiving different N loads (e.g. Butterbach-Bahl et al., 1998, 2002; see Tab. 2-5). Therefore, N_2O emissions may be negligible in temperate forests receiving low N depositions, which is in line with published N_2O emissions from such forests (e.g. Bowden et al., 1991; Castro et al., 1993; Macdonald et al., 1997) compared to forests receiving high N depositions (e.g. Brumme and Beese, 1992; Papen and Butterbach-Bahl, 1999).

7.1.2. Origin of NO_x emissions

It was concluded that **chronic high N depositions** were probably **solely responsible for NO_x emissions** from temperate forest soils (chapter 3). Long-term reduction of N depositions to a pre-industrial level in the spruce stand at Solling, turned the forest soil from a net source of NO_x (0.62 kg N ha^{-1} yr^{-1}) into a net sink (−0.33 kg N ha^{-1} yr^{-1}). Net emissions of NO_x were further observed from the unroofed Solling spruce stand (0.04 kg N ha^{-1} yr^{-1}) and from the Solling beech stand (0.11 kg N ha^{-1} yr^{-1}), but which were low due to predominance of denitrification (chapter 2.4.3.). Our finding was corroborated by studies from coniferous and deciduous temperate forests receiving low N depositions, which exhibited close to zero NO fluxes (Johansson et al., 1984; Kitzler et al., 2006b; Venterea et al., 2003). Whereas fertilisation of forest soils exposed to low N depositions significantly increased NO emissions (Johansson et al., 1984; Venterea et al., 2003). Uptake of NO_x as was observed in the present

study under reduced N depositions indicated that turnover of leaf litter N does not represent a net source of NO_x at least in temperate forests receiving low N depositions.

7.2. Application of emission factors to N oxides

Emission factors (EF) for N oxides have been frequently used to quantify the contribution of N depositions to N oxide emissions (e.g. Butterbach-Bahl et al., 1998; Johansson et al., 1984; Venterea et al., 2003). However, in the first study (chapter 2) **difficulties and errors** arising from the use of different methods to calculate EF were **discussed for the first time in literature**. In brief, EFs have been calculated using (i) the **regression approach** (EF_R; e.g. Butterbach-Bahl et al., 1998), (ii) **N fertilisation** experiments (EF_{FB}; e.g. Brumme and Beese, 1992), and (iii) the **fraction method** (EF_F; e.g. Oura et al., 2001; see chapter 2). These methods are subject to the following limitations: (i) The regression approach demands for a strong relationship between N oxide emissions and N depositions. This approach further overestimates EF when N oxide emissions and N depositions both show a positive relationship to a third control parameter (e.g. temperature, soil moisture). (ii) N fertilisation may result in artificially high N oxide emissions because the pulse in mineral N concentrations does not reflect the true atmospheric N depositions and may also cause positive priming effects on the N cycling in soils. In forest ecosystems exposed to chronic high N depositions, N oxide emissions measured after N fertilisation events may only account for emissions that are additionally emitted from fertilised N but not emissions resulting from depositions, since fluxes of an unfertilised control are subtracted. (iii) The fraction method assumes that N depositions represent the sole source for N oxide emissions. Thus, contribution of e.g. turnover of litter N to N oxide emissions is not accounted for.

The comparison of EFs revealed that EFs calculated with different methods significantly differed and were not comparable (chapter 2; as an example see Fig. 7-1). Hence, EF values calculated with the three frequently used methods may even be incorrect. Application of EFs for emission inventories need to be regarded with great care, since the development of many policy actions to reduce N oxide emissions is based on these inventories.

In the present study, **[15]N labelling techniques** were applied **for the first time** to calculate EFs for N_2O emissions from temperate forest soils (EF_{15N}; chapter 2). This method **proved to be a precise approach** for quantifying the contribution of atmospheric N depositions to emissions of N_2O. Emission factors calculated by the ^{15}N tracer approach reflected the contribution of N depositions to N_2O emissions over a period of one year (recent N

depositions). However, emissions resulting from N depositions of former years (remobilised accumulated N depositions) were not accounted for. The **annual EFs** for **N_2O from recent N depositions** were low with **0.1% for the spruce** stand and **0.6% for the beech** stand at Solling (Fig. 7-1). The **fraction method** (EF_F) may provide a good estimate for an EF value that considers the impact of **recent and accumulated N depositions**, assuming that the contribution of leaf litter N to N_2O emissions is marginal. Assuming that EF_{R2} and EF_{F2} (Fig. 7-1) are the best estimators of the true EF, then approx. **11%** of total deposited N (recent and accumulated) was released as N_2O from the Solling **beech** forest soil in 2007–08 compared to 0.6% of recent N depositions. In the **spruce** stand, the corresponding **EF**s were approx. **0.9%** and 0.1%. Hence, **release of N deposition as N_2O was one order of magnitude higher in the beech stand than in the spruce stand**. High rates of nitrate leaching from the Solling spruce stand and also from other German spruce forests have been reported (e.g. Borken and Matzner, 2004; Feng et al., 2008). In contrast, nitrate leaching played a minor role for N losses from the Solling beech stand (Brumme et al., 2009b; Brumme and Khanna, 2009b; Corre et al., 2003). Conditions favouring soil anaerobicity and N_2O production in the beech stand may be responsible for different pathways of N losses in the investigated stands (chapter 7.3.2.).
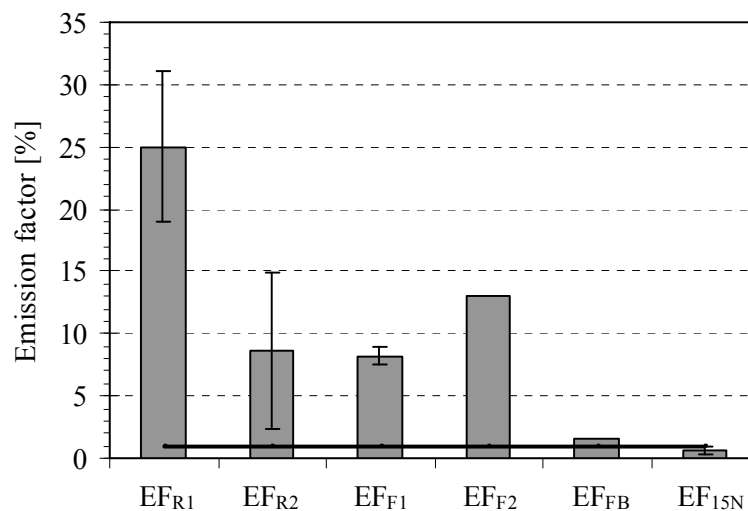
Figure 7-1: Emission factors (EF) for N_2O emissions derived from N depositions for the Solling beech stand using different approaches. The IPCC default EF of 1% is indicated as a black line. Standard errors are given where available. EF_{R1}: regression method, value for 2007–08; EF_{R2}: regression method considering the positive relationship of temperature with N_2O emissions and N depositions, value for 2007–08; EF_{F1}: fraction method, mean value for 1990–98; EF_{F2}: fraction method, value for 2007–08; EF_{FB}: N fertilisation experiment in 1988; EF_{15N}: ^{15}N labelling method, value for 2007–08.

An **extrapolation of EFs for German forests** considering seasonal and background emission patterns of N_2O as proposed by Schulte-Bisping et al. (2003) yielded an **EF** of approx. **1.6%, including recent and accumulated N depositions** (Tab. 7-1). This EF value is similar in magnitude but higher than the default value of 1% suggested by the IPCC (2006).

The **huge contribution of accumulated N depositions to N_2O emissions** implies that even a drastic decline in N depositions in Germany would probably not result in a decrease in N_2O emissions in the next decade(s).

Table 7-1: Extrapolation of emission factors (EF) for German forest soils. Areas and N_2O emissions were based on data used by Schulte-Bisping et al. (2003). German forest were classified into seasonal emission patterns (SEP) and background emissions patterns of N_2O (BEP; Brumme et al., 1999; Schulte-Bisping et al., 2003). Deposition was assumed to be 20 kg N ha^{-1} yr^{-1} (Brumme and Khanna, 2008). EF_F was calculated according to the fraction method.

	Area [km^2]	N_2O [Gg N yr^{-1}]	N_2O [kg N ha^{-1} yr^{-1}]	EF_F [%]
Deciduous forest	22,400	1.49	0.67	3.35
BEP	18,500	0.69	0.37	1.85
SEP	3,900	0.80	2.05	10.25
Coniferous forest				
BEP	57,700	0.96	0.17	0.85
Mixed forest	21,400	0.81	0.38	1.90
Total	101,500*	3.26	0.32	1.60

Note: * The total forest area in Germany amounts to 110,758 km² (Second National Forest Inventory; BMVEL, 2004).

Emission factors for NO_x using the fraction method were **0.1%** for the Solling **spruce** stand, **0.6%** for the **beech** stand, and 1.8% for the "control" roof plot. However, the fraction method assumes zero fluxes at low N depositions, which was presumably not the case as was indicated by the strong NO_x uptake in the "clean rain" roof plot. Considering the uptake, the EF value of the **"control" roof** plot increased to **2.7%**. Beier et al. (2001) reported an EF value for NO_x of < 4% for a Danish beech forest exposed to high N depositions. In general, EFs calculated for NO ranged from 0.2% to 8% for coniferous and deciduous forests (e.g. Johansson et al., 1984; Kitzler et al., 2006b; Venterea et al., 2003). High NO emissions and an EF of 14% were observed by Skiba et al. (1998, 1999) at a forest soil receiving high NH_3 depositions of 80 kg N ha^{-1} yr^{-1} close to a poultry farm.

In conclusion, the **impact of N depositions on N oxide emissions strongly depends on the forest ecosystem** as was already suggested by Liu and Greaver (2009). **Temperate forests** exhibiting seasonal emission patterns of N_2O may be **considerable sources for N_2O**

in regions receiving **chronic high N depositions**. Similar to N_2O, NO_x emissions may be considerable from highly N-affected forest soils favouring nitrification or chemodenitrification instead of denitrification. Hence, the application of a single EF value that is independent of forest type, as proposed by IPCC (2006), is not reasonable. In case of EFs for N_2O, a division into deciduous and coniferous forests and SEP and BEP is required similar to the suggestion in Tab. 7-1.

7.3. What are the factors regulating N_2O and NO_x emissions from temperate forest soils and which production processes are involved?

7.3.1. Processes of N oxide production

The first study (chapter 2) indicated that **denitrification** was the main process responsible for **N_2O** emissions at the Solling spruce stand, which exhibited low annual N_2O emissions (0.3 kg N ha^{-1} yr^{-1}), and at the Solling beech stand, which exhibited high and seasonal N_2O emissions (2.6 kg N ha^{-1} yr^{-1}). Ambus et al. (2006) and Wolf and Brumme (2002) came to the same conclusion for 11 European forest soils and the beech forest soil at Solling, respectively. In contrast, **NO** emissions were probably the result of **nitrification** as was indicated by the second and the fourth study (chapter 3 and 5). Nitrification was also reported to be the main source of NO emissions in other European forests (e.g. Gasche and Papen, 1999; Rosenkranz et al., 2006). The fifth study (chapter 6) further revealed that **chemodenitrification** (van Cleemput and Baert, 1984; van Cleemput and Samater, 1996) possibly played a major role for **NO and NO_2** production in the acid Solling forest soils where emissions of NO_2 were measured. Likewise, Schindlbacher et al. (2004) suggested that chemodenitrification was important for NO production in acid temperate forest soils.

7.3.2. Regulation of N oxide fluxes

The present thesis demonstrated the complexity of the regulation of N oxide fluxes on the ecosystem level. As postulated by the conceptual "hole in the pipe" (HIP) model (Firestone and Davidson, 1989), two levels of regulation of N oxide emissions from soils could also be confirmed by the present study. According to the HIP model, the sum of total N oxide production is a function of N cycling rates, whereas the ratio of released gases (NO, N_2O, N_2) is a function of environmental factors such as O_2 availability (Davidson et al., 2000). Availability of O_2 is controlled by gas diffusivity of the soil and O_2 consumption by micro-organisms and roots (Bollmann and Conrad, 1998; Brumme et al., 1999; Smith et al., 2003). Soil gas diffusivity in turn depends on soil texture, soil moisture, and air-filled porosity

(chapter 5). Thus, the ratio of released gases often depends on soil moisture (Davidson et al., 2000). In the six investigated German beech stands, fluxes of **N_2O and NO_x** were controlled by **N turnover** (net N mineralisation and net nitrification rates) and **soil gas diffusivity** (chapter 5). In the one year experiment at the Solling beech stand, fluxes of N_2O were regulated by soil nitrate contents and **soil moisture** (chapter 6). At the spruce stand of the Solling roof project, NO_x emissions depended on soil nitrate contents and net nitrification rates (chapter 3). Net nitrification and excess NO_3^- in soil solution were not detectable under the roof after long-term reduction of N depositions. This was corroborated by Corre and Lamersdorf (2004) who found tight microbial N cycling and neither gross nor net nitrification under reduced N depositions at this spruce stand. Soil moisture did not seem to play a role for NO_x emissions from the roofed spruce stands, which exhibited significantly different NO_x fluxes at similar water contents. However, comparison of N_2O and NO_x fluxes from the "control" roof (chapter 3) and from the adjacent unroofed spruce stand (chapter 6) demonstrated that significantly higher water contents as observed in the unroofed plot resulted in significantly higher N_2O but lower NO_x emissions compared to the "control" roof plot at similar nitrate contents. Thus, NO was presumably consumed by denitrification in the unroofed plot. However, unfavourable conditions for denitrification resulted in similar N_2O emissions under both roofs independent of significant differences in N depositions and nitrification rates (chapter 3). Differences in soil moisture between the "control" roof plot and unroofed plot at the Solling spruce were ascribed to **roof effects**. The water sprinkler system at the roof plots did not reproduce the temporal and spatial variability of natural throughfall and rain events < 1 mm could not be simulated resulting in increased rain intensity and decreased number of rain events (Gundersen et al., 1998). Further roof effects were described in detail by Gundersen et al. (1998).

Dependence of N oxide fluxes on mineralisation and nitrification rates (e.g. Gasche and Papen, 1999; Rosenkranz et al., 2006) and soil moisture (e.g. Rosenkranz et al., 2006; Schindlbacher et al., 2004) have been frequently reported and were discussed in detail in chapter 3, 5, and 6. In addition, N_2O and NO_x fluxes from the spruce and beech stands investigated in the present study significantly increased with **soil temperature** (chapter 3, 5, and 6). Soil temperature, which on the one hand influences microbial activity (e.g. N turnover) and on the other hand influences soil anaerobicity due to its effect on soil respiration and water-solubility of gases, has been frequently described as key driver for N oxide emissions as well (e.g. Brumme and Borken, 2009; Gasche and Papen, 1999; Schindlbacher et al., 2004).

The hypothesis that **German forest soils** are in general characterised by **high N turnover rates** and N availability due to chronic high N depositions had to be **rejected**. Nitrogen turnover not only depended on N deposition rates but also on litter quality (e.g. C/N ratio, pH) and soil properties (e.g. soil texture). In general, higher N turnover rates, especially nitrification rates, have been reported from deciduous than coniferous forests (e.g. Ambus et al., 2006). It could **not** be **confirmed** that the tightly-packed **laminar beech leaf litter layer** of moder type humus generally **resulted in low gas diffusivity** (chapter 5). **But high annual precipitation** and soil moisture in combination with a **loamy soil texture and moder type humus caused anaerobicity** at the Solling beech stand during times of high soil respirations (chapter 5). In contrast, similar conditions (precipitation, WFPS, texture, humus type) did not result in anaerobicity at the Solling spruce stand (chapter 6; Ball et al., 1997). Therefore, **litter structure finally decides about soil anaerobicity**. This was also indicated by a litter exchange experiment at the Solling spruce and beech stands (Brumme and Borken, 2009; Brumme et al., 1999). These observations explained why background emission patterns of N_2O appear in coniferous and in deciduous forests whereas seasonal emission patterns have only been reported from acid beech forests (Brumme et al., 1999). However, the fourth study (chapter 5) also indicated that soil anaerobicity alone does not result in high N_2O emissions but that high N turnover rates are additionally necessary for seasonal emission patterns of N_2O in beech forests as was the case for the Solling beech stand. In addition, the assumption that forest soils having BEP of N_2O emit higher NO amounts than forest soils having SEP could not be verified in the present study (chapter 5 and 6). This was most likely due to net $NO + NO_2$ measurements and the dominance of abiotic processes controlling NO_x production and consumption in these acid forest soils. The **key driver for NO_x** fluxes was the **NO_x concentration in forest air** (chapter 3, 5, and 6).

In conclusion, the reported **forest type effect** on N_2O and NO emissions (e.g. Brumme et al., 1999; Butterbach-Bahl et al., 1997) resulted from the influence of **litter quality and structure on N turnover rates and soil gas diffusivity**. However, forest type alone did not decide on N oxide emissions. Davidson and Verchot (2000) confirmed the applicability of the HIP model, which was developed for the tropics, across a broad range of sites including tropical and temperate climates. We further demonstrated that the **HIP model** is also **valid to** explain regulation of N oxide fluxes from **European temperate forest soils receiving chronic high N depositions**.

7.4. Conclusions

The ^{15}N tracer technique turned out to be a precise tool to investigate the origin of N_2O emissions from temperate forest soils receiving chronic high N depositions. The first hypothesis that N depositions are mostly responsible for N_2O and NO_x emissions was accepted. However, the largest part of total N_2O emissions obviously derived from N depositions of former years, which were accumulated in soils and remobilised, whereas recent N depositions of the last year only accounted for approx. 10% of total N_2O emissions. Leaf litter N was involved in N_2O emissions but contribution to total N_2O emissions during the first ten years after litterfall was low indicating that leaf litter N in general may represent a minor source for N_2O and probably also for NO_x emissions.

Hypotheses (1):

- Nitrogen depositions are mostly responsible for N_2O and NO_x emissions. (✓)
- Turnover of leaf litter N represents a minor source for N_2O emissions. (✓?)

The second research topic addressed the regulation of N_2O and NO_x fluxes. The present study demonstrated that the conceptual HIP model, which was developed for the tropics, can also be applied to explain the regulation of N oxide fluxes from temperate forest soils exposed to chronic high N depositions. The hypothesis that German forest soils generally have high N turnover rates and N availability due to chronic high N depositions was rejected. Releases of N_2O and NO_x from soils strongly vary due to differences in gas diffusivity. However, low gas diffusivity of the surface organic layer alone does not result in high seasonal N_2O emissions. A combination of high N turnover rates and soil anaerobicity due to high annual precipitation, loamy soil texture, and moder type humus with a laminar beech leaf litter layer is necessary for high seasonal emissions from beech forests. Therefore, the hypothesis that deciduous forests with moder type humus generally exhibit SEP of N_2O was rejected. Moreover, the present study suggested that high seasonal N_2O emissions probably are less common in German beech forests than previously assumed. Deciduous forests with mull type humus may also exhibit SEP whereas coniferous forests exhibit BEP. The hypothesis that NO_x emissions are higher from forests showing BEP of N_2O than from forests showing SEP of N_2O was rejected.

Hypotheses (2):

- German forest soils generally have high N turnover rates and N availability due to chronic high N depositions. (×)

- Releases of N_2O and NO_x from soils strongly vary due to differences in gas diffusivity of the surface organic layer. (✓)

- Deciduous forests with moder type humus generally exhibit seasonal emission patterns of N_2O. (×)

- Coniferous forests belong to the background emission type. (✓)

- Forests having BEP of N_2O have higher NO_x emissions than forests having SEP. (×)

7.5. Suggestions for future research

In the first study (chapter 2), ^{15}N labelling techniques were used for the first time to determine the contribution of N depositions to N_2O emissions from European temperate forest soils over one year. It was shown that this method represents a precise tool, which may improve estimates of the contribution of N deposition to N_2O emissions. However, the replication of such experiments is required. The ^{15}N tracer experiments revealed that N depositions of the last year only played a minor role for total N_2O emissions whereas N depositions from former years that were accumulated in soil and subsequently remobilised obviously played the major role. However, further long-term ^{15}N tracer studies are necessary to quantify the contribution of remobilised N depositions. In particular, ^{15}N studies in beech stands exhibiting SEP compared to beech stands exhibiting BEP are of great interest. Such studies may further contribute to find unbiased emission factors. The first study demonstrated that emission factors that have been used so far are not comparable and may be highly biased. Further research on emission factors and their standardisation has absolute priority due to their high political relevance.

In addition, research on production and consumption processes of N_2O and NO should be intensified. Hereby, research may benefit from utilisation of isotope ratios (e.g. $^{14}N/^{15}N$ and $^{16}O/^{18}O$) and isotopomer ratios (intramolecular site preference of ^{15}N; e.g. Baggs, 2008).

Important future investigations might deal with the development of a method to measure ^{15}N in NO from forest soils to investigate production processes as well as the contribution of N depositions to NO emissions similar as was done for N_2O in the first study. First ideas and results have already been developed in the framework of the present study (Appendix A).

A roof project such as the one at Solling represents a great opportunity to investigate the long-term impact of N depositions and their reductions on forest ecosystems (chapter 3). With regard to N_2O emissions, a roof would be especially interesting in the beech stand due to high seasonal N_2O emissions. One experiment could be as follows: continuous $^{15}NH_4^{15}NO_3$-labelling of N depositions under one subplot of each roof and weekly measurements of ^{15}N-N_2O for at least 15 years to investigate the contribution of accumulated and remobilised N. The contribution of recent N depositions could be determined in the first year of labelling and after 15 years when labelling the other subplot of each roof for the first time. However, the Solling beech stand may be unsuitable for a roof project due to its recent age. Furthermore, roof projects are accompanied by high material and labour costs.

Further research is also needed on the contribution of leaf litter N turnover to N_2O and NO emissions (chapter 4). Therefore, decomposition rates and ^{15}N distribution in leaf litter and soil should be determined simultaneously and every year at least over a 10-year period. It would be interesting to compare leaf litter with high and low N concentrations in forests with high and low N depositions due to the effect of high N availability on early stage and later stage decomposition (Berg and McClaugherty, 2003).

The conclusion about the regulation of N_2O emissions by N turnover and gas diffusivity is mainly based on one forest stand, namely the Solling beech stand, which was the only forest exhibiting SEP of N_2O (chapter 5). Hence, future research should concentrate on other beech stands exhibiting SEP.

In the fifth study (chapter 6), the importance of abiotic processes for production and consumption of NO and NO_2 in acid forest soils was indicated. Future research might deal with these processes with particular emphasis on quantifying the contribution of chemodenitrification. In addition, separate measurements of NO and NO_2 seem to be more appropriate to draw a conclusion about dynamics, production, and consumption of both gases.

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Appendix A

Development of a method to determine ^{15}N in soil-derived nitric oxide and nitrogen dioxide in forest ecosystems by in-situ N accumulation

Motivation

Tracer studies using the stable isotope ^{15}N could contribute to the process understanding of NO production in soils and to the examination of the significance of N deposition for NO emissions in forest ecosystems, similarly as it is already the case for N_2O (see chapter 2; Wolf und Brumme, 2002). To date only Russow et al. (2008) successfully measured ^{15}NO during an agricultural fertilisation experiment in the field. However, the partly very low NO concentrations of < 10 ppbv in the ground-level forest air and the high reactivity of NO so far impeded determination of ^{15}N in soil-derived NO in forests. Direct determination of the isotopic ratio of NO in the laboratory has been conducted by accumulating ^{15}N using a molecular sieve and/or a cryo trap with liquid N_2 (e.g. Lauf and Gebauer, 2001; Russow et al., 2009; Sich and Russow, 1999; Wolf and Russow, 2000). However, it was shown that the application of the molecular sieve resulted in an inaccuracy, which was ascribed to a "memory effect" as result of a remaining quantity of absorbed NO. Further, utilisation of liquid N_2 is hardly or not practicable in forests. In medicine, an infrared-laser spectroscopy was developed to determine ^{15}NO in very low concentrations (magnitude of ppt; Heinrich et al., 2009). Thus, for laboratory measurements a lossless transport and storage of NO in glass bottles or inert gas bags is required to exclude fractionation processes, but which is not possible. Contrary to the mentioned on-line techniques, off-line techniques have been used where NO was converted into a salt by chemical reactions with guaiacol or hydrogen peroxide. These techniques were particularly applied in investigations of exhausts of vehicles and coal-burning power plants where NO concentrations of > 1000 ppm occurred (e.g. Ammann et al., 1999; Heaton, 1990). Thus, the aim of my study was to develop an in-situ method to determine ^{15}N in low concentrations of soil-derived NO and NO_2.

Method development

The idea was to lead NO in an oxidant that oxidise the gas completely to NO_3^- of which the ^{15}N value could be determined. A quantitative conversion is required to avoid fractionation. Several oxidants were tested of which potassium persulphate ($K_2S_2O_8$)

performed most convenient due to being a powerful oxidant but also being easily disposed under oxygen release by autoclaving. I used 50 g $K_2S_2O_8$ and 15 g sodium hydroxide (NaOH) per litre. The oxidation took place at room temperature in an alkaline milieu (pH ~13). The pH value was lowered during autoclaving. Since NO_3^- concentrations of the final sample solutions were low (Tab. A-1), I chose the conversion of NO_3^- via nitrite (reduction with cadmium) to N_2O (reduction with azide; McIlvin and Altabet, 2005) of which the ^{15}N value was determined. The method is applicable for N concentrations of 0.5–40 µM. Analyses of N_2O and ^{15}N of N_2O were conducted with a gaschromatograph (Fractovap 400, Carbo Erba, Milano, Italy) and an isotope ratio mass spectrometer coupled with a pre-concentration unit (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany), respectively. The Bunsen absorption coefficient, which provides a measure for the solubility of gases in a fluid, was used to calculate the theoretical concentration of N_2O of the samples. One N atom of N_2O derived from nitrite and one from azide. Thus, the ^{15}N value of NO_3^- was received from the ^{15}N value of N_2O as follows (Eq. A.1; McIlvin and Altabet, 2005):

$$^{15}NO_3^- = \frac{(^{15}N\text{-}N_2O - b)}{m_{Std}}$$
(A.1)

where

b	expected y-axis intercept of the relationship of theoretical $^{15}NO_3^-$ values of the standards plotted against the measured ^{15}N-N_2O values (the expected value is situated between the isotopic values of nitrite and azide).
m_{Std}	Observed linear slope of the above mentioned relationship (the expected value is 0.5).

Two experiments were conducted: (i) I determined ^{15}N of a standard NO gas which was mixed with NO-free room air and (ii) I determined ^{15}N of self-produced NO with known isotopic composition.

In the *first experiment*, I mixed a NO standard gas (3000 ppbv NO in N_2; Air Liquide GmbH, Germany) with NO-free room air, which was produced using an activated-carbon filter. The gas mixture then bubbled through a washing flask (frit of porosity I) with 40 mL $K_2S_2O_8$ solution for some hours. Several gas flux rates and NO concentrations were tested. A potential break-through of NO could be detected with a Scintrex LMA-3 chemiluminescence detektor (Scintrex Unisearch, Ontario, Canada), which was connected downstream of the washing flask. Due to the greater water solubility of NO_2 compared to NO, NO was converted to NO_2 using a chromium trioxide catalyst in some tests. Subsequently, the solution was autoclaved for 30 minutes. Additionally, I autoclaved ^{15}N-labelled NO_3^- standard solutions to test for possible N losses and fractionation processes during autoclaving. Analysis of NO_3^-

concentrations was carried out using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, The Netherlands).

In the *second experiment*, NO was produced in a N_2 and O_2-free environment (production was conducted by Jens Dyckmans). Thus, determination of the ^{15}N value of NO was possible. The production underlay the following redox reaction of nitric acid (HNO_3) with copper (Cu) (Eq. A.2).

$$3\,Cu + 8\,HNO_3 \rightarrow 3\,Cu(NO_3)_2 + 2\,NO + 4\,H_2O \tag{A.2}$$
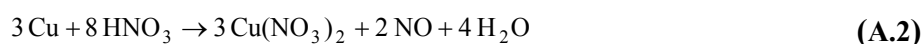
The self-produced NO (approx. 13000 ppm NO) was slowly injected (3 mL) in 40 mL $K_2S_2O_8$ solution using an air-tight syringe. Subsequently, the solution was shook for five minutes before autoclaving for one hour. Finally, NO_3^- was again converted to N_2O as described above.

First results and discussion

Results presented for the *first experiment* were derived from a test series where 14.5 ml NO min^{-1} were led in the $K_2S_2O_8$ solution for 14–15 hours (Tab. A-1). The theoretical N concentration of N_2O calculated from the theoretical N concentration of NO_3^- was 5 μg N L^{-1}, which fitted well to the mean measured N concentration of 5.03 ± 0.47 μg N L^{-1} ($n = 15$). Hence, the recovery of NO and NO_2 as N_2O on average was 100%. This implied that both gases were completely solved in the oxidant, which was also supported by the connected detector, which indicated no break-through of NO and NO_2.

However, the concentrations of NO_3^- measured before the reduction to N_2O were lower than the theoretically calculated N concentrations of the solutions (0.5 mg N L^{-1}). A possible reason was an inaccuracy of the applied NO_3^- analysis due to the composition of the sample solution and to N concentrations close to the detection limit.

The ^{15}N values of the NO tests (-11.9 ± 1.4‰) were significantly lower than the values of the NO_2 tests (-1.0 ± 4.2‰; $P < 0.01$, Student's t-test). Due to the similarly high recovery of both gases as N_2O, we can exclude an incomplete conversion. Thus, fractionation processes during the conversion of NO to NO_2 by the catalyst were probably responsible for the differences. However, a final conclusion can only be drawn when the ^{15}N value of the NO standard gas has been analysed.

The reproducibility of ^{15}N values was lower for the NO_2 tests than for the NO tests, which could be ascribed to a higher variability of N_2O concentrations in the NO_2 tests, since ^{15}N values were significantly correlated with N concentrations (Spearman's rank correlation coefficient $R = -0.7$, $P < 0.01$).

Table A-1: Results of five laboratory tests where NO$_2$ and NO (14.5 ml NO min^{-1}) were led in 40 ml K$_2$S$_2$O$_8$ solution (50 g L^{-1} K$_2$S$_2$O$_8$ and 15 g NaOH L^{-1}) for 14–15 hours (equivalent to approx. 0.5 mg N L^{-1}). Given are the pH values and the NO$_3^-$ concentrations after autoclaving, the theoretical and measured N$_2$O concentrations after the conversion of NO$_3^-$ to N$_2$O, the measured δ ^{15}N values of N$_2$O, and the calculated δ ^{15}N values of NO$_3^-$. Samples were replicated three times for the conversion of NO$_3^-$ to N$_2$O.

Description	pH	NO$_3^-$-N theoretical [mg L^{-1}]	NO$_3^-$-N measured [mg L^{-1}]	N$_2$O-N theoretical [µg L^{-1}]	N$_2$O-N measured [µg L^{-1}]	δ^{15}N-N$_2$O measured [‰]	δ^{15}N-NO$_3^-$ calculated [‰]
	7	0.5	0.305	3.062	5.96	2.280	-2.627
					3.39	n.d.	n.c.
					6.46	2.497	-2.230
NO$_2$ in K$_2$S$_2$O$_8$	7	0.5	0.295	2.962	3.00	2.697	-1.864
					7.99	-0.193	-7.153
					4.13	0.885	-5.180
	6	0.5	n.d.	n.d.	3.24	5.528	3.317
					1.94	6.351	4.824
					3.32	5.174	2.669
	8–9	0.5	0.150	1.506	5.52	-2.599	-11.556
					6.28	-3.406	-13.033
NO in K$_2$S$_2$O$_8$					7.38	-3.369	-12.966
					5.33	-2.944	-12.188
	8–9	0.5	0.150	1.506	5.30	-1.359	-9.287
					6.26	-3.106	-12.484

Note: n.d. not detected, n.c. not calculated.

The reproducibility of ^{15}N values of five samples and of the laboratory replicates in the *second experiment* was satisfying (–32.4 ± 0.6‰; Tab. A-2).

However, the ^{15}N values of NO$_3^-$ markedly differed from ^{15}N values of the used NO (–46‰). A reason for this difference could be fractionation processes during the injection. However, it is more likely that an error occurred during the ^{15}NO determination.

The linear regressions on theoretical ^{15}NO$_3^-$ concentrations of the standards plotted against measured ^{15}N-N$_2$O values yielded the following parameters for the two laboratory tests: $b = -3.7154‰$; $m_{Std} = 0.5464$ (*first experiment*) and $b = -2.2971‰$; $m_{Std} = 0.4488$ (*second experiment*).

Table A-2: Measured ^{15}N values of N$_2$O and calculated ^{15}N values of NO$_3^-$ given for five samples where 3 mL NO (δ ^{15}N of –46‰; approx. 13000 ppm) were led in 40 mL K$_2$S$_2$O$_8$ solution (approx. 0.5 mg N L^{-1}). Samples were replicated three times for the conversion of NO$_3^-$ to N$_2$O. The pH values after autoclaving were 7–8 for all samples.

Sample-ID	δ^{15}N-N$_2$O measured [‰]	δ^{15}N-NO$_3^-$ calculated [‰]
	-15.68	-29.81
1	-16.96	-32.67
	-16.94	-32.63
	-15.58	-29.59
2	-16.93	-32.60
	-16.95	-32.64
	-17.97	-34.91
3	-18.54	-36.19
	-18.56	-36.23
	-16.96	-32.67
4	-17.32	-33.46
	-17.09	-32.95
	-15.88	-30.26
5	-16.06	-30.65
	-15.35	-29.07

Conclusion and outlook

The first experiments provided promising results. I am confident that an in-situ method to determine ^{15}N of NO in forest ecosystems can be developed on the basis of the present idea. However, at first the development in the laboratory has to receive priority to obtain satisfying reproducibility and consistence between ^{15}N values of NO and NO$_3^-$. Eventually, the developed method has to be applied to conditions in the forest. A pre-test at the Solling spruce stand revealed that further difficulties may occur. First, temperatures close to room temperature are necessary to avoid crystallisation of the salt, which requires for a heating system. Second, high carbon dioxide emissions resulting from soil respiration may lower the pH of the K$_2$S$_2$O$_8$ solution and thus may make the oxidant useless. Concluding, there is still a lot to be done!

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Appendix B

The number of control chambers for ^{15}N-N$_2$O measurements was reduced in favour of treated chambers, since temporal as well as spatial variability of emitted natural ^{15}N in our studied forest stands was low compared to the expected variability in emitted ^{15}N in the labelled chambers, which we concluded from previous measurements in the beech stand (Fig. A-1). For assessment of temporal variability, mean values of the control chambers between different experiments within a stand (purple columns) have to be compared. To assess spatial variability, the control chambers among each other within an experiment (blue columns) as well as with the values of other chambers used to measure natural ^{15}N emissions (yellow columns) have to be compared. As an example, in the one-year experiment 2007–08, the coefficients of variation of the control chambers ($n = 3$) were 0.9% and 2.7% in the beech and spruce stands, respectively. The corresponding coefficients of variation for the NH$_4^+$-labelled and the NH$_4$NO$_3$-labelled chambers (each $n = 5$; data not shown) were 15.0% and 20.1% in the beech stand and 10.2%, and 23.9% in the spruce stand.
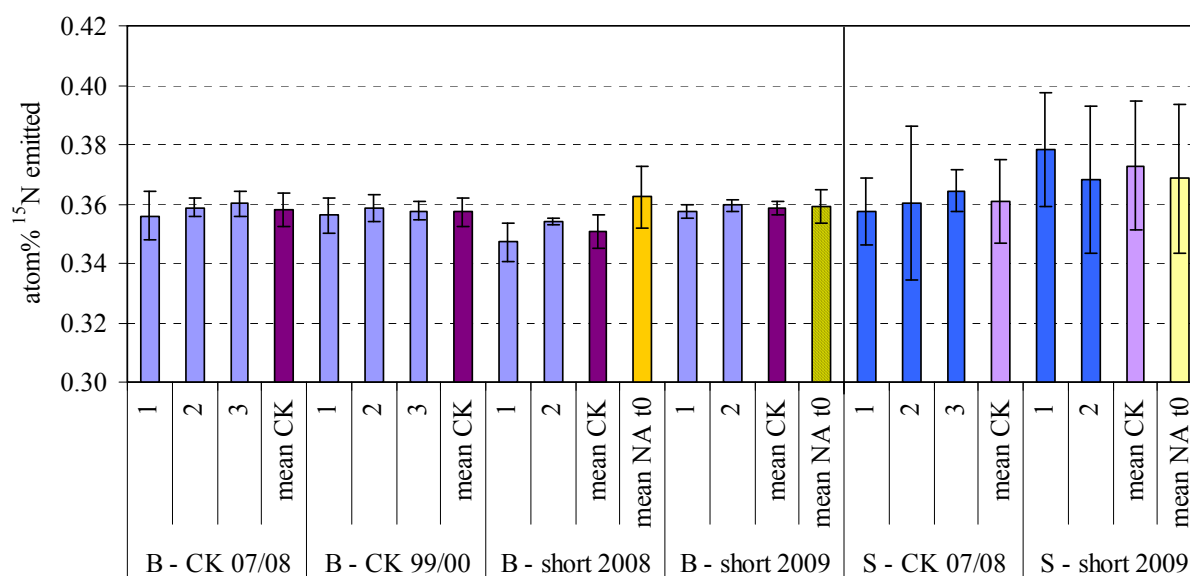

Figure A-1: Natural abundance of ^{15}N emitted (mean ± standard deviation) of the beech (B) and spruce (S) stands at the Solling. Data derived from our one-year experiments in the beech and spruce stands in 2007–08 (CK 07/08), from another long-term experiment in our beech stand conducted by us in 1999–2000 (CK 99/00), from a short-term (3 weeks) experiment in our beech stand, which we conducted in summer 2008 (short 2008), and from our short-term experiment in summer 2009 in the beech and spruce stands (short 2009). We used the same control chambers (CK 1–3; blue columns) per stand for every experiment. The purple columns indicate the means of these control chambers of each experiment. The yellow columns indicate the mean of chambers where the natural abundance was measured before labelling (mean NA t0; each $n = 8$). Please note that the y-axis does not start at zero.

Declaration of Originality and Certificate of Authorship

I, Nadine Eickenscheidt, hereby declare that I am the sole author of this dissertation entitled "Origin and regulation of soil N_2O and NO_x fluxes from coniferous and deciduous temperate forests exposed to chronic high N depositions". 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, 4, 5, and 6 have been written by me as first author.

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