Dynamics and underlying processes of N_2O and NO soil-atmosphere exchange under extreme meteorological boundary conditions

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SUMMARY

Climate models predict an increasing frequency and intensity of summer drought periods with subsequent heavy rainfall or soil frost and thaw events in mountain regions of Central Europe. These indirect effects of global warming may considerably influence soil microbial processes and in consequence emissions of climate-relevant trace gases. Regarding the nitrogen cycle, N_2O and NO emissions are of concern, since they are involved in climate warming and soils represent a main source for these two gases. In spite of a growing number of studies on this subject, knowledge on effects of climate change on soil N_2O and NO emissions is still scarce. This is mainly due to a hitherto poor mechanistic understanding of underlying processes within soil.

In this thesis, the impact of extreme meteorological boundary conditions on N_2O and NO fluxes in a Norway spruce forest and an acidic fen in the Fichtelgebirge area was investigated. The summer drought period and precipitation were experimentally increased in the forest and the fen over a 2-year span. Soil frost was induced in the forest by removal of the natural snow cover. The experiments were run in three replicates each and non-manipulated plots served as controls. Throughout the experiments, N_2O and NO fluxes were recorded in weekly to monthly intervals. In addition, N_2O concentrations and isotope signatures in soil air were measured along soil profiles to identify and localise the underlying biogenic production and consumption processes.

Prolonged drought continuously reduced the N_2O emission from the forest soil and even turned the soil temporarily into a sink for atmospheric N_2O. Soil freezing and thawing caused a burst of N_2O release contributing 84 % of the annual emission.

Soil air N_2O concentration and stable isotope profiles provide a new mechanistic explanation tool for all of these findings. N_2O concentration in the soil air decreased in most cases exponentially from the subsoil to the soil surface. This observation identifies microbial activity in the subsoil (at ≥ 70 cm soil depth) as an additional source for N_2O and diffusion to the soil surface along a concentration gradient. Furthermore, isotope abundance analysis identified simultaneous microbial N_2O consumption (reduction to N_2). Drought reduced the source strength of the organic layers for N_2O while simultaneously the sink function of the mineral soil for N_2O remained active. This resulted in a net N_2O sink func-

tion of the forest soil under severe drought. Frost in the topsoil was the only exception for these trends in N_2O concentration and isotope signature along soil profiles. Under conditions of soil frost the topsoil served no longer as a sink for N_2O, thus leading to the observed burst in N_2O emission.

NO emissions from the forest soil exceeded the N_2O emissions by up to two orders of magnitude. Prolonged drought in- or decreased NO emissions depending on the soil moisture content of the organic layers. Wetting after long-lasting drought periods – which turned out to be of less importance regarding N_2O fluxes – strongly increased biogenic NO emissions and contributed 44 % to the annual loss. In contrast to the forest soil, NO fluxes from the fen were always one to two orders of magnitude lower than the N_2O fluxes. These results support earlier findings that this highly reactive gas is either only marginally produced in the fen soil or undergoes chemical conversion before escaping from the soil surface. Nevertheless, water table reduction resulted in significantly increased net NO emission.

Regarding N_2O, this thesis suggests that summer drought periods may drastically increase emissions from minerotrophic fens depending on the reduction of water table height. Furthermore, heavy rainfall following drought periods caused short lived, but strong N_2O peaks having significant impact on the annual N_2O loss, that have not been reported so far. ^{15}N and ^{18}O isotope data provide evidence that these N_2O peaks are due to newly produced N_2O in the upper soil.

This thesis documents the huge impact of extreme weather events on soil N_2O and NO emissions and provides so far scarcely considered mechanistic explanations for these observations. A major outcome of this work is the finding of a hitherto unconsidered sink function of forest soils for atmospheric N_2O, when soil net N_2O production is compensated for by net consumption during long-lasting droughts. This work underlines the importance of investigating the fate of N_2O within soil profiles next to flux measurements to improve the current knowledge on the complex interactions between meteorological boundary conditions and soil biogenic processes and thus help further upgrading global N_2O balances.

ZUSAMMENFASSUNG

Klimamodelle prognostizieren eine zunehmende Häufigkeit und Intensität von Sommertrockenheit und Starkregenereignissen sowie von Bodenfrost und Auftauereignissen für Bergregionen Mitteleuropas. Diese indirekten Effekte der globalen Erwärmung werden mikrobielle Prozesse im Boden und somit Emissionen klimarelevanter Spurengase beträchtlich beeinflussen. Bezüglich des Stickstoffkreislaufes sind besonders N_2O- und NO-Emissionen von Bedeutung, da beide Spurengase an der Klimaerwärmung beteiligt sind und Böden eine Hauptquelle für beide darstellen. Trotz zunehmender Forschungstätigkeit zu diesem Thema ist der derzeitige Wissensstand über die Auswirkungen des Klimawandels auf bodenbürtige N_2O und NO Emissionen jedoch gering. Ursache dafür ist vor allem mangelnde Kenntnis über die zugrunde liegenden Prozesse im Boden.

In dieser Arbeit wurden die Auswirkungen extremer meteorologischer Randbedingungen auf N_2O- und NO-Flüsse in einem Fichtenwald und in einem Niedermoor im Fichtelgebirge untersucht. In zwei aufeinander folgenden Jahren wurden Sommertrockenheit und Niederschlag im Wald und im Niedermoor experimentell verstärkt. Bodenfrost wurde im Wald durch Entfernung der natürlichen Schneedecke induziert. Die Experimente wurden in jeweils drei Wiederholungen durchgeführt; nicht-manipulierte Flächen dienten als Kontrollen. Zusätzlich zu den N_2O- und NO-Flussmessungen in wöchentlichen bis monatlichen Intervallen wurden Konzentrationen und Isotopensignaturen des N_2O in der Bodenluft entlang von Bodenprofilen analysiert, um die zugrunde liegenden biogenen Produktions- und Konsumptionsprozesse zu identifizieren und zu lokalisieren.

Andauernde Sommertrockenheit verringerte kontinuierlich die N_2O-Emissionen vom Waldboden und wandelte den Boden sogar vorübergehend in eine Senke für atmosphärisches N_2O. Gefrieren und Auftauen des Bodens verursachten N_2O-Emissionen, die 84 % der jährlichen Emission umfassten.

N_2O-Konzentrations- und Isotopenprofile der Bodenluft liefern essentiell neue mechanistische Erklärungen für diese Beobachtungen. Die N_2O-Konzentration in der Bodenluft nahm in den meisten Fällen exponentiell vom Unterboden zur Bodenoberfläche ab. Dies verweist auf mikrobielle Aktivität im Unterboden (in ≥ 70 cm Bodentiefe) als zusätzliche Quelle für N_2O sowie auf Diffusion zur Bodenoberfläche entlang des Konzentrationsgra-

dienten. Isotopenanalysen zeigten weiterhin simultane mikrobielle N_2O-Konsumption (Reduktion zu N_2) auf. Dürre verringerte die Quellenstärke der organischen Auflage für N_2O, während die Senkenfunktion des Mineralbodens für N_2O aktiv blieb. Dies führte zu einer Netto-N_2O-Senkenfunktion des Waldbodens unter extremer Trockenheit. Frost im Oberboden war die einzige Ausnahme für diesen Verlauf der N_2O-Konzentration und Isotopensignatur entlang von Bodenprofilen. Unter Bodenfrost-Bedingungen fungierte der Oberboden nicht mehr als Senke für N_2O, was zu den beobachteten N_2O-Emissionsspitzen führte.

Die NO-Emissionen des Waldbodens überstiegen die N_2O-Emissionen um bis zu zwei Größenordnungen. Trockenheit erhöhte oder verringerte die NO-Emissionen in Abhängigkeit vom Bodenwassergehalt der organischen Auflage. Wiederbefeuchtung nach andauernden Dürreperioden - die sich hinsichtlich der N_2O-Emissionen als wenig bedeutsam herausstellte – erhöhte deutlich die NO-Emissionen mit einem Beitrag von 44 % an der jährlichen Emission. Im Gegensatz zu dem Waldboden waren die NO-Flüsse im Niedermoor immer um ein bis zwei Größenordnungen niedriger als die N_2O-Flüsse. Dies bestätigt, dass dieses hochreaktive Gas entweder kaum im Moorboden produziert wird oder chemischen Umwandlungen ausgesetzt ist, bevor es dem Boden entweicht. Dennoch hatten Wasserstandsabsenkungen signifikant erhöhte NO-Emissionen zur Folge.

Zum N_2O wird in dieser Arbeit gezeigt, dass Sommertrockenheit Emissionen von nährstoffreichen Niedermooren in Abhängigkeit von der Wasserstandsabsenkung um ein Vielfaches steigern kann. Starke Niederschläge verursachten im Anschluss an Dürreperioden kurzfristige, aber stark erhöhte N_2O-Emissionen mit signifikanter Auswirkung auf die jährliche N_2O-Emission. Dies war bisher unbekannt. ^{15}N- und ^{18}O-Isotopendaten belegen, dass diese N_2O-Peaks auf oberflächennahe Neubildung von N_2O zurückgehen.

Diese Arbeit dokumentiert einen starken Einfluss extremer Wetterereignisse auf bodenbürtige N_2O- und NO-Emissionen und bietet bislang zu wenig betrachtete mechanistische Erklärungen für diese Beobachtungen. Ein Hauptresultat dieser Arbeit ist der Beleg einer bisher unbeachteten Senkenfunktion des Waldbodens für atmosphärisches N_2O, sobald bei andauernder Trockenheit die Netto-N_2O-Produktion des Bodens durch den Nettoverbrauch kompensiert wird. Die Ergebnisse dieser Arbeit verdeutlichen die Relevanz von Untersuchungen zur N_2O-Dynamik im Boden zusätzlich zu N_2O-Flussmessungen, um die komplexen Interaktionen zwischen meteorologischen Randbedingungen und biogenen Bodenprozessen besser verstehen und in globale N_2O-Bilanzen einbinden zu können.

CHAPTER 1

On this thesis

Background

Soil N_2O and NO emissions

Despite its low atmospheric concentration of 319 ppbv (global mean concentration in 2005; IPCC 2007a), N_2O has been identified as the third most important greenhouse gas following CO_2 and CH_4, contributing about 6 % to the total observed global warming at present (WMO 2006). Its global warming potential per molecule is around 320 times larger compared to CO_2 on a 100-year time span – partly due to its extremely long atmospheric lifetime of approximately 114 years (Rodhe 1990). N_2O is further on of special concern, since atmospheric concentration of this greenhouse gas has risen by about 18 % from pre-industrial values and increases nearly linearly since the past few decades by a rate of 0.2 to 0.3 % a^{-1} (IPCC 2007a). It also plays a significant role in atmospheric photochemical reactions that contribute to stratospheric ozone depletion (Cicerone 1987). Up to now, these reactions – resulting in destruction of atmospheric N_2O in the stratosphere – are considered to be the only sink for N_2O in global models (IPCC 2007a).

NO is a relatively short-lived molecule with a mean lifetime of approximate 1.5 d (Yamulki *et al.* 1995) and with a concentration in clean air of less than 1 ppbv (Carroll *et al.* 1992, Harrison *et al.* 2000). It plays, however, a crucial role in tropospheric chemistry (Crutzen 1979, Logan *et al.* 1981). NO is indirectly involved in global warming by taking part in reactions leading to the production of tropospheric ozone, a radiatively active greenhouse gas (Hall *et al.* 1996). Additionally, NO contributes, due to wet and dry deposition, significantly to the N-saturation of natural ecosystems (Aber *et al.* 1989) in turn leading to concomitant emissions of N trace gases (Davidson & Kingerlee 1997, Fenn *et al.* 1996, Papen & Butterbach-Bahl 1999, Pilegaard *et al.* 2006).

In spite of considerable uncertainties, there is substantial evidence that both N_2O and NO are to a large amount produced in soils. With regard to N_2O, up to 10 Tg N a^{-1} (Olivier *et al.* 1998) of the N_2O emitted into the atmosphere are estimated to originate from soils, corresponding approximately 70 %, with natural soils being considered the largest source. However, the sum of all known N_2O sources at present does not balance the stratospheric N_2O sink and the tropospheric accumulation of N_2O as it's not accounting for the lack of about 3 Tg N_2O-N a^{-1} from the atmosphere (IPCC 2007a). This indicates that currently either the N_2O sources are overestimated or that the N_2O sink is underestimated. The quantification of global NO emissions from soils is even more uncertain, ranging between 5.5 to 21 Tg N a^{-1}, the upper figure being equal to the amount of NO originating from burning of fossil fuels (Davidson & Kingerlee 1997, Holland *et al.* 1999).

Soil N_2O and NO emissions primarily originate from microbial nitrification, nitrifier denitrification and denitrification (see Fig. 1; Poth & Focht 1985, Remde & Conrad 1990, Granli & Bøckman 1994, Kester *et al.* 1997, Wrage *et al.* 2001), although other processes have been observed, too (Papen *et al.* 1989, Anderson *et al.* 1993). Nitrification and nitrifier denitrification are both mainly carried out by autotrophic nitrifying bacteria. The role of ammonia-oxidising Archaea, which were recently found to contribute to nitrification in soils, is still matter of debate (Treusch *et al.* 2005, Leininger *et al.* 2006). In the first stage of nitrification, NH_4^+ is oxidised to NO_2^-, whereby N_2O (Jiang & Bakken 1999) and possibly also NO (Hooper & Terry 1979) may be formed by the spontaneous disintegration of unstable intermediates. NH_4^+ oxidation requires O_2 and thus takes place in aerobic microsites of soils. Nitrifier denitrification is thought to occur under short-term O_2 limitation whereby NO_2^- is used as electron acceptor (Poth & Focht 1985, Remde & Conrad 1990). This reduction is considered to be similar to denitrification of heterotrophic denitrifiers, which reduce NO_3^- under low O_2 conditions. Both N_2O and NO are intermediates, and may thus form possible end products or are consumed during denitrification (including nitrifier denitrification).

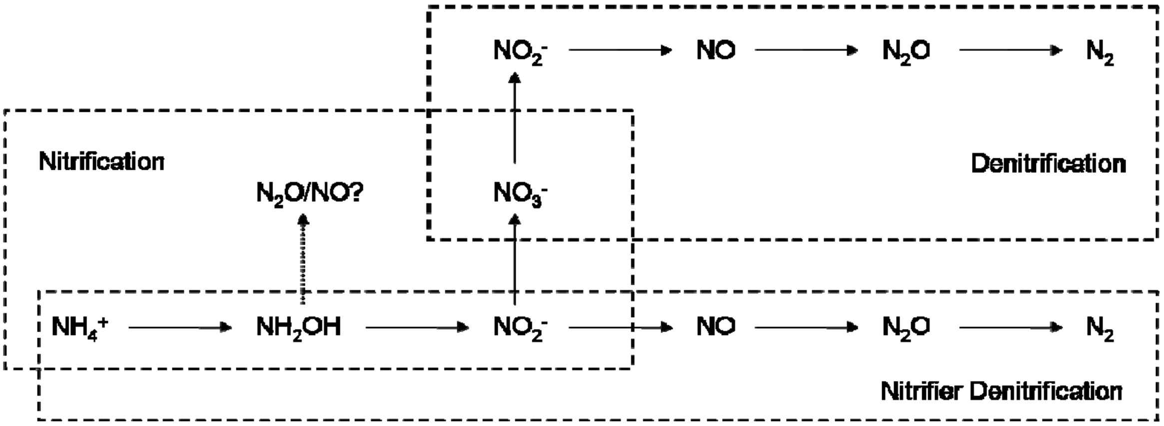

Figure 1. Overview of the processes producing N_2O and NO in soils (after Kool *et al.* 2009). The dotted arrow indicates possible formation of both N trace gases.

Nitrification as well as denitrification and thus the quantity of N_2O and NO fluxes between soil and atmosphere are heavily dependent on soil physical factors such as soil temperature and water content. Emissions of both N_2O (Smith *et al.* 1998) and NO (Slemr & Seiler 1984) increase with increasing soil temperature due to enhanced rates of enzymatic processes as long as other factors (e.g. moisture or substrate) are not limiting. Soil water acts as transport medium for the substrates NH_4^+ (nitrification) and NO_3^- (denitrification) and influences the rate of O_2 supply, thereby controlling whether aerobic processes such as nitrification or anaerobic processes such as denitrification dominate within the soil. An increase of drastic changes in meteorological conditions, as expected for this century (see below), is therefore thought to strongly influence N_2O and NO emissions from soils.

This study focuses on N_2O and NO emissions from two different (semi-) natural ecosystem types that are common in Central Europe – a Norway spruce forest and an acidic fen. In Europe, forest ecosystems which cover about $1.9*10^6$ km^2 or 28 % of the total land area (Ambus *et al.* 2006) have been identified to be significant sources for both N trace gases and may therefore have significant impact on European N_2O and NO emissions (Davidson & Kingerlee 1997, Pilegaard *et al.* 2006).

Peatlands cover only 3 % of the total land surface, but constitute important storage pools for organic carbon and nitrogen compounds with around 30 % of the world's terrestrial reserves (Martikainen *et al.* 1993). In contrast to ombrotrophic peatlands (bogs), minerotrophic types (fens) which are mainly widespread in Europe (Aselman & Crutzen 1989) have considerably high contents of nitrogen (up to 3 % N in dry peat substrate; Kuntze 1993). Thus, they are potential sources of nitrogen oxides, but conditions in waterlogged natural peatlands limit nitrification, which is a prerequisite for the formation of both N_2O and NO, either from nitrification or denitrification.

Climate change as expected from climate models

There is convincing evidence that the global climate is changing at an unprecedented rate (Free & Robock 1999, Levitus *et al.* 2001, IPCC 2007b). While exact predictions of future climate are uncertain, major computer models predict an average temperature increase between 1.8 and 4.0°C for the next century (IPCC 2007b). The reasons for present climate change have been discussed for decades. A large proportion of the observed rise in global temperatures has been attributed to human activities. The changes in the albedo of the earth's surface due to land use changes and the increasing greenhouse gas emissions have been identified as the main causes for global climate warming (Houghton 1994, Crowley 2000, IPCC 2007a). Besides temperature, climate change affects other weather parameters. Climate model simulations are generally in agreement with the hypothesis that the hydrologic cycle should intensify and become more variable with the climate change (Trnka *et al.* 2008), and extreme weather events (floods, hurricanes, droughts, etc.) are likely to occur more frequently. However, considerable uncertainty exists with regard to the specific character of climate change impacts, because most impacts will vary widely in scale, intensity and time of occurrence among different regions (IPCC 2007b).

Regional models of future climate change predict an increasing frequency and serverity of exceptional summer droughts and heatwaves in various parts of Central Europe (Kunstmann *et al.* 2004, Rowell 2005, Frei *et al.* 2006), in particular in sub-continental and continental regions (Wetherald & Manabe 2002, Schär *et al.* 2004, Good *et al.* 2006, IPCC 2007c). Less rainfall in combination with higher summer temperatures and elevated evaporative demand will increase soil water deficits and drought exposure. Furthermore, heavy precipitation events are very likely to increase in severity at least in some parts of the year (IPCC 2007c).

Meteorologists furthermore predict increases in winter temperatures for many regions of Central Europe (Hulme & Sheard 1999, Christensen & Christensen 2003, Johns *et al.* 2003, Rowell & Jones 2006). However, global warming in the frame of climate change might influence the intensity and frequency of soil frost depending on regional climate conditions. Whereas the intensity and frequency of soil frost will be reduced in some regions due to increased temperatures, decreasing snow cover in winter may result in an increase of the frequency and/or intensity of frost events at higher latitudes and in mountainous regions even though average winter air temperatures are increasing (Cooley 1990).

Potential feedbacks of soil N_2O and NO emissions to climate change

In terms of the predicted global climate change the balance of climate-relevant trace gas emissions has become more important in both scientific and political discussion. An important question in this context is: What impacts do potential feedback effects of the expected climate change have on the global balance of climate-relevant gases?

In the few available field studies on effects of the above mentioned predicted extreme weather events on N trace gas emissions from soils, it has been shown that drought/wetting and frost/thaw events might strongly influence N_2O and NO emissions from forest soils (see also Matzner & Borken 2008, Borken & Matzner 2009). With respect to wetlands, water table fluctuations seem to be of importance regarding exchange of N trace gases with the atmosphere, but there are too few (N_2O) or even no (NO) studies available to make valid conclusions. In the following paragraphs the hitherto state of knowledge on effects of these extreme meteorological changes on the fluxes of both trace gases between soil and atmosphere is outlined.

Forest soils – drought/wetting. Drought and wetting contrarily affect both N_2O and NO emissions from soils. Long-lasting drought periods generally reduce microbial activity in soils and thereby emissions of N_2O and NO (Kieft *et al.* 1987, Yamulki *et al.* 1995, Davidson *et al.* 2004, van Haren *et al.* 2005, del Prado *et al.* 2006). Against, rewetting of dry soils has often been found to result in emission peaks of these gases (Anderson & Levine 1987, Groffman & Tiedje 1988, Rudaz *et al.* 1991, Davidson 1992, Davidson *et al.* 1993, Scholes *et al.* 1997, Jørgensen *et al.* 1998, Xu *et al.* 1998, Ludwig *et al.* 2001, Priemé & Christensen 2001) and therefore bears the possibility of an additional significant gaseous N loss. This general trend seems to be true for a wide range of ecosystem types including temperate forests (Ludwig *et al.* 2001, Borken & Matzner 2009).

Both carbon and nitrogen mineralisation rates are generally enhanced for a few days after rewetting of dry soil (Birch 1958, Bloem *et al.* 1992, Cui & Caldwell 1997, Franzluebbers *et al.* 2000). A sudden increase of the microbial activity after rewetting has been so far attributed to a reconstituting mineralisation of soil organic matter. Death of microorganisms during drought or rewetting or exposure of previously protected organic matter may additionally lead to an increase of easily decomposable organic material following rewetting (Groffman & Tiedje 1988, van Gestel *et al.* 1991). The N_2O and NO production may thus be stimulated by the increased availability of nitrogen and in the case of denitrification additional by easily available organic carbon (Mummey *et al.* 1994, Włodarczyk 2000). So far, it is still a matter of debate whether drying or wetting has the larger potential to influence cumulative N trace gas emissions from soils. Nevertheless, the majority of studies in this context have focused on the often strong, but short-lived emission bursts following rewetting, although there is increasing evidence that prolonged drought events might be of greater relevance – at least regarding N_2O emissions – for the annual budget (Borken & Matzner 2009).

Forest soils – frost/thaw. In general, microbial activities, including nitrification and denitrification, are greatest during seasons with high temperatures (Sommerfeld *et al.* 1993). However, N_2O emissions have shown a great temperature anomaly and several studies have demonstrated the triggering effect of freezing and thawing on the emissions of N_2O from soils (Burton & Beauchamp 1994, Papen & Butterbach-Bahl 1999, Teepe *et al.* 2000, Teepe & Ludwig 2004, Groffman *et al.* 2006).

Explanations for N_2O emission peaks occurring during soil frost are often contradictory in the literature. With regard to agricultural soils, explanations range from N_2O production in unfrozen subsoil and the escape of N_2O (Burton & Beauchamp 1994, Kaiser *et al.* 1998) to microbial activity together with N_2O production in unfrozen compartments at the soil surface (Goodroad & Keeney 1984, Teepe *et al.* 2001). Regarding forest soils it is generally assumed that N_2O production occurs predominantly in the organic layers (Menyailo & Huwe 1999, Pihlatie *et al.* 2007). Accordingly, N_2O peaks during soil frost have been attributed to enhanced N_2O production in the organic layers. That was explained by substrate accumulation in small water films resulting in enhanced microbiological activity in the unfrozen soil water (Papen & Butterbach-Bahl 1999, Teepe *et al.* 2000). Peak emissions during forest soil thawing have until now been explained by stimulated microbial activity due to an enhanced supply of nutrients caused by death of microbial biomass and/or disruption of aggregates during soil frost (Papen & Butterbach-Bahl 1999, Neilsen *et al.* 2001, Teepe *et al.* 2000).

The quantitative importance of forest soil freezing and thawing for annual stand-level N_2O budgets is highly uncertain. Nonetheless, estimates from the few field investigations indicate that such events may contribute up to 70 % to the annual N_2O emissions (Papen & Butterbach-Bahl 1999, Teepe *et al.* 2000).

In contrast, results from laboratory experiments investigating topsoil fluxes of similar forest soil types, in some cases even from the same study site, showed much smaller N_2O bursts than observed in field studies during soil frost events (Neilsen *et al.* 2001, Teepe & Ludwig 2004, Goldberg *et al.* 2008). These contradictory findings from field and laboratory investigations may be due to experimental designs in laboratory experiments that do not reflect natural conditions (Henry 2007). But they may also indicate a minor contribution of the organic layers to the total soil N_2O emissions from at least several temperate forest soils during freezing and/or thawing (Teepe & Ludwig 2004, Goldberg *et al.* 2008).

Regarding NO only very few studies on freeze/thaw effects have been done so far, but peaks of NO emissions during soil freezing and thawing – as reported for N_2O – have not been observed (Koponen *et al.* 2006, Goldberg *et al.* 2008). These studies showed that in contrast to N_2O, NO emissions from soil were strongly temperature-driven. Furthermore, the high reactivity of NO and its short lifetime (Yamulki *et al.* 1995) impedes accumulation of NO in unfrozen water or under the frozen topsoil in contrast to the chemically rather inert gas N_2O.

Peatlands – water table fluctuations. Although peatlands are large storage pools for nitrogen and carbon, and thus have the potential to emit N_2O and NO, conditions in waterlogged natural peatlands inhibit emissions of both N trace gases. However, long-term drainage of minerotrophic peat soils for agriculture or forestry has been shown to drastically increase N_2O (Martikainen *et al.* 1993, Augustin *et al.* 1998, Merbach *et al.* 2001) and NO emissions (Lång *et al.* 1995). This is due to enhanced nitrification and in consequence possibly also denitrification rates with increasing aeration of peat soils (Updegraff *et al.* 1995).

Groundwater levels are predicted to lower during the summer months at northern midlatitudes in future due to enhanced evaporation and decreased precipitation as a result of climate warming (Manabe & Wetherald 1986). The groundwater table is an important regulator for N_2O and NO emissions due to its influence on oxygen concentration and the diffusion of trace gases produced in the soil into the atmosphere.

So far, effects of short-term water table fluctuations on N trace gas emissions from wetlands are far from being well understood. To my knowledge only two studies on N_2O emissions during short-term water table reduction of virgin fens exist (Freeman *et al.* 1993, Regina *et al.* 1999). They suggest a low potential of fens for significant increase of the N_2O source function due to short dry periods. Both studies reported on a sudden drop of N_2O emissions associated with the rising water table in the drained peats. However, these studies were conducted on boreal peatlands, and may be not indicative for temperate fens in Central Europe.

Research challenges

So far, only few manipulative experiments have been undertaken *in-situ* to assess the changes of N_2O and NO emissions from soils following drying/wetting and freezing/thawing periods. Some conclusions may be deduced from natural observations although many interacting parameters vary between different years and comparison to a control is not possible.

A growing number of laboratory studies have been performed during recent decades to assess the effects of drying and wetting or soil freezing and thawing on soil N gas emissions. However, results from laboratory studies are mainly inconsistent, most likely because of varying experimental designs, soil properties and treatments (Henry 2007).

Furthermore, flux measurements of N_2O between soil surface and atmosphere do not suffice to trace back to the underlying processes within the soil. Whereas broad evidence exists that very shallow surface layers are the primary production zone for the highly reactive trace gas NO (Johansson & Granat 1984, Ludwig *et al.* 1992, Rudolph *et al.* 1996, Rudolph & Conrad 1996, Yang & Meixner 1997), this is obviously not the case with regard to the chemically rather inert gas N_2O (Burton & Beauchamp 1994, Pérez *et al.* 2000, van Groenigen *et al.* 2005, Rock *et al.* 2007, Goldberg *et al.* 2008). The N_2O fluxes between soil and atmosphere thus represent the net effect of production and/or consumption processes that may occur simultaneously in different soil depths, of diffusion of N_2O along concentration gradients, and of N_2O dissolving in or degassing from soil water. A mechanistic interpretation of N_2O surface fluxes therefore requires solid information about underlying soil processes.

Large variations of *in-situ* N_2O and NO fluxes between soil and atmosphere in different types of ecosystems emphasise the need of supplementary field data. It is furthermore of outstanding importance to improve our knowledge about soil processes controlling aboveground fluxes for revision of regional and global N_2O and NO budgets and for a better understanding of the effects of climate change on N_2O and NO emissions from soils.

Use of N_2O isotope abundance analyses within soil profiles. Since different biological and physical processes are characterised by distinct changes in the isotopic composition of the soil N_2O pool (Pérez 2005), $\delta^{15}N$ and $\delta^{18}O$ abundance analysis together with N_2O concentration measurements along soil profiles has been suggested as a suitable tool to elucidate N_2O governing processes and their dynamics within soils (van Groenigen *et al.* 2005, Rock *et al.* 2007). Enrichment factors (isotope enrichment of a reaction product relative to that of the substrate) of the microbial processes are generally assumed to be larger than for physical processes (Pérez 2005). Bacterial production of N_2O by nitrification and denitrification yields N_2O depleted in ^{15}N relative to its respective source, whereas reduction of N_2O to N_2 enriches the remaining N_2O in both ^{15}N and ^{18}O (Pérez 2005, Barford *et al.* 1999). Diffusion also fractionates against the heavy isotopes (Pérez *et al.* 2000, Pérez 2005), resulting in stepwise ^{15}N and ^{18}O depletion of N_2O along a concentration gradient. Shifts in $\delta^{18}O$ during production processes are currently under debate (Pérez 2005, Toyoda *et al.* 2005). Most recent findings that the ^{18}O signature of N_2O from both nitrification and denitrification is mainly determined by H_2O due to O exchange between nitrogen oxides and H_2O further impedes interpretation of $\delta^{18}O$ values of soil N_2O (Kool *et al.* 2009).

So far only few stratified N_2O concentration and isotope abundance analyses have been conducted in soils to investigate N_2O dynamics within soils (Mandernack *et al.* 2000, Pérez *et al.* 2000, van Groenigen *et al.* 2005, Rock *et al.* 2005). However, localisation of production and consumption processes within soils is essential to investigate effects of climate change events on N_2O emissions, since knowledge about depths of these processes will allow for more specifically relating them to soil environmental factors.

Objectives

This study was conducted within the framework of the Research Group 562 ‘Dynamics of soil processes under extreme meteorological boundary conditions’ of the German Research Foundation (DFG), comprising soil scientists, hydrologists, microbiologists and plant ecologists with the major aim of investigating the consequences of extreme meteorological boundary conditions for element turnover in two different (semi-) natural ecosystems, a Norway spruce forest and a temperate acidic fen. The specific objectives of this thesis are:

(1) to propose and test suitable methods for gas sampling for further analyses on N_2O concentrations and isotope abundances under extremely divergent conditions; in a water-saturated fen and in a well aerated forest soil and to analyse the applicability of this tool for process identification within these soils

CHAPTER 2

(2) to quantify the effect of summer drought followed by heavy rainfall on the fluxes of N_2O and NO between a Norway spruce forest soil and the atmosphere – and to investigate the processes governing the N_2O pools within soil profiles and their temporal dynamics

CHAPTER 3

(3) to quantify N_2O fluxes during soil freezing/thawing – and to identify underlying reasons for the phenomenon of N_2O bursts during soil freezing and thawing

CHAPTER 4

(4) to quantify the effects of water table fluctuation due to long-lasting summer drought and subsequent heavy rainfall on the exchange of the trace gases N_2O and NO between a temperate acidic fen and the atmosphere – and to identify and localise the underlying biogenic processes along the soil profile

CHAPTER 5

The following field experiments were carried out by the Research Group and aided me in achieving my objectives 2-4:

(2) Summer drought was induced by roof constructions over a period of 46 days in two consecutive years. In the first year of the experiment, the soil was subsequently rewetted by artificial throughfall solution, simulating a heavy rain event. In the second year wetting occurred naturally. The experiment was run in three replicates and three non-manipulated plots served as controls.

(3) In a below-average cold winter, the natural snow cover was removed on three experimental plots to induce soil frost. Three plots with natural snow cover served as controls.

(4) Summer drought was induced in the fen over periods of 6 and 10 weeks, respectively, over a 2-year span by means of a roof construction and a drainage system on three treatment plots. At the end of the drought experiments, the plots were rewetted, simulating a heavy rain event. Three control plots were not treated at all. Due to a moisture gradient within the peatland the three drought (D) and control (C) plots could not be treated as true replicates. This gradient resulted in reduction of water table heights between 10 and 30 cm (C1-D1=10 cm, C2-D2=20 cm, C3-D3=30 cm reduction). Because of these differences in water level heights the parameters for C1-D1, C2-D2 and C3-D3 were compared.

Soil N_2O and NO fluxes between soil and atmosphere were recorded throughout the experimental time (2 years) in weekly to monthly intervals (see CHAPTERS 3-5).

In addition to the N_2O flux measurements, in each plot N_2O concentrations and isotope signatures in soil air were measured in six different soil depths from 5 to 70 cm at the forest site to identify and localise processes governing the N_2O pool and N_2O dynamics within the soil. In the fen, dissolved N_2O concentrations in the soil solution (water-saturated conditions) or in soil air (water-unsaturated conditions), respectively, and isotope abundances were measured along soil profiles in six different depths from 10 to 60 cm.

Synopsis

N_2O concentrations and isotope signatures along soil profiles (CHAPTER 2)

Despite the obvious need to examine soil as a three-dimensional body regarding production, consumption, transport processes and storage of N_2O, there exist only few studies which investigated N_2O dynamics within soils (see Clough *et al.* 2005, Kellman & Kavanaugh 2008) and to my knowledge only four studies which used stratified N_2O concentration and isotope abundance analyses (Mandernack *et al.* 2000, Pérez *et al.* 2000, van Groenigen *et al.* 2005, Rock *et al.* 2007). The manuscript in CHAPTER 2 is the first to report on time series of N_2O concentration gradients and respective N_2O stable isotope signatures at natural abundance level along soil profiles in (semi)natural ecosystems.

One difficulty regarding the isotope abundance analysis of N_2O in air samples is the required considerably large sample size of about 50 ml, if concentrations are close to ambient air (Brand 1995). The soil gas samplers proposed in this manuscript were constructed to meet these and the different requirements of a water-saturated and an unsaturated soil, respectively. In addition to the spatio-temporal distribution of N_2O and $\delta^{15}N$ and $\delta^{18}O$ signatures of N_2O in soil profiles, net N_2O production/consumption was calculated to identify the predominant process (production, consumption or diffusion) controlling the soil N_2O pool for individual depth intervals as proposed by Rock *et al.* (2007) for verification.

In the waterlogged fen, with a diffusion coefficient of N_2O being four orders of magnitude smaller than in air (Heincke & Kaupenjohann 1999, and references therein), and thus allowing for only very low N_2O exchange rates between different soil depths, isotopic shifts together with changes in N_2O concentrations suggested overarching processes regarding soil N_2O pools as confirmed by the turnover calculations. Highest net N_2O production occurred at 30 to 50 cm soil depth. From the strong correlation between $\delta^{15}N$ and $\delta^{18}O$ of N_2O in the fen soil throughout the year, it was concluded that at any time N_2O is to a large amount further reduced to N_2, since production processes do not result in correlation between both parameters (Sutka *et al.* 1998, Pérez *et al.* 2000, Toyoda *et al.* 2005, Menyailo & Hungate 2006, Kool *et al.* 2009) in contrast to consumption (Menyailo & Hungate 2006). This is in agreement with earlier findings that N_2 emissions from waterlogged soils could have a major share in total N gaseous loss and N_2O emissions might only be of minor importance (Well *et al.* 2001, Mander *et al.* 2003, Velty *et al.* 2007).

In the forest soil, a clear vertical pattern of N_2O concentration profiles was observed. Throughout the year, N_2O concentrations peaked at the deepest investigated soil depth (at about 70 cm). This coincided with lowest $\delta^{15}N$ values observed in this horizon, suggesting N_2O production being the predominant process in the subsoil of the forest site. However, calculated net N_2O production was up to 20-fold lower here compared to the topsoil and the high concentrations were presumably a result of both, small pore volume and low diffusivity in the subsoil. The findings of steadily increasing $\delta^{15}N$ values together with decreasing N_2O concentrations towards the topsoil throughout the year suggests consumption of N_2O during upward diffusion of N_2O from the site of production. Indeed, integrated turnover calculations indicated consumption to be the dominating process in the mineral soil towards the border to the organic layers throughout the year, but they also revealed that there was a more complex pattern of net production and consumption processes. Considering the inherent uncertainty of the calculated diffusion coefficients for unsaturated soils, due to problems in determination of the exact volumetric gas content of the soil (Jin & Jury 1996), rapid exchange of soil gas with the atmosphere may presumably have obscured larger differences in the isotopic signatures as expected from net production/consumption processes within the soil profile. Only in summer, coinciding with the highest N_2O net production in the topsoil, diffusive fluxes could not equal out $\delta^{15}N$ and $\delta^{18}O$ of topsoil and atmospheric N_2O.

This study documents the value of N_2O concentration and isotope abundance analysis along soil profiles as a tool for localisation and identification of N_2O governing processes. This new tool may extend our knowledge on processes affecting surface N_2O fluxes, but the results of this study additionally suggest that isotope effects of small turnover processes can be obscured by diffusive exchange. So far, the few studies on stratified N_2O concentration and isotope abundance analysis did not or just marginally consider distinct diffusive fluxes along soil profiles. This study underlines that a detailed understanding of N_2O turnover in soils also requires knowledge about diffusive fluxes and surface exchange – especially in aerated soils with low N_2O pools. The results of this study, while not allowing quantification of gross production and consumption of N_2O, provide evidence for an important role of N_2O consumption processes within both soil types on net surface fluxes that is hardly considered so far, at least regarding aerated soils. These observations emphasise the potential of these and probably also other soil and ecosystem types to act as sinks for atmospheric N_2O, which may have important implications for global budgets.

Forest soil N_2O and NO emissions as affected by drought/rewetting (CHAPTER 3)

In the first year of the throughfall exclusion experiment (2006; CHAPTER 3A), drought turned the Norway spruce forest soil from an N_2O source to a transient sink for atmospheric N_2O. This N_2O sink function even increased with ongoing soil drought and was also observed on the controls during natural drought. This remarkable result may have considerable importance for quantitative estimates of N_2O emissions from soils (see also Billings 2008). These findings of a soil sink function for N_2O are exciting for two reasons:

(1) So far the only sink for N_2O considered in global models is the destruction of atmospheric N_2O in the stratosphere through photolysis and photooxidation (IPCC 2007a). Despite this there is increasing evidence from the literature that soils can serve – at least temporarily – as sinks for atmospheric N_2O (Ryden 1981, Minami 1997, Wrage *et al.* 2004, Flechard *et al.* 2005, Neftel *et al.* 2007). However, in many publications, negative N_2O fluxes were neglected and not discussed or were treated as analytical errors (e.g. Verchot *et al.* 1999, Kamp *et al.* 2000, Glatzel & Stahr 2001, Erickson *et al.* 2002).

(2) Quantitatively relevant microbial N_2O consumption is only known to occur during denitrifying processes, i.e. during the reduction of N_2O to N_2. Thus, N_2O fluxes from the atmosphere into soils should mainly occur at high soil moisture contents, since the enzyme N_2O-reductase that is responsible for the reduction of N_2O during denitrification is more sensitive to O_2 than the other denitrification enzymes (Knowles 1982).

Very few reports in the literature point to a soil N_2O sink function under dry conditions (e.g. Donoso *et al.* 1993, Yamulki *et al.* 1995, Klemedtsson *et al.* 1997, Verchot *et al.* 1999, Flechard *et al.* 2005), and underlying reasons have not yet been identified.

The course of N_2O concentrations was – as described in the paragraph above – exponentially decreasing from sub- to topsoil. Shifts in the isotopic signatures of soil N_2O indicated further reduction of N_2O to N_2 being the process governing the N_2O pool in the top mineral soil. However, in this study net negative N_2O fluxes were accompanied by sub-ambient N_2O concentrations in the topsoil. But in this first study a definite explanation for the reason of this soil sink function during drought could not be given.

Throughfall exclusion in the second year (2007; CHAPTER 3B) again strongly affected N_2O fluxes from soil to atmosphere, resulting in continuously reduced N_2O fluxes from soil to atmosphere, but net negative fluxes have not been observed throughout the second experimental drought. In this year, N_2O concentration and isotope abundance analysis were continuously analysed to elucidate the effect of drought on N_2O dynamics within soil.

Isotope abundance analyses on N_2O again pointed N_2O consumption taking place throughout the soil profile. But in contrast to the year before N_2O concentrations in the top mineral soil were never below ambient in 2007. Since net N_2O consumption along the soil profile during throughfall exclusion were similar for both years, as deduced by turnover calculations, the different net N_2O fluxes between soil and atmosphere must have been due to different N_2O production in the organic layers in these two consecutive years. The difference in N_2O topsoil production between both years was most probably due to different soil moisture. Measurements revealed that the water-filled pore space (WFPS) in 6 cm depth of one TE plot (volumetric soil water contents were just measured on one plot) at the end of the experiment in 2007 was twice as high as in 2006 (28 % *versus* 13 % WFPS). In 2006, the experiment even started with a WFPS as high as at the end of the experiment in 2007.

N_2O production in the organic layers apparently decreased during drought in 2007, as indicated by continuously decreasing N_2O fluxes. Although N_2O consumption decreased as well with ongoing drought, it turned out to be the dominating process along the whole soil profile until the border to the organic layers throughout throughfall exclusion. The net sink strength of the mineral soil for N_2O is obviously less affected by drought as net production of N_2O in the organic layers, which is most likely due to the larger effect of drought on the water potential in the organic layers. Thus, it is concluded that this Norway spruce forest soil acts as a sink for atmospheric N_2O, when net N_2O production in the topsoil decreases below the level of the net N_2O consumption capacity of the soil. This is schematically visualised in Fig. 2.

Because of these results the question arises, whether drought may lead to transient N_2O uptake in other ecosystem types, too. Net N_2O consumption has been found to be the N_2O governing process within various soil types (Pérez *et al.* 2001, van Groenigen *et* *al.* 2005, Rock *et al.* 2007, Kellman & Kavanaugh 2008). Thus, it has to be investigated if the schematic model shown in Fig. 2 is also applicable to other ecosystems.

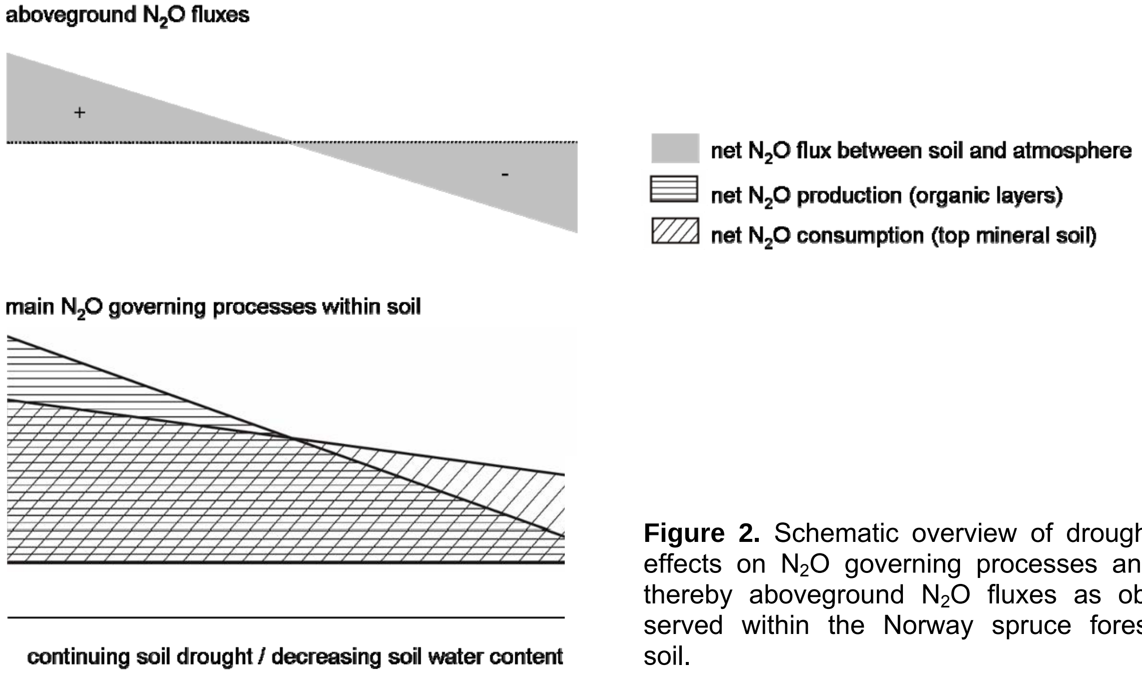

Figure 2. Schematic overview of drought effects on N_2O governing processes and thereby aboveground N_2O fluxes as observed within the Norway spruce forest soil.

Wetting of the dry soil immediately increased N_2O fluxes between soil and atmosphere in both years, but the effect of rewetting could not compensate for effects of the preceding drought period. The wetting periods did not enhance cumulative N_2O emissions compared to the controls, whereas throughfall exclusion resulted in significantly decreased total emissions by 23 %.

Regarding NO emissions (CHAPTER 3B), a different behaviour concerning soil drought was observed compared to N_2O: In 2006, NO fluxes continuously decreased throughout the throughfall exclusion, whereas NO fluxes at first increased with ongoing drought in 2007, but decreased at the end of the throughfall exclusion. This behaviour was related to the WFPS in 6 cm soil depth. NO fluxes reached a maximum at around 33 % WFPS and decreased towards higher and lower values. This is a reasonable result, since the relation between NO fluxes and water content is more complex compared to fluxes of N_2O, which in general increase at higher water contents as a result of higher denitrifying microbial activity (Wolf & Russow 2000, Pilegaard *et al.* 2006). At high soil moisture, gas diffusivity is limited and the aerobic microbial activity decreases with further wetting of the soil. Additionally, increasing residence time because of increasing soil moisture and slower diffusion facilitates the conversion of the highly reactive NO. At low soil moisture the microbial activity decreases with decreasing soil moisture due to limited substrate diffusion (Skopp *et al.* 1990, Pilegaard *et al.* 2006). Several studies presented maximum NO emissions at intermediate soil moisture in various ecosystem types including forest soils (Cardenas *et al.* 1993, Grundmann *et al.* 1995, Sullivan *et al.* 1996, Martin *et al.* 1998, Parsons *et al.* 1996, Pilegaard *et al.* 1999, van Dijk & Duyzer 1999, Peirce & Aneja 2000, van Dijk & Meixner 2001), but with a wide range of optimum moistures between 20 % and 85 % WFPS.

Whereas drought differently affected NO emissions due to dissimilar intensity, wetting following prolonged summer drought in both years resulted in large NO emission peaks contributing 44 % of the total emission of NO on the TE plots. These findings are in agreement with other studies that report on NO peaks due to addition of water to very dry soil (Yamulki *et al.* 1995, Scholes *et al.* 1997, Ludwig *et al.* 2001). These emission peaks seem to occur because of nutrient accumulation during soil drought and dormant, drought-stressed microorganisms that immediately utilise these nutrients after rewetting (Ludwig *et al.* 2001). This is confirmed by the fact that other natural rain events throughout the year did not increase NO emissions.

The annual emissions of N_2O and NO observed in these studies are within the range reported for other European forest soils (Butterbach-Bahl *et al.* 1997, Meixner *et al.* 1997, Pilegaard *et al.* 2006, and references therein). NO emissions were up to two orders of magnitude higher than N_2O emissions, at least in summer, as already reported for temperate forest soils (Butterbach-Bahl *et al.* 1997, Goldberg *et al.* 2008, Muhr *et al.* 2008).

Forest soil N_2O and NO emissions as affected by freezing/thawing (CHAPTER 4)

In contrast to drought/wetting, which contrarily affected fluxes of N_2O between soil and atmosphere, both soil freezing and thawing resulted in almost 10-fold enhanced N_2O fluxes on the snow removal (SR) plots contributing 84 % to the annual N_2O emissions. In the subsequent winter without soil frost N_2O emissions were not enhanced. This study supports the few hitherto findings of a huge relevance of soil frost and thaw periods on

N_2O losses from temperate forest soils to the atmosphere and emphasises that such winter fluxes have to be taken into account in global N_2O models.

The N_2O concentration profiles affected by soil frost differed from the general trend. While concentrations were still highest in subsoil and decreased towards the topsoil, N_2O concentrations in the soil atmosphere were by one to two orders of magnitude higher under conditions of frozen topsoil, compared to unfrozen control plots. The higher N_2O concentrations in the soil of the SR plots were asserted to be due to: 1) Continuing N_2O production in the (unfrozen) subsoil and 2) a decreased N_2O consumption during upward movement of subsoil-derived N_2O in the top mineral soil. This was shown by a shift in ^{15}N and ^{18}O in N_2O towards the topsoil as indicative for pure diffusion. The drop of N_2O consumption in the topsoil is most probably due to a reduced activity of the enzyme N_2O reductase, as observed for soil temperatures around and below 0°C (Holtan-Hartwig *et al.* 2002). 3) Additionally, hindered N_2O exchange between topsoil and atmosphere due to frozen soil water as diffusion barrier in the topsoil resulted in smaller detected N_2O fluxes between soil and atmosphere as to be expected from N_2O concentrations in the top mineral soil.

These findings of subsoil production of N_2O together with reduced N_2O consumption in the topsoil during soil frost contradict previous mechanistic explanations of soil frost effects on N_2O emission. This emphasizes the necessity to investigate N_2O dynamics along soil profiles in various temperate forest ecosystem types. Identification of parameters inducing a subsoil N_2O production and impeding N_2O consumption in the topsoil is necessary to improve the mechanistic understanding of N_2O freeze/thaw fluxes from these soils. Furthermore, our results give a hint for the reason of different findings in laboratory mesocosm experiments and field investigations on N_2O emissions associated with frost/thaw events in forest soils: Laboratory investigations focussed mostly on topsoil processes, and therefore, excluded potential subsoil N_2O production.

The observed N_2O emissions during soil frost in this study were of similar importance for the annual N_2O budget as N_2O emissions in the subsequent thawing period. It was suggested that the largest amount of N_2O released from the investigated spruce forest during soil thawing was due to a slow release of subsoil N_2O along the concentration gradient because of limited diffusion during soil thawing. Furthermore a delayed activation of N_2O reductase in the topsoil after soil frost is likely due to still low soil temperatures. Enhanced nutrient supply as a result of soil freezing can be excluded as a reason for the enhanced N_2O emissions, as reflected by concentration measurements of dissolved organic carbon and nitrogen solutes carried out throughout the experiment (Hentschel *et al.* 2009). This is also confirmed by Muhr *et al.* (2009), who did not observe enhanced CO_2 fluxes during soil freezing and thawing.

Fen soil N_2O and NO emissions as affected by water table (CHAPTER 5)

Experimental lowering of the water table level in the fen similarly affected N_2O and NO emissions compared to the non-manipulated controls and depended on the extent of the water table reduction. Whereas the plot with least lowering of the water table (10 cm; D1) did hardly reveal any effect regarding both N_2O and NO emission, a strong increase in N_2O (530 %) and NO emissions (270 %) during both drought periods were observed in the plot with the largest (30 cm; D3) water table reduction compared to the control.

In contrast to the existing studies on boreal peatlands, where an increase in N_2O emissions has not been observed until 14 weeks after lowering the water table (Freeman *et al.* 1993, Regina *et al.* 1999), water table drawdown in this study increased N_2O and NO emissions within four to five weeks – at least in the driest plot D3 – and thus within a notably shorter time scale. Furthermore, emission peaks of 16 μmol m^{-2} h^{-1} in this study exceed those reported by Freeman *et al.* (1993) and Regina *et al.* (1999) during drying of 2 μmol m^{-2} h^{-1}. Such differences may be related to the long-term N deposition at our site, which has been estimated at 20.1 kg N ha^{-1} a^{-1} (Schulze 2000). Whereas dry and wet N deposition has been reported to severely affect the N status of temperate ecosystems, i.e. leading to N-saturation (Aber *et al.* 1989), this appears to be less pronounced in the boreal zone (Tamm 1991). It may thus be speculated that temperate fens with high N content or input from the surrounding watershed have a high potential for nitrogen oxides emissions due to water table fluctuations.

Average flux rates of N_2O from the controls (0.3 to 0.8 kg N_2O-N ha^{-1} a^{-1}) coincided with rates reported earlier in studies from undisturbed minerotrophic fen mires with high groundwater level (0.04 to 1.2 kg N_2O-N ha^{-1} a^{-1}; Martikainen *et al.* 1993, Martikainen *et al.* 1995, Merbach *et al.* 2001). The increased N_2O flux observed upon water table drawdown was in agreement with findings from mires that were drained for forestry or agriculture by ditching several years before (Martikainen *et al.* 1993, Augustin *et al.* 1998, Merbach *et al.* 2001). Mean N_2O flux rates in D3 reached up to 5 kg N_2O-N ha^{-1} a^{-1}, if averaged over the total measurement period, thus exceeding rates reported for undisturbed fens and almost reaching those values reported for a drained, slightly degraded German fen rich in N (6 to 28 kg N_2O-N ha^{-1} a^{-1}; Augustin *et al.* 1998, Merbach *et al.* 2001). This study documents for the first time that a short term manipulation of water table levels in near-natural fens may produce temporarily a similar N_2O emission as observed in fens subjected to a constant drainage and anthropogenic interferences.

Largest N_2O emissions generally were observed when NO_3^- concentrations were elevated in 5 cm depth corresponding with high N_2O net production rates in 10 cm depth. The partly elevated nitrate concentrations in C3 and D3 compared to the C1 and D1 plots were presumably also related to the observed moisture gradient. This would also explain a high potential denitrifying activity in these plots, causing higher N-losses in form of N_2O.

Rewetting caused a short-lived, but strong increase in soil N_2O emission in D2 and D3 that lasted about 15 days in both years. Based on the entire experimental period (537 days) these events of 30 days altogether contributed 40 % (D2) and 20 % (D3), respectively, to the total N_2O emissions. Isotope abundance analyses give evidence that this N_2O is newly produced and not due to physical displacement. Up to now such N_2O peaks after rewetting of drained or water table manipulated fens have not been reported. Contrarily, in the study of Regina *et al.* (1999), the authors found an immediate reduction of N_2O fluxes from peat mesocosms after rewetting. The short-lived nature and significance of the emissions on the annual scale underline the importance of such short term water table fluctuations and the need for highly resolved measurements in calculating N trace gas budgets of peatlands. Flux estimates neglecting water table fluctuations could severely underestimate N_2O emissions.

N_2O concentrations in the fen increased substantially during winter times, suggesting that low temperatures impeded further reduction. However, since the largest part of the measured cumulative N_2O fluxes were emitted at lowered water table levels during summer times, fluxes and soil pool sizes were obviously decoupled. A positive relation between N_2O concentrations in the topsoil and N_2O surface fluxes suggests that the N_2O pool in the uppermost soil, albeit small and difficult to assess, is relevant for the N_2O fluxes between soil and atmosphere.

NO emissions were also enhanced during experimental drought, except for D1. However, NO fluxes were up to two orders of magnitude lower compared to N_2O emissions, as would be expected in moist soils (Tilsner *et al.* 2003, Yamulki *et al.* 1995, Regina *et al.* 1998). NO emission peaks were neither related to NH_4^+ nor NO_3^- concentrations. Our results show that in contrast to N_2O emissions, for which the nutrient status of the peatland is an important driving parameter for an increase during drought (Martikainen *et al.* 1993), even minerotrophic peatlands that are nutrient rich have only a low potential for increased NO emission even after water table drawdown.

Concluding remarks

This work demonstrates for the first time the outstanding importance of net N_2O consumption for its N_2O balance within a temperate forest soil. It was shown that severe drought may even result in a net uptake of atmospheric N_2O due to decreased net production together with less affected net consumption. The hitherto unbalanced global N_2O budget underlines the likelihood of a so far unconsidered sink function of soils for atmospheric N_2O. It has to be further investigated, if this behaviour holds true for other ecosystem types, too. In contrast, soil freezing resulted in strongly increased N_2O emissions from the same type of forest soil due to a decline of N_2O consumption within soil and ongoing net N_2O production. Thus, a major aim for future studies should be to improve our knowledge on soil N_2O consumption regarding involved organisms, reactions and environmental factors.

In temperate fens a drought appears to substantially increase N_2O emissions to levels as already described for their long-term drainage. The effect of drought was intensified by additional biogenic emission related to the subsequent intense rainfall events that had a significant impact on the annual N_2O loss. Ratios of N_2O/NO fluxes from the forest and the fen were converse, as expected (see Table 1). NO fluxes of both the forest and the fen were sensitive to drought and rewetting. Drought in general increased emissions of NO, though revealing a more complex relation compared to N_2O. Heavy rainfall in contrast seems to increase NO emissions from temperate forests but decreases those from fens.

In conclusion, the findings of this thesis underline the importance of short term extreme weather events for soil N_2O and NO emissions (see Table 1) and the need for high-resolution measurements in calculating N trace gas budgets. Models neglecting such events as drought, heavy rainfall, soil frost/thaw or water table fluctuations could severely miscalculate N_2O and NO emissions from soils.

Table 1. Mean, minimum and maximum flux rates of N_2O and NO as observed during the field experiments (throughfall exclusion [TE], snow removal [SR] and drought [D]) of this thesis in comparison to those from similar ecosystem types. Data from the literature refer to mean annual emissions, if not stated otherwise.

References	Ecosystem	Specifics	N_2O flux [μmol m^{-2} h^{-1}]		NO flux [μmol m^{-2} h^{-1}]	
			mean	min-max	mean	min-max
this thesis	Temperate spruce forest	controls	0.1	-0.2 - 0.8	0.8	<0.1 - 4.8
(Chapters 3+4)	(Germany)	TE plots	0.0	-0.1 - 0.7	1.0	<0.1 - 9.3
		SR plots	0.3	-0.2 - 3.5	not measured	
Butterbach-Bahl *et al.* 1997	Temperate spruce forest		0.2	1.4 - 6.7	6.4	16.3 - 106.0
	Temperate beech forest		0.7	3.2 - 41.6	2.9	5.0 - 38.3
	(both Germany)					
Butterbach-Bahl *et al.* 2002	Temperate pine forest		0.2	<0.0 - 0.5	0.2	-0.5 - 0.9
	(Germany)					
Kitzler *et al.* 2006	Temperate, central-montane		0.2	-0.1 - 1.5	<0.1[a]	<0.1 - 0.5
	spruce-fir-beech forest					
	(Austria)					
this thesis	Temperate acidic fen	controls	0.2	-0.9 - 3.6	0.1	<0.1 - 0.8
(Chapter 5)	(Germany)	D 3[b]	2.0	-0.4 - 36	0.1	<0.1 - 1.1
Merbach *et al.* 2001	Temperate, slightly degraded	virgin[c,d]	0.5/0.3	<0.0 - 2	not measured	
	graded fen (Germany)	drained	2.4/11	0.2 - 15		
		for forestry[d]				
Martikainen *et al.* 1993 (N_2O),	Boreal fen	virgin[c]	<0.1[e]		<0.1[e]	
Lång *et al.* 1995 (NO)	(Finland)					
Regina *et al.* 1998		drained	2.1	<0.1 - 3.4	0.9[f]	<0.1 - 1.5[f]
		for forestry				
Regina *et al.* 1996 (N_2O),	Boreal fen (Finland)	drained	7.5	<0.0 - 11.8	4.6	0.3 - 58.6
Lång *et al.* 1995 (NO)		for agriculture				

[a] Based on measurements between May and November.
[b] Results from the Drought plot with largest water table reduction.
[c] 'Virgin' means not drained.
[d] Data from two different years.
[e] Below detection limit.
[f] Based on measurements during the growth season (May-September).

Record of contributions to this thesis

CHAPTER 1 and the summary of this thesis were written by me. This dissertation includes five publications that are written by myself and are already published, accepted for publication in or submitted to international peer-reviewed journals. The contribution of me and all co-authors is listed below.

CHAPTER 2 Goldberg SD, Knorr K-H, Gebauer G (2008) N_2O concentration and isotope signature along profiles provide deeper insight into the fate of N_2O in soils. *Isotopes in Environmental and Health Studies*, 44, 377-391.

Goldberg SD: 65 % (concepts, field and laboratory work, interpretation, discussion and presentation of results, manuscript preparation)
Knorr K-H: 10 % (calculation of turnover rates, discussion)
Gebauer G: 25 % (concepts, discussion of results, contribution to manuscript preparation)

CHAPTER 3A Goldberg SD, Gebauer G (2009) Drought turns a Central European Norway spruce forest soil from an N_2O source to a transient N_2O sink. *Global Change Biology*, doi: 10.1111/j.1365-2486.2008.01752.x, in press.

Goldberg SD: 70 % (concepts, field and laboratory work, interpretation, discussion and presentation of results, manuscript preparation)
Gebauer G: 30 % (concepts, discussion of results, contribution to manuscript preparation)

CHAPTER 3B Goldberg SD, Gebauer G (2009) N_2O and NO fluxes between a Norway spruce forest soil and atmosphere as affected by prolonged summer drought. *Soil Biology & Biochemistry* (submitted).

Goldberg SD:	85 % (concepts, field and laboratory work, interpretation, discussion and presentation of results, manuscript preparation)
Gebauer G:	15 % (concepts, discussion of results, contribution to manuscript preparation)

CHAPTER 4 Goldberg SD, Borken W, Gebauer G (2009) N_2O emission in a Norway spruce forest due to soil frost – Concentration and isotope profiles shed a new light on an old story. *Biogeochemistry* (submitted).

Goldberg SD:	75 % (concepts, field and laboratory work, interpretation, discussion and presentation of results, manuscript preparation)
Werner Borken:	5 % (field site coordination, contribution to manuscript preparation)
Gebauer G:	20 % (concepts, discussion of results, contribution to manuscript preparation)

CHAPTER 5 Goldberg SD, Knorr K-H, Blodau C, Lischeid G, Gebauer G (2009) Impact of experimental drying and rewetting on N_2O and NO turnover and emissions from a temperate acidic fen. *Global Change Biology* (submitted).

Goldberg SD:	65 % (concepts, field and laboratory work, interpretation, discussion and presentation of results, manuscript preparation)
Knorr K-H, Blodau C:	15 % (calculation of turnover rates, discussion, supply of data on NO_3^- and NH_4^+ concentrations, contribution to manuscript writing)
Lischeid G:	5 % (field site coordination, preparation of water table level data)
Gebauer G:	15 % (concepts, discussion of results, contribution to manuscript preparation)

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CHAPTER 2

N_2O concentrations and isotope signatures along soil profiles

N_2O concentration and isotope signature along profiles provide deeper insight into the fate of N_2O in soils

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Abstract

Nitrous oxide is an important greenhouse gas and its origin and fate are thus of broad interest. Most studies on emissions of nitrous oxide from soils focused on fluxes between soil and atmosphere and hence represent an integration of physical and biological processes at different depths of a soil profile. Analysis of N_2O concentration and isotope signature along soil profiles was suggested to improve the localisation of sources and sinks in soils as well as underlying processes and could therefore extend our knowledge on processes affecting surface N_2O fluxes. Such a mechanistic understanding would be desirable to improve N_2O mitigation strategies and global N_2O budgets.

To investigate N_2O dynamics within soil profiles of two contrasting (semi)natural ecosystem types (a temperate acidic fen and a Norway spruce forest), soil gas samplers were constructed to meet the different requirements of a water-saturated and an unsaturated soil, respectively. The samplers were installed in three replicates and allowed soil gas sampling from six different soil depths. We analyzed soil air for N_2O concentration and isotope composition and calculated N_2O net turnover using a mass balance approach and considering diffusive fluxes.

At the fen site, N_2O was mainly produced in 30 to 50 cm soil depth. Diffusion to adjacent layers above and below indicated N_2O consumption. Values of $\delta^{15}N$ and $\delta^{18}O$ of N_2O in the fen soil were always linearly correlated and their qualitative changes within the profile corresponded with the calculated turnover processes, suggesting further reduction of N_2O. In the spruce forest, highest N_2O production occurred in the topsoil, but there was also notable production occurring in the subsoil in 70 cm depth. Changes in N_2O isotope composition as to be expected from local production and consumption processes within the soil profile did hardly occur, though. This was presumably caused by high diffusive fluxes and comparatively low net turnover, as isotope signatures approached values measured for ambient N_2O towards the topsoil. Our results demonstrate a highly variable influence of diffusive versus production/consumption processes on N_2O concentration and isotope composition, depending on the type of ecosystem. This finding indicates the necessity of further N_2O concentration and isotope profile investigations in different types of natural and anthropogenic ecosystems in order to generalise our mechanistic understanding of N_2O exchange between soil and atmosphere.

Keywords: denitrification; N_2O production; N_2O consumption; ^{15}N; ^{18}O; soil profiles

Introduction

Nitrous oxide is a greenhouse gas of special concern, since its global warming potential per molecule is about 300 times larger compared to CO_2 over a time span of 100 years – partly due to its extremely long atmospheric lifetime of approximately 114 years – and its atmospheric concentrations are steadily increasing by currently 0.26 % a^{-1} (IPCC 2007). Furthermore, N_2O is involved in the destruction of the stratospheric ozone layer. To our current knowledge, about 70% of the global N_2O emissions originate from soils, with natural soils producing the largest amount (IPCC 2007). Hence, N_2O exchange between soil and atmosphere, especially in natural ecosystems, is of outstanding importance for its regional and global budget.

N_2O production in soils mainly arises from biogenic nitrification and denitrification. However, N_2O can also be consumed in soils through denitrification processes (Granli & Bøckman 1994). The N_2O fluxes between soil and atmosphere represent the net effect of different production and/or consumption processes that may occur simultaneously in different depths in a soil, of diffusion of N_2O along concentration gradients, and of N_2O dissolving in or degassing from soil water. A mechanistic interpretation of N_2O surface fluxes therefore requires solid information about underlying soil processes. In some studies, N_2O concentration analysis along soil profiles was used to provide deeper insights into the origin and fate of N_2O in soils (Mosier & Hutchinson 1981, Burton & Beauchamp 1994, Davidson *et al.* 2004, van Haren *et al.* 2005). However, concentration gradients alone do not suffice to understand the underlying processes as multiple interactions of biological and physical processes occur.

Another approach to obtain information about the origin – and partly the history – of soil-emitted N_2O is the analysis of isotope abundance of ^{15}N and ^{18}O in N_2O collected from the headspace of flux chambers (Kim & Craig 1993, Pérez *et al.* 2000, Pérez *et al.* 2001, Tilsner *et al.* 2003, Goldberg *et al.* 2008). Stable isotope analysis may provide information about the fate of N_2O in a soil, since the different biological and physical processes are characterised by distinct changes in the isotopic composition of the soil N_2O pool (e.g. Pérez 2005). Nonetheless, the isotope signature of N_2O accumulating in chamber headspaces reflects an integration over the various processes occurring at different soil depths and therefore does not yield information about their localisation within the soil profile. Additionally, this method requires a certain threshold value of N_2O fluxes to avoid biases by calculation (Tilsner *et al.* 2003) and thus cannot be applied in ecosystems with low or even negative N_2O flux rates (Goldberg *et al.* 2008).

Large variations of *in-situ* N_2O fluxes between soil and atmosphere in different types of ecosystems and a yet poor understanding of underlying mechanisms indicate that our knowledge about soil processes controlling aboveground N_2O fluxes is still limited. Stratified N_2O concentration and isotope analysis along soil profiles could help to improve the mechanistic understanding of N_2O fluxes measured at the soil surface and thus to revise regional and global N_2O budgets.

Up to now, very few investigations have analysed $\delta^{15}N$, $\delta^{18}O$ and concentration gradients of N_2O in soils simultaneously (Pérez *et al.* 2000, Mandernack *et al.* 2000, van Groenigen *et al.* 2005, Rock *et al.* 2007) and the majority of these studies was conducted in agricultural soils. Knowledge about N_2O profiles and underlying turnover in more natural ecosystems is, however, still limited (Pérez *et al.* 2000, Goldberg & Gebauer 2009).

Therefore, the objectives of this study were (1) to provide insight into the processes controlling the N_2O pool in soil profiles of two contrasting natural ecosystems, (2) to report on accompanying changes in $\delta^{15}N$ and $\delta^{18}O$ values of soil N_2O, and (3) to propose suitable methods for gas sampling under extremely divergent conditions: in a water-saturated fen and in a well aerated forest soil.

Materials and methods

Experimental sites. Both sites, the fen site (50°08'N, 11°52'E; 700 m a.s.l.) and the Norway spruce forest stand (50°08'N, 11°52'E; 770 m a.s.l.), are located in the Lehstenbach catchment (size: 4.5 km^2, Fichtelgebirge, northeastern Bavaria, Germany). The catchment is dominated by Norway spruce forest, the annual mean air temperature is +5.3°C and the mean annual precipitation is about 1160 mm (Foken 2003)

The weakly acidic (pH 3.5 - 5.5) minerotrophic fen is dominated by graminoids (i.e. *Molinia coerulea*, *Agrostis* sp., *Carex rostrata*). Peat accumulation ranges from 40-70 cm. The von Post index of peat decomposition (Stanek & Silc 1977) ranges from 3 on a scale from 1 to 10 at depths of 0-10 cm to 7-9 at depths below (Knorr *et al.* 2008). The soil of the fen was classified as a Fibric Histosol on granite bedrock; for detailed soil characteristics see Table 1. The groundwater flow direction at the site is from the north to the south (Paul *et al.* 2006).

Table 1. Chemical properties, bulk density, porosity and von Post index for peat decomposition at the fen site in the Fichtelgebirge (northeastern Bavaria, Germany).

depth	C	N	bulk density	porosity	von Post scale
[cm]	[%]		[g cm^{-3}]	[%]	
-10	31.1	1.8	0.29	85.5	3
-20	26.5	1.5	0.24	85.1	7
-30	28.3	1.3	0.22	83.3	8
-40	31.4	1.2	0.49	78.8	9
-50	36.5	1.3	0.35	80.2	9

The soil of the mature Norway spruce forest (*Picea abies* (L.) Karst.) has a sandy loam texture and was classified as a Haplic Podsol with a mor-like forest floor of 6 to 10 cm depth comprising Oi, Oe and Oa horizons. For a detailed description of the soil characteristics see Table 2 (data from Zuber (2007) and Hentschel *et al.* (2007)). The forest floor is almost completely covered with ground vegetation, mainly *Deschampsia flexuosa* and *Calamagrostis villosa*.

Table 2. Chemical properties, granulometric composition, bulk density and porosity of the soil at the Norway spruce site in the Fichtelgebirge (northeastern Bavaria, Germany) after Zuber (2007) and Hentschel *et al.* (2007).

horizon	depth	C	N	sand	silt	clay	bulk density	porosity
	[cm]	[%]					[g cm^{-3}]	[%]
EA	-10	7.4	0.4	37	46	16	0.63	74.3
Bh	-12	5.5	0.3	30	47	22	0.76	68.2
Bs	-18	3.4	0.2	33	41	24	0.81	67.5
Bw	-55	1.3	0.1	31	48	20	0.86	65.7
Cv	<-55	0.4	<0.05	32	46	19	1.27	51.0

Mean groundwater level is about 6 to 8 m below the surface at this experimental site. Measurements from 1988 to 2003 at a groundwater well in 200 m distance from our experimental forest site revealed DOC, NO_3^- and NH_4^+ concentrations in the groundwater to be in the range of 0.5 to 2 mg l^{-1}, 0.1 to 0.2 mmol l^{-1} and 0.5 to 4 μmol l^{-1}, respectively (G. Lischeid, personal comm.). NO_3^- leaching from nitrification in the topsoil as well as from atmospheric deposition is known to contribute considerably to the NO_3^- found in the groundwater in this area (Durka *et al.* 1994).

Gas sampling devices. The sampling devices were built for soil N_2O concentration and concomitant isotope analysis. It has to be noted that for the isotope abundance analysis of N_2O in air samples, considerably large sample sizes are required (about 50 ml, if concentrations are close to ambient air).

At the fen site, with a high water table throughout the whole year, the sampling device had to fulfil two requirements: (1) being water-tight and (2) having a high gas permeable surface. For this purpose, silicone tubes were used, since N_2O diffuses quickly through this material (Nielsen *et al.* 1997, Jacinthe & Groffman 2001, Kammann *et al.* 2001). Equilibrium time was not experimentally determined in this study, but has been proven to be in the range of hours to days due to thin wall thickness and a high exposed area (Jacinthe & Groffman 2001). The sampling device installed in the fen allowed gas sampling from different depths at a single location in a relatively undisturbed soil profile. In each of three fen plots one sampling tube containing 6 cylinders was vertically installed. Since the fen is waterlogged nearly the whole year vertical installations are not affected by mass flow along the outside walls, as occurring in terrestrial ecosystems during drought periods. Six PVC cylinders (ID: 7 cm, AD: 7.9 cm; 10 cm height) with perforations (∅ 0.5 cm) in a 2.5 cm thick band at their middles were stringed together and connected by an inner 1 m long PVC tube (AD: 2.5 cm). In each cap a 5 m long silicone tube (ID: 3 mm; AD: 5 mm) with a total sampling volume of 35.34 ml was coiled and both ends were connected at the border to the inner PVC tube with a gas impermeable polyurethane (PUR) tube (ID: 1.8 mm; AD: 3 mm) of required length for gas sampling above the soil/water surface (Fig. 1). These PUR tubes were fitted with stopcocks (Luer Lock, Value Plastics, Fort Collins, USA) that were used to extract samples from the silicone tubes. The cap connections and the connections between caps and the mid-PVC tube were sealed with O-rings and silicone. The connections between silicone tubes and gas impermeable PUR tube (diminishing pipe 1.6/3.2, Novodirect, Illkirch, France) at the mid-PVC tube were sealed with silicone. Before field installation connections were checked for leakage using water. The selected inner diameter and wall thickness of the silicone tube fulfilled the required stability criteria to avoid bending of the tube. Additionally, the device should not exceed the chosen diameter to exclude disturbance in the soil profile. Thus, the obtained sampling volume in the tubes was the maximum achievable with these requirements.

A hole was cored using a gouge bit slightly smaller (OD: 6 cm) than the OD of the cap pipe to ensure a good contact between the device and the surrounding soil to 65 cm depth. Then the device was placed in the hole. Soil gas samples therefore were collected from 9 to 11 cm, 19 to 21 cm, 29 to 31 cm, 39 to 41 cm, 49 to 51 cm and 59 to 61 cm soil depth (hereafter 10, 20, 30, 40, 50, 60 cm).

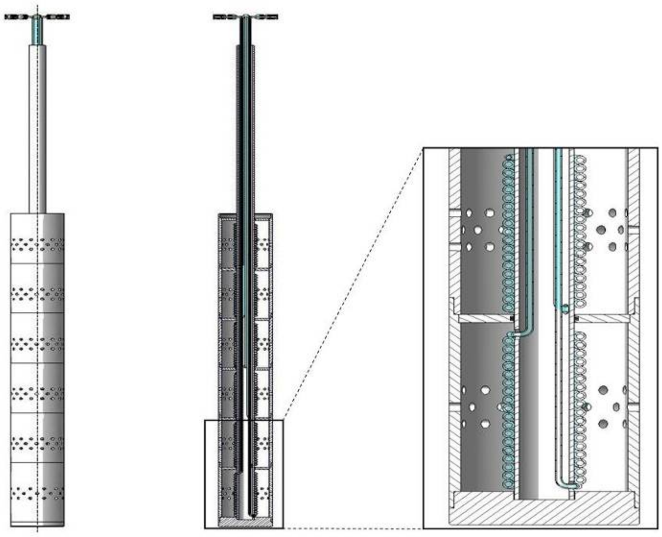

Figure 1. Design of the vertically installed gas sampling device at the fen site. Each soil gas collector consists of six well-defined cylinders, each with 5 m of a silicone tube inside. The silicone tubes are connected to a gas impermeable tube that allows sampling of soil gas above the soil surface.

Soil air tubes at the forest site were installed between ca. 7 to 70 cm soil depth (at about 7, 11, 20, 28, 43, 70 cm soil depth) in three replicates each. The gas sampling tubes were inserted horizontally into undisturbed soil from a trench, and the trench was back-filled. Before the installation of the gas samplers, a hole was cored using a gouge bit slightly smaller (OD: 1.8 cm) than the OD of the sampling tubes to ensure minimal disturbance of the soil. The tubes were positioned at the borders between the soil horizons EA, Bh, Bs, Bw, Cv. In the middle of the Bw horizon an additional soil air tube was installed, because of the thickness of this horizon. The horizontal orientation of the gas sampling tubes was chosen to prevent mass flow along their outside walls. The samplers were constructed of 50 cm long polyvinylchloride (PVC) tubes with a 1.6 cm inner diameter (corresponding to a sampling volume of 100.5 ml) and perforations at the front 20 cm (Fig. 2). Both ends of the tubes were sealed. Right-angled stainless steel tubes (ID: 1.5 mm, AD: 3.0 mm) connected the tubes in the soil with the soil surface and were fitted with stopcocks (Luer Lock, Value Plastics, Fort Collins, USA) that were used to extract samples from the tubes.

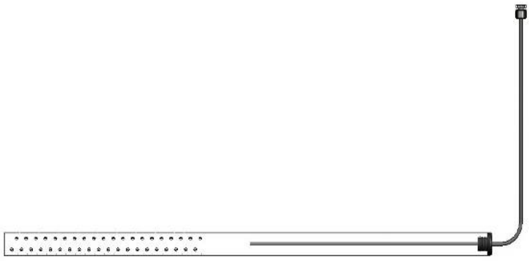

Figure 2. Design of the 50 cm long gas sampling tubes with perforations at the front 20 cm as installed in a Norway spruce forest soil. Sampling tubes were installed in six different soil depths. The stainless steel tubes (for gas sampling) of analogous length were closed by stopcocks.

Soil gas sampling procedure. For the manual soil air gas sampling glass bottles (100 ml, with an inlet, an outlet, and a septum) were used. The glass bottles were first flushed with N_2 and than evacuated using a membrane vacuum pump. The vacuum in the glass bottles was measured using a pressure gauge (TensioCheck TC 03S, Tensio-Technik, Geisenheim, Germany) and then gas samples from the various soil depths were taken. Samples of ambient air were collected (n = 3) on the respective sampling dates at 50 cm above the soil surface.

Soil gases from the fen site were sampled from the silicone samplers replacing the extracted volume with ambient air. To this end, one end of a sampling tube was connected to an evacuated gas vessel and subsequently opened. Thereafter the gas vessel and the second end of the sampling tube were opened. This was done to avoid cavitations of the silicone tube, to maintain sufficient gas within the tube for the next sampling and to gain the whole sampling air. In the fen, gas samples were collected on 24 July, 6 August, 22 August, 12 September, 29 October, 12 November and 3 December 2007. At the forest site, sampling was done by opening the stopcock of a sampling tube and connecting an evacuated gas vessel. In the forest gas samples were collected on 16 January, 7 March, 7 May, 8 July, 3 September and 1 November 2007.

Measurement and calculation of N_2O isotope ratios and N_2O concentrations. $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of N_2O as well as N_2O concentrations were measured using a gas chromatograph-isotope ratio mass spectrometer coupling with a pre-GC concentration interface (PreCon-GC-C-IRMS) (Hewlett-Packard GC 5890 series II, Wilmington, USA; Combustion Interface II and gas-IRMS delta V, both Finnigan MAT, Bremen, Germany) as described by Brand (1995). As a laboratory standard, N_2O gas (99.9990 %, Linde, Munich, Germany) was used that was previously calibrated with N_2 and N_2O reference gas. The internal reproducibility of the measurement system is typically ± 0.15 ‰ for N and ± 0.30 ‰ for O. Isotope ratios are presented as δ-values, which are defined as:

$$\delta x\,[‰] = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000 \qquad (1)$$

where δx is the δ-value of the heavy isotope x (^{15}N or ^{18}O, respectively) and R is the ratio of heavy isotope (atom percent, at %) to light isotope (at %). The international standards are N_2 in ambient air for $\delta^{15}N$ (Mariotti 1983) and standard mean ocean water (Vienna-SMOW) for $\delta^{18}O$, respectively.

Since the gas vessels from the fen contained a mixture of sample air and ambient air, N_2O concentrations as well as δ values had to be corrected for N_2O in the ambient air. N_2O concentrations were corrected by subtracting the fraction of the ambient air from the measured N_2O concentration of a sample. δ values were corrected according to:

$$\delta x_{soil\ N_2O}\,[‰] = \frac{\delta x_{measured} \cdot c_{measured} - \delta x_{ambient\ air} \cdot c_{ambient\ air}}{c_{measured} - c_{ambient\ air}} \qquad (2)$$

where δx is the δ value of the heavy isotope x [‰], c is the N_2O concentration [ppbv] and the indices "soil N_2O", "measured" and "ambient air" indicate soil N_2O sampled from various depths, total N_2O in the gas vessel and N_2O in ambient air, respectively (Tilsner *et al.* 2003).

N_2O concentrations were calculated from the total sample size and the peak area on mass 44 with the help of a calibration line. The reproducibility of N_2O quantification was 4 ppb.

With the technique used in the waterlogged fen, N_2O of the gas phase in equilibrium with the solution was measured. Dissolved nitrous oxide (N_2O) concentrations in the soil solution of the fen site were obtained using solubility data of Weiss & Price (1980) recalculated for measured *in-situ* temperature and salinity (data not shown).

Calculation of N_2O turnover within the soil profiles. Net turnover R_N of N_2O (pmol cm^{-3} d^{-1}) in the individual depth layers in the fen and the forest soil was calculated from mass balances of diffusive fluxes and changes in storage over time according to Eq. (3).

$$R_N = \frac{\Delta S_{N_2O}}{\Delta t} + \left[D_A \frac{\Delta c_{N_2O,upper}}{\Delta x} \right]_{upper} \cdot z^{-1} - \left[D_A \frac{\Delta c_{N_2O,lower}}{\Delta x} \right]_{lower} \cdot z^{-1} \tag{3}$$

The term $\Delta S_{N2O}/\Delta t$ represents the change in storage of N_2O in a layer; the left-hand expression in parenthesis represents the diffusive flux of N_2O at the upper boundary, the right-hand expression at the lower boundary of a layer (D_A: apparent diffusion coefficient in soil, $\Delta c_{N2O}/\Delta x$: concentration gradient at upper or lower end of segment, z: thickness of the layer).

The diffusion coefficients D_A for N_2O in the pore-water of the fen were calculated for *in-situ* temperature following Wilke & Chang (1955), and were corrected for porosity using $D = D_0 \phi^2$ (Lerman 1988). Diffusion coefficients D_A for N_2O in the unsaturated forest soil were obtained using the temperature corrected gaseous diffusion coefficient (Pritchard & Currie 1982) and a correction function $\alpha(a) = a^2 \phi^{-2/3}$ (α: correction factor at air content a, ϕ: soil porosity) (Jin & Jury 1996). At the forest site, volumetric gas content was derived from total porosity and volumetric water content recorded with TDR soil moisture probes (IMKO, Germany).

Statistical analysis. N_2O concentrations and $\delta^{15}N$ and $\delta^{18}O$ signatures are given in the text as means of n = 3 ± standard error of the mean, if not noted otherwise. Analysis of correlation between two parameters over depth for different sampling dates and over time for different soil depths were performed by using the correlation test after Pearson. N_2O concentrations were log-transformed for both ecosystem types and each depth and date, respectively, before executing the analysis to attain a linear relationship between the two according variables. In case of correlations between $\delta^{15}N$ and $\delta^{18}O$ values, ratios of $\delta^{18}O/\delta^{15}N$ were calculated by regression analysis.

Results

N_2O profiles in the fen. During the sampling period from July to December 2007, the water level in the fen ranged from 13 cm below to 15 cm above ground, indicating anaerobic conditions for a major part of the fen soil throughout the year. The water table level was always higher than 10 cm below soil surface on all sampling dates.

In the fen, mean dissolved N_2O concentrations varied over three orders of magnitude from 16 nmol l^{-1} to 6.2 μmol l^{-1} (Fig. 3), while mean ambient air N_2O concentrations were fairly constant, adjusting between 10 and 11 nmol l^{-1}.

In contrast to the forest site, the soil N_2O concentration in the fen did not follow one continuous trend throughout the entire sampling period. While highest concentrations occurred at intermediate depths of 30 cm until sampling in August, maxima of N_2O concentrations subsequently shifted to 40-50 cm depth. Net N_2O production also peaked in 30 cm soil depth on the first three sampling dates, in July and August, with values between 20 and 45 pmol cm^{-3} d^{-1}. Thereafter, N_2O net turnover decreased in almost all soil depths to rates between -2 and 1 pmol cm^{-3} d^{-1}. In October, maximum net N_2O production shifted to 40 cm soil depth and in November and December net N_2O production peaked at 50 cm soil depth with mean rates of 47 and 20 pmol cm^{-3} d^{-1}, respectively. Net negative N_2O turnover, i.e. further reduction of N_2O to N_2, was most pronounced in July and August at 20 cm and 40 cm depth, ranging from -6 to -21 pmol cm^{-3} d^{-1}.

$\delta^{15}N$ values of soil N_2O in the fen varied between -21 ‰ and +1 ‰. Values of $\delta^{15}N$ of N_2O in ambient air varied between +4 ‰ and +5 ‰ (Fig. 3). Differences in $\delta^{15}N$ values of N_2O from different soil depths ranged between 10 ‰ and 18 ‰. With only few exceptions, the most negative $\delta^{15}N$ values coincided with the highest N_2O concentrations and *vice versa*. $\delta^{15}N$ values of N_2O were always in a negative logarithmic manner correlated with the soil N_2O concentrations over depth for the different sampling dates as well as over time for different depths (except for 20 cm soil depth).

The ^{18}O signatures of soil N_2O showed values between +10 ‰ and +45 ‰ (Fig. 3). The differences in $\delta^{18}O$ values of N_2O between the different soil horizons at individual sampling dates were even larger than those of the $\delta^{15}N$ values (15 ‰ to 27 ‰). $\delta^{18}O$ values of ambient air N_2O were in a narrow range between +39 ‰ and +45 ‰. The $\delta^{18}O$ values of N_2O in the fen soil were also mostly correlated in a negative logarithmic manner with the soil N_2O concentrations if correlated over time and were always linearly correlated with the $\delta^{15}N$ values of soil N_2O. The ratios of ^{18}O to ^{15}N ranged between 0.8 and 1.5 for individual sampling dates and between 0.6 and 1.2 for individual depths.

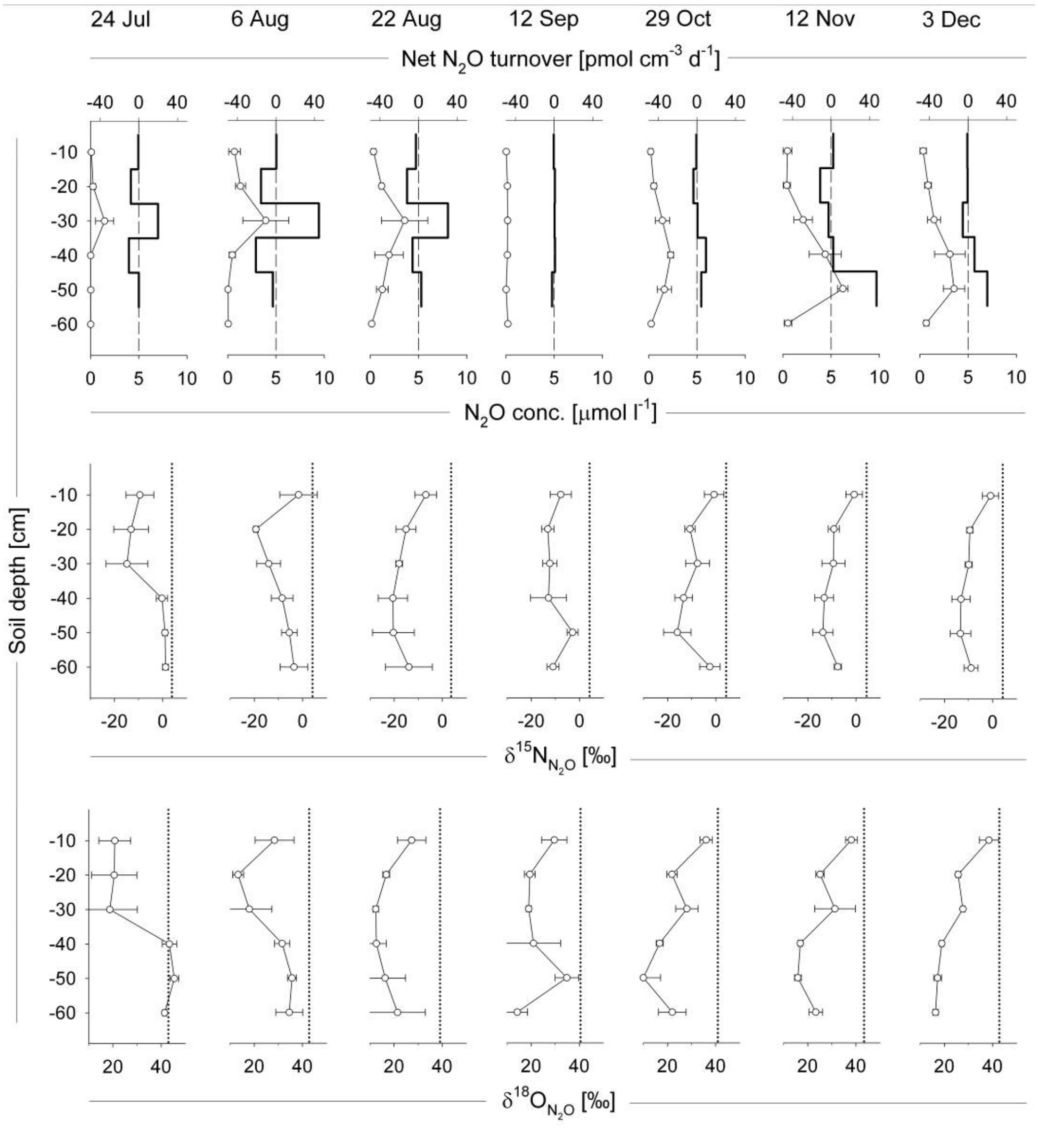

Figure 3. N_2O concentrations and corresponding turnover rates (top), $\delta^{15}N$ (middle) and $\delta^{18}O$ values (bottom) of dissolved N_2O in soil water in a fen in the Fichtelgebirge. In the plots on the top, the thick solid lines represent calculated mean N_2O turnover rates (top x axis); concentration profiles are given with open circles, connected by solid lines (bottom x axis). Error bars denote standard errors of the means (n = 3). The dotted line reflects the respective $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in the ambient atmosphere. The water level was above -10 cm soil depth on each sampling date.

N_2O profiles in the Norway spruce forest soil. Mean N_2O concentrations in soil air at the Norway spruce forest site varied by two orders of magnitude from 8 to 208 nmol l^{-1} (Fig. 4), while mean ambient air N_2O concentrations remained again fairly constant with values between 9 and 10 nmol l^{-1}.

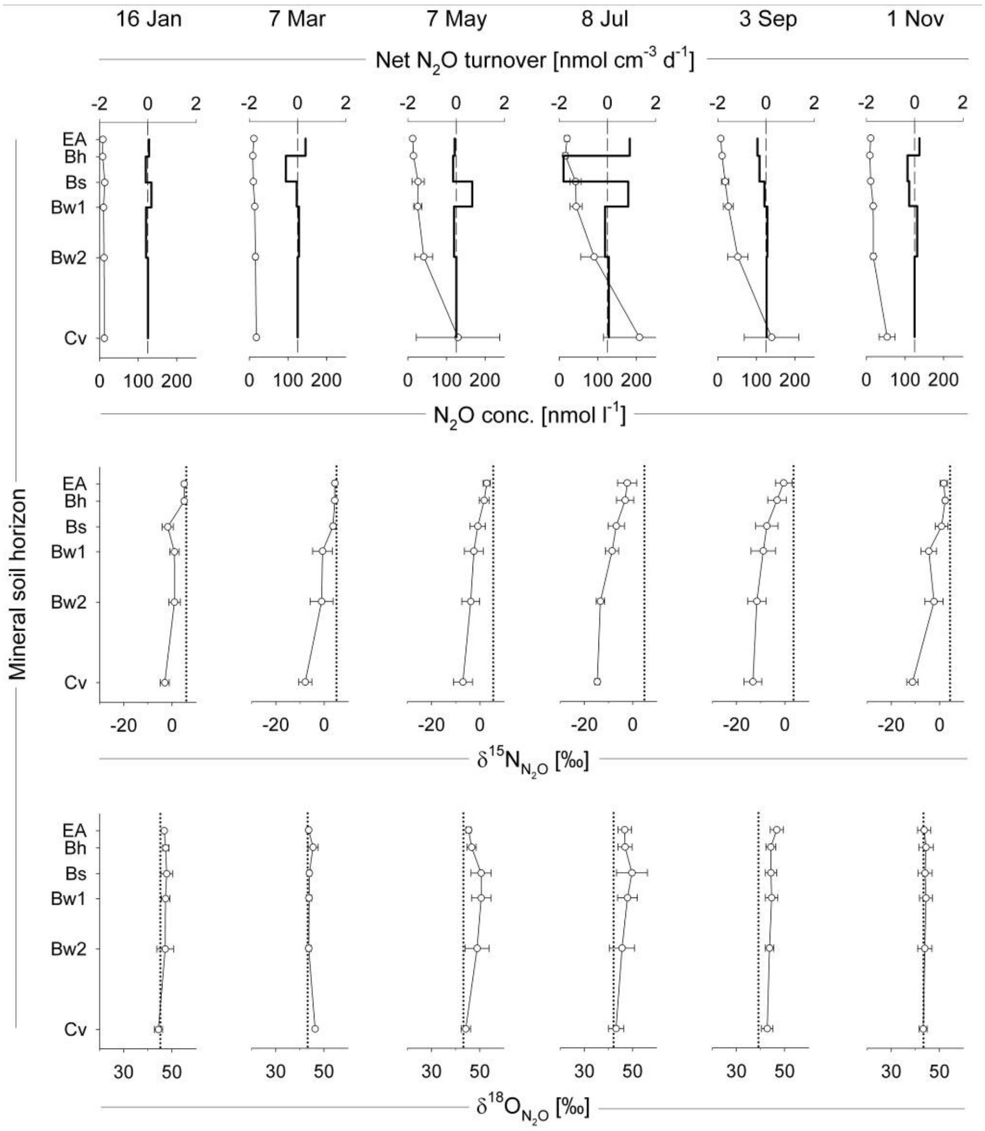

Figure 4. N_2O concentrations and corresponding turnover rates (top), $\delta^{15}N$ (middle) and $\delta^{18}O$ values (bottom) of N_2O in soil air along soil profiles at six measurement depths in a Norway spruce forest in the Fichtelgebirge. In the plots on the top, the thick solid lines represent calculated mean N_2O turnover rates (top x axis); concentration profiles are given with open circles, connected by solid lines (bottom x axis). Error bars denote standard errors of the means (n = 3). The dotted line reflects the respective $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in the ambient atmosphere. The space between different soil horizons reflects their mean distance along the vertical profile.

Throughout the year, highest N_2O concentrations occurred in the Cv horizon at about 70 cm soil depth, accompanied with always low net N_2O production (2 to 56 pmol cm^{-3} d^{-1}). Concentrations decreased in a logarithmic manner towards the topsoil, approaching N_2O concentrations close to ambient air.

Net N_2O production mostly peaked in the EA horizon, and in the Bs horizon, with mean rates ranging from 49 to 922 pmol cm^{-3} d^{-1} at these depths, and reached its maximum in summer. Negative net N_2O turnover also reached maximum negative values in summer, and was most pronounced in the horizon in between zones of high production (Bh), approaching values from -86 to -1886 pmol cm^{-3} d^{-1}.

Mean $\delta^{15}N$ values of the Norway spruce forest soil N_2O were in a similar range as found for the fen site, ranging from -15 ‰ to +4 ‰. $\delta^{15}N$ of N_2O in ambient air varied between +4 ‰ and +6 ‰ (Fig. 4). Most negative $\delta^{15}N$ values were observed for N_2O in the Cv horizon with values between -15 ± 1 ‰ and -3 ± 2 ‰. Values of $\delta^{15}N$ of soil air N_2O increased towards the surface as N_2O concentrations decreased and were thus in a negative logarithmic manner correlated with the soil N_2O concentrations over depth as well as over time.

Mean $\delta^{18}O$ values of N_2O in the forest soil ranged from +40 ‰ to +54 ‰ and were thus more positive and in a narrower range than values observed in the fen soil. Values of $\delta^{18}O$ of N_2O in ambient air varied between +39 ‰ and +45 ‰ (Fig. 4). In general, $\delta^{18}O$ values did not follow an obvious trend, presumably due to the small range (3 ‰ to 10 ‰) compared to the $\delta^{15}N$ values. $\delta^{18}O$ values and soil N_2O concentrations were never correlated over depth for the different sampling dates as well as over time. Furthermore, only weak correlation between $\delta^{18}O$ and $\delta^{15}N$ of N_2O in the forest soil was found.

Discussion

Processes controlling the concentration and isotope signature of N_2O within soil profiles. In our study, we evaluated the spatio-temporal distribution of N_2O in soil profiles and calculated net N_2O production to identify the predominant process (production, consumption or diffusion) controlling the soil N_2O pool. This was done for individual depth intervals as proposed by Rock *et al.* (2007). In addition, we monitored $\delta^{15}N$ and $\delta^{18}O$ of soil N_2O and compared isotopic shifts qualitatively with the calculated turnover processes.

Our study clearly demonstrated an impact of biological but also of diffusive processes on concentrations and isotopic composition of the soil N_2O pool.

Changes in N_2O concentrations and their isotope signatures within a soil profile result from physical (especially diffusion) and microbial processes (production through microbial denitrification and nitrification, and consumption through denitrification). Each process leads to concomitant and distinct shifts of the isotopic composition of the produced, remaining, or diffusing N_2O, due to fractionation against the heavy isotopes. Enrichment factors (isotope enrichment of a reaction product relative to that of the substrate) of the microbial processes are generally assumed to be larger than for physical processes (Pérez 2005). Bacterial production of N_2O by nitrification and denitrification yields N_2O depleted in ^{15}N relative to its respective source, whereas reduction of N_2O to N_2 enriches the remaining N_2O in both ^{15}N and ^{18}O (Pérez 2005, Barford *et al.* 1999). Shifts in $\delta^{18}O$ during production processes are currently under debate and thus, reported enrichment factors are contradictory (Pérez 2005, Toyoda *et al.* 2005). Diffusion also fractionates against the heavy isotopes (Pérez *et al.* 2000, Pérez 2005), resulting in stepwise ^{15}N and ^{18}O depletion of N_2O along a concentration gradient.

Several authors have tried to assess the production and/or consumption of N_2O present within the soil by combining isotope data of N_2O, its respective substrates and isotope enrichment factors for the various reactions (Mandernack *et al.* 2000, Pérez *et al.* 2001, Tilsner *et al.* 2003, Pérez 2005, Menyailo & Hungate 2006). However, under field conditions, the $\delta^{15}N$ and $\delta^{18}O$ of N_2O produced from both nitrification and denitrification processes, can show a large spectrum of both $\delta^{15}N$ and $\delta^{18}O$ values as observed in this study and elsewhere, due to the actual synthesis conditions (Schmidt *et al.* 2004), varying fractionation with process rates (Vieten *et al.* 2007), and micro-site heterogeneity of the soil substrates (Parkin *et al.* 1987). The same holds true for $\delta^{15}N$ and $\delta^{18}O$ values of N_2O affected by consumption processes, since they equally depend on the fraction reduced to N_2. Thus, isotope enrichment factors for ^{15}N reported to date also show a broad range, impeding mechanistic interpretation of natural abundance isotope analysis under field conditions. Even more difficulties in interpretation may arise from high exchange rates of soil N_2O with atmospheric N_2O as may be expected for our forest site, obscuring isotopic shifts from biological processes within the soil.

Process identification and localisation in the fen. In the fen, the concentration gradients were steep throughout the entire sampling period – except for September – and N_2O

concentrations in the pore water exceeded the values observed in the soil air at the forest site by 1-2 orders of magnitude. Nevertheless, the concentrations of dissolved N_2O in soil water of our study are within the range reported for different aqueous systems (Heincke & Kaupenjohann 1999, and references therein, Hiscock *et al.* 2003, Deurer *et al.* 2008).

Highest net N_2O production occurred at 30, 40 and 50 cm soil depth, with a shift from 30 cm in the summer to 50 cm in the winter. Therefore, we assume microbial denitrification to be responsible for N_2O production in the fen profile, since anaerobic conditions in the waterlogged soil make microbial nitrification rather unlikely (Davidson 1991) – at least in soil depths below the groundwater level.

The course of the N_2O production in the fen differed from the forest throughout the year, as N_2O production was even still occurring during winter. However, the diffusion coefficient of N_2O in water is four orders of magnitude smaller than in air (Heincke & Kaupenjohann 1999, and references therein) and thus allows for only very low N_2O exchange rates between different soil depths in the fen as compared to the forest soil. Whereas a complete turnover of the N_2O pool in the forest took approx. 2 days, N_2O pools in the fen were much larger with turnover rates lasting for weeks to months. However, it has to be noted that net N_2O turnover derived from our calculations is likely to be much smaller compared to gross N_2O turnover, i.e. total denitrification, since saturated fens typically allow rapid denitrification, if nitrate is present (Zak & Grigal 1991).

With our current knowledge it remains unclear, why largest N_2O production in the fen shows a spatial change during the investigation time from 30 to 50 cm soil depth. Furthermore, nitrate concentrations were always low in the fen, with maximum values of 50 μmol l^{-1} (unpublished data). According to a recent study using intact peat monoliths of this particular fen site, the peat seems to be rather heterogeneous in terms of redox zonation and microbial reductive processes likely occur in microenvironments (Knorr *et al.* 2008). Therefore, one may propose fluctuating redox conditions to be responsible for these shifts in production zones, presumably induced by extreme rain events.

Upward as well as downward diffusion of N_2O from production zones along concentration gradients in most cases was accompanied by an increase in $\delta^{15}N$ and $\delta^{18}O$ values, suggesting N_2O consumption, as confirmed by the turnover calculations. This is a reasonable result, as a prompt reduction of N_2O may well be expected in a peat matrix characterised by ferric iron, sulphate, and CO_2 reduction (Knorr *et al.* 2008).

The covariance between $\delta^{15}N$ and $\delta^{18}O$ values of soil N_2O in the fen suggested further reduction of N_2O to N_2, since both elements of the substrate N_2O are affected during this process (Menyailo & Hungate 2006). Values of $\delta^{18}O$ and $\delta^{15}N$ of soil N_2O in the fen were in most cases highly correlated and showed ratios of $\delta^{18}O/\delta^{15}N$ between 0.6 and 1.5. These ratios fall into the range of values reported for $\delta^{18}O/\delta^{15}N$ ratios of N_2O found in different soil types, including aquatic systems, ranging from 0.3 to 2.7 (Mandernack *et al.* 2000, Pérez *et al.* 2001, Menyailo & Hungate 2006, Ostrom *et al.* 2007, Yamagishi *et al.* 2007). A linear relationship between $\delta^{18}O$ and $\delta^{15}N$ with a slope of about 2.5 has been proposed to indicate N_2O reduction as the predominant process (Menyailo & Hungate 2006, Vieten *et al.* 2007), whereas smaller slopes, as found in this study, have been associated with dominance of N_2O production (Menyailo & Hungate 2006). However, the latter ratios are based on laboratory incubation studies and may not be applicable to our data. Large variations in the ratio of $\delta^{18}O/\delta^{15}N$ among different field studies could arise from different environmental conditions, and/or differences in denitrifying organisms (Menyailo & Hungate 2006). Furthermore, the exchange of O between NO_3^- and soil water could potentially bias an interpretation of $\delta^{18}O/\delta^{15}N$ ratios (Kool *et al.* 2007) and most recently it was assumed that there is even an O-exchange in N_2O with water (N. Wrage, personal comm.). The individual contribution of N_2O production or further reduction may thus not be derived from the available data. This ambiguity underlines the difficulty of comparing N_2O stable isotope data, e.g. $\delta^{18}O/\delta^{15}N$ ratios, among various ecosystems or even among different soil depths.

Only in some cases, diffusion of N_2O was not accompanied by further reduction to N_2. This was typically indicated by slightly more isotopically depleted N_2O in spite of lower N_2O concentrations. Such trends were not accidental, but could be found simultaneously on all investigated replicates.

Process identification and localisation in the Norway spruce forest soil. In the forest soil, both a clear vertical and a clear annual pattern of N_2O concentration profiles were observed. N_2O concentrations always peaked at the deepest investigated soil depth (at about 70 cm, in the Cv horizon) for all sampling dates. This coincided with lowest $\delta^{15}N$ values observed in this horizon, suggesting N_2O production being the predominant process in the subsoil of the forest site throughout the year. However, N_2O net production was up to 20-fold lower here compared to the topsoil and the high concentrations were presumably a result of both, small pore volume and low diffusivity in the subsoil. Since no data from deeper soil horizons are available, we cannot exclude that N_2O was produced even deeper in the soil. We propose microbial denitrification to be responsible for the N_2O

production in the subsoil for two reasons: (1) Occurrence of anaerobic conditions favouring denitrification is likely at this soil depth and (2) activity of nitrifying microorganisms seems to be unlikely in the subsoil as deflected by minute NH_4^+ availability as substrate for nitrification (Frank 1996).

Our findings of steadily increasing $\delta^{15}N$ values together with decreasing N_2O concentrations along the soil profiles throughout the year would suggest consumption of N_2O during upward diffusion of N_2O from the site of production. However, the turnover calculations revealed that there was a more complex pattern of production and/or consumption processes as well as large diffusive fluxes underlying the measured N_2O profile. Considering the inherent uncertainty of the calculated diffusion coefficients for unsaturated soils, the Bw horizon was neither a consistent source nor sink for N_2O throughout the year according to our calculations, while in the Bs horizon, net production of N_2O was the predominant process, especially in late spring and summer. Net N_2O consumption determined the N_2O pool in the Bh horizon at 12 to 18 cm depth according to our calculations, with the largest net N_2O consumption in summer. Largest net N_2O production occurred in the EA horizon, with again maximum turnover rates in summer.

Thus, steadily increasing $\delta^{15}N$, approaching values measured for atmospheric N_2O, and also a $\delta^{18}O$ signature close to that of atmospheric N_2O towards the soil surface rather suggest a rapid exchange of soil gas with the atmosphere. This rapid exchange may presumably have obscured larger differences in the isotopic signatures as one would have expected from production and consumption processes within the soil profile.

Only in summer, coinciding with the highest N_2O net production in the topsoil, the $\delta^{15}N$ values in the EA horizon significantly differed from that of atmospheric N_2O. We suspect substrate availability to be the driving factor for this observed annual course with N-mineralisation and nitrification being highest during the warm summer months (Granli & Bøckman 1994). Therefore, during high production in summer, diffusive fluxes could not equal out $\delta^{15}N$ of topsoil and atmospheric N_2O. Accordingly, largest shifts of $\delta^{18}O$ values were observed in May and July, also coinciding with the highest net N_2O turnover.

However, the $\delta^{18}O$ values did never show distinct gradients along the forest soil profile as the $\delta^{15}N$ values. Diffusion of N_2O together with consumption processes along the soil profile should have affected the $\delta^{18}O$ values in the same way as the $\delta^{15}N$ values. The fact that no correlation between $\delta^{18}O$ and $\delta^{15}N$ values of N_2O existed at any depth was presumably due to the very small range of N_2O-$\delta^{18}O$ values in the forest soil. Probably, processes affecting the ^{18}O signature of N_2O were smaller compared to ^{15}N or they cancelled out each other. It has to be noted, though, that $\delta^{18}O$ values of N_2O are not yet well understood and interpretation is still challenging, for example due to biochemical O exchange of N_2O precursors with soil water, that affects $\delta^{18}O$ values of N_2O (Kool *et al.* 2007).

Nevertheless, patterns of isotopic composition of the N_2O in the forest site differed substantially from patterns observed at the fen site. We attribute these differences not only to different biological processes governing the fate of N_2O in the forest soil, but also to a predominance of rapid exchange of soil N_2O and atmospheric N_2O in the topsoil.

Conclusions

This study is the first to analyse time series of N_2O concentration gradients and respective N_2O stable isotope signatures at natural abundance level along soil profiles in two types of (semi)natural temperate ecosystems: a waterlogged northern acidic fen and a Norway spruce forest with a well aerated soil. Our results suggest that only in a soil with small diffusivity and/or large N_2O turnover rates (i.e. the water-saturated fen soil) isotope changes consistently reflected dominating biological processes. Contrarily, in a soil with high diffusivity but small N_2O turnover rates (i.e. the unsaturated forest soil), exchange with atmospheric N_2O may diminish isotopic changes as to be expected from reported enrichment factors. This has implications for interpretation of concentration and isotope profiles in aerated soils, since isotope effects of small turnover processes can be obscured by diffusive exchange. Thus, in soils with low N_2O pools, i.e. rapid turnover times as observed for the forest soil, a detailed understanding of N_2O turnover requires not only analysis of N_2O concentrations and isotope composition but also knowledge about diffusive fluxes and surface exchange.

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CHAPTER 3

Forest soil N_2O and NO emissions as affected by drought/rewetting

PART A:

Drought turns a Central European Norway spruce forest soil from an N_2O source to a transient N_2O sink

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Abstract

Based on current climate scenarios, a higher frequency of summer drought periods followed by heavy rainfall events is predicted for Central Europe. It is expected that drying/rewetting events induce an increased matter cycling in soils and may contribute considerably to increased emissions of the greenhouse gas N_2O on annual scales.

To investigate the influence of drying/rewetting events on N_2O emissions in a mature Norway spruce forest in the Fichtelgebirge area (northeastern Bavaria, Germany), a summer drought period of 46 days was induced by roof installations on triplicate plots, followed by a rewetting event of 66 mm experimental rainfall in two days. Three non-manipulated plots served as controls. The experimentally induced soil drought was accompanied by a natural drought.

During the drought period, the soil of both the throughfall exclusion and control plots served as an N_2O sink. This was accompanied by sub-ambient N_2O concentrations in upper soil horizons. The sink strength of the throughfall exclusion plots was doubled compared to the control plots. We conclude that the soil water status together with the soil nitrate availability was an important driving factor for the N_2O sink strength. Rewetting quickly turned the soil into a source for atmospheric N_2O again, but it took almost four months to turn the cumulative soil N_2O fluxes from negative (sink) to positive (source) values. N_2O concentration and isotope analyses along soil profiles revealed that N_2O produced in the subsoil was subsequently consumed during upward diffusion along the soil profile throughout the entire experiment.

Our results show that long drought periods can lead to drastic decreases of N_2O fluxes from soils to the atmosphere or may even turn forest soils temporarily to N_2O sinks. Accumulation of more field-scale data on soil N_2O uptake as well as a better understanding of underlying mechanisms would essentially advance our knowledge of the global N_2O budget.

Keywords: $\delta^{15}N$; $\delta^{18}O$; N_2O consumption; N_2O sink; spruce forest soil; summer drought

Introduction

Enhanced warming of the earth's surface together with an increasing frequency of extreme meteorological events like drought and heavy rainfalls in temperate regions is expected for this century (IPCC 2007). Given these prognoses, the question arises how these predicted climate changes will influence the global budget of greenhouse gases. N_2O, with a net greenhouse effect per unit mass about 320 times stronger than CO_2 on a 100-year time span (Rodhe 1990), is in this context of particular interest. During the past few decades, atmospheric N_2O concentrations increased nearly linearly by a rate of 0.2 to 0.3 % a^{-1} and reached 319 ppbv in 2005 (IPCC 2007).

Soils act as the main sources for atmospheric N_2O. Total annual emissions from natural soils have been estimated to be 6.0 Tg N a^{-1}, those from cultivated soils 3.5 Tg N a^{-1} and total global N_2O emissions from all sources 14 Tg N a^{-1} (Olivier *et al.* 1998). The principal sources of N_2O in soils are microbial nitrification, nitrifier denitrification and denitrification, with the latter ones also being able to consume N_2O (Granli & Bøckman 1994, Schmidt *et al.* 2004). All of these processes are mainly driven by soil temperature, soil moisture and substrate availability. Drastic changes in meteorological conditions are, therefore, thought to strongly change N_2O emissions from soils.

In general, drought favours soil aeration and is thus thought to make conditions unfavourable for N_2O production by denitrification (Castaldi 2000) or nitrifier denitrification. Furthermore, drought limits the overall activity of soil microorganisms, reducing the amount of N cycled in the ecosystem (Kieft *et al.* 1987). Rewetting of dry soils in most studies resulted in sudden increases in soil N_2O emissions (Anderson & Levine 1987, Groffman & Tiedje 1988, Rudaz *et al.* 1991; Davidson 1992, Davidson *et al.* 1993, Scholes *et al.* 1997, Jørgensen *et al.* 1998). This could be due to several reasons. Decline of microorganisms due to drought stress leads to an accumulation of organic material (van Gestel *et al.* 1991), which is available for surviving microorganisms after rewetting. Additionally, an increase of easily available organic material can take place by desorption from the soil matrix (Seneviratne & Wild 1985), as well as by breakdown of soil aggregates during drought and the following rewetting, whereby physically protected organic material is exposed (Adu & Oades 1978, Lundquist *et al.* 1999). Both carbon and nitrogen mineralisation rates are generally enhanced for a few days after rewetting of dry soil (Birch 1958, Bloem *et al.* 1992, Cui & Caldwell 1997, Franzluebbers *et al.* 2000). The N_2O production is stimulated by the increased availability of nitrogen and in the case of denitrification additional by easily available organic carbon (Mummey *et al.* 1994, Włodarczyk 2000).

The majority of studies on drying/rewetting effects on N_2O emissions from soils have focused on the often strong, but short-lived response of the N_2O emissions to rewetting. Our objectives in this field study were to assess – next to the effect of rewetting – the effect of summer drought on the fluxes of N_2O between soil and atmosphere and to investigate the fate of N_2O along soil profiles by analysing simultaneously N_2O concentration and isotope abundance gradients.

Materials and methods

Experimental site. The experiment was conducted in a mature Norway spruce forest (Picea abies (L.) Karst.) in the Fichtelgebirge, Germany (50°08'N, 11°52'E; 770 m a.s.l.). This site (Coulissenhieb II) belongs to the Lehstenbach catchment (size: 4.5 km^2), which is almost completely covered by Norway spruce forest. The experimental site is exposed to WNW with a slope of about 3 %. Mean groundwater level is about 6 to 8 m below the surface at this experimental site. Measurements from 1988 to 2003 at a groundwater well in 200 m distance from our experimental forest site revealed DOC, NO_3^- and NH_4^+ concentrations in the groundwater to be in the range of 0.5 to 2 mg l^{-1}, 0.1 to 0.2 mmol l^{-1} and 0.5 to 4 μmol l^{-1}, respectively (G. Lischeid, personal comm.). NO_3^- leaching from nitrification in the topsoil as well as from atmospheric deposition is known to contribute in this area considerably to the NO_3^- found in the groundwater (Durka *et al.* 1994).

The average annual air temperature in this region is +5.3°C and the mean annual precipitation is about 1160 mm (Foken 2003). The soil has a sandy loam texture and is classified as a Haplic Podsol according to the FAO soil classification (IUSS Working Group WRB 2006) with a mor-like forest floor of 6 to 10 cm depth comprising Oi, Oe and Oa horizons. The C and N contents of the soil decrease with increasing depth from Oa horizon to Bw horizon from 18 % to 0.4 % and from 1.0 % to <0.05 %, respectively. The pH (H_2O) ranges from 4.0 in the organic layers to 4.5 in the Bw horizon. For a detailed description of the soil see Hentschel *et al.* (2007). The forest floor is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* and *Calamagrostis villosa*.

Design of the experiment. The control (C) and the throughfall exclusion plots (TE plots; three of each) were established in summer 2005. At each TE plot, 400 m^2 (20 m x 20 m) woody roof constructions were installed below the forest canopy and about 2.5 to 3 m above the forest floor. The roofs were covered on 24 June in 2006 with transparent polycarbonate to prevent soil infiltration of natural rainfall. A 0.4 m deep trench was excavated around the perimeter of the treatment plots, to reduce the potential for lateral movement of soil water from the surrounding forest into the plots. With the help of the roof construction, 67 mm throughfall were excluded during 46 days from 24 June to 9 August 2006. Subsequent to the throughfall exclusion, the soil was rewetted for two days with an intensity of 33 mm rain per day, using a sprinkler system. We applied artificial throughfall with the following components (μmol l^{-1}): Na^+ 133, K^+ 41, Ca^{2+} 134, Mg^{2+} 55, NH_4^+ 2, NO_3^- 174, SO_4^{2-} 172, PO_4^{3-} <5, Cl^- 81 and a pH of 5.0 (K. Schulze, personal comm.). After rewetting, the roofs were detached.

All measurements reported here were taken at least 5 m from the trench edge to guard against edge effects. The C plots without roofs and of the same size were not manipulated. The soil matric potential was recorded every ten minutes with tensiometers installed in 20 cm soil depth. Rainfall was measured on the C plots by using throughfall collectors at 1.5 m above ground level. Air temperatures were recorded hourly at 2 m above ground level. The roofs on the TE plots did not cause differences in the air temperature between C and TE plots.

Measurements of N_2O fluxes between soil and atmosphere. On each of the 6 plots, 3 stainless steel cylinders with a total height of 8 cm and an inner diameter of 19.5 cm were permanently installed 3 cm deep into the soil for N_2O flux measurements. N_2O fluxes were measured weekly during the throughfall exclusion period, in 1- to 3-day intervals for two weeks after rewetting and in weekly to monthly intervals during the following four months. Concentrations of N_2O, CO_2 and H_2O in the headspace were determined using a closed chamber technique in conjunction with a photoacoustic infrared gas analyser (Multigas Monitor 1312, INNOVA, Denmark, see Yamulki & Jarvis 1999). Both CO_2 and water vapour wavelengths are close to those of N_2O, thus resulting in interferences. Therefore, the Multigas Monitor offers the option of applying H_2O- and so called cross-interference compensation corrections. To exclude still occurring influences of changing CO_2 and H_2O concentrations on N_2O concentration measurements (e.g. Yamulki & Jarvis 1999), the air was pumped through a CO_2 trap filled with soda lime pellets (Merck KGaA, Darmstadt, Germany) and a water trap filled with Drierite (98 % $CaSO_4$, 2 % $CoCl_2$, 8 mesh, W.A. Hammond Drierite Co. LTD, Xenia, USA) before passing the monitor. CO_2 and water vapour concentrations were thus kept constant at <10 ppmv and 7000 to 8000 ppmv, respectively. N_2O concentrations were measured with a precision of ±15 ppbv for a single N_2O concentration measurement.

At each measurement, six chambers were connected to a self-constructed 10 channel multiplexer in a closed loop air circulation with two 25 meter long Teflon tubes (ID: 4 mm). In this way, gas fluxes on two plots, one TE and one C plot, were measured simultaneously. The multiplexer automatically switched from one valve to the next every 2 minutes. Thus, for each chamber, N_2O concentrations in the headspace were analysed 5 times over the total period of one hour. Gas flux rates were calculated from the linear increase or decrease in the gas concentrations in the chamber headspace with headspace volume and time. To estimate cumulative N_2O fluxes over the whole measuring period, sum curves were created by multiplying mean emission rates of two consecutive gas flux rates with the corresponding time period and summarising these time weighted means.

Gas sampling along soil profiles. Six sub-surface soil air tubes were installed horizontally at each plot between ca. 5 to 70 cm soil depth (at about 5, 15, 20, 25, 45 and 70 cm soil depth) at the transition to the soil horizons EA, Bh, Bs, Bw, Cv. In the middle of the Bw horizon an additional soil air tube was installed, because of the thickness of this horizon. These samplers were constructed of 50 cm long polyvinylchloride (PVC) tubes with a 1.6 cm inner diameter and were perforated by drill holes (∅ 0.3 cm) in the backmost 20 cm, thereby allowing free gas exchange between the cylinder volume and the soil atmosphere. Both ends of the tubes were sealed. Stainless steel tubes (ID: 1.5 mm) were connected at right angles with the tubes and led to the soil surface. They were fitted with stopcocks (Luer Lock, Value Plastics, Fort Collins, USA) that were used to extract samples from the tubes. Glass bottles (100 ml) were used for the manual gas sampling. The bottles were first flushed with N_2 and than evacuated. The vacuum in the glass bottles was verified with the help of a pressure gauge (TensioCheck TC 03S, Tensio-Technik, Geisenheim, Germany) and then gas samples were taken. Soil gas samples were taken two weeks before and two weeks after starting the throughfall exclusion (12 June and 10 July, respectively) and four months after rewetting (16 December). Samples of ambient air (n = 3) were also collected on the respective sampling dates.

Measurement of N_2O isotope ratios and N_2O concentrations. $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of N_2O gas as well as N_2O concentrations were measured using a gas chromatograph-isotope ratio mass spectrometer coupling with a pre-GC concentration interface (PreCon-GC-C-IRMS) (Hewlett-Packard GC 5890 series II, Wilmington, USA; Combustion Interface II and gas-IRMS delta S, both Finnigan MAT, Bremen, Germany) as described by Brand (1995). As a laboratory standard, N_2O gas (99.9990 %, Linde, Munich, Germany) was used. The N isotope ratio of the N_2O reference gas was calibrated against an N_2 reference gas after reduction to N_2 on a Ni (99.98 %) surface at 1150°C in the combustion interface II. The N_2 reference gas was previously calibrated against the reference substances N1 and N2 provided by the IAEA (Vienna, Austria). The $\delta^{18}O$ in our N_2O standard had been calibrated to an additional N_2O reference gas provided by T. Röckmann at the MPI for Nuclear Physics in Heidelberg with a mass spectrometer with double inlet system. The internal reproducibility of the measurement system is typically ± 0.15 ‰ for N and ± 0.30 ‰ for O. Isotope ratios are presented as δ-values, which are defined as:

$$\delta x\,[‰] = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \cdot 1000 \qquad (1)$$

where δx is the δ-value of the heavy isotope x (^{15}N or ^{18}O, respectively) and R is the ratio of heavy isotope (atom percent, at %) to light isotope (at %). The international standards are N_2 in ambient air for $\delta^{15}N$ (Mariotti 1983) and standard mean ocean water (Vienna-SMOW) for $\delta^{18}O$, respectively.

N_2O concentrations were calculated from the sampled gas volume and the peak area on mass 44 with the help of a calibration curve. The reproducibility of N_2O quantification based on this method is ± 4 ppb.

Determination of NH_4^+ and NO_3^- pools in the soil. Data on soil NH_4^+ and NO_3^- pools are provided by K. Schulze (Department of Soil Ecology, University of Bayreuth), who used the *in-situ* coring method after Adams *et al.* (1989). Soil cores (3.1 cm diameter) were collected in three replicates from the TE and C plots, respectively, down to 20 cm soil depth and separated into organic layer and mineral soil. Soils were shaken with 1 M KCl for one hour, followed by colorimetric determination of the NH_4^+ and NO_3^- concentrations in the filtered extracts. Soil NH_4^+ and NO_3^- pool sizes were determined on six different dates from May to October 2006 and were calculated on a soil surface basis.

Statistical methods and calculations of N_2O fluxes. Differences between NH_4^+ and NO_3^- pools at different sampling dates were tested using the non-parametric Kruskal-Wallis test. When the Kruskal-Wallis test was significant at $\alpha=0.05$, the Mann-Whitney U-test was used as a post-hoc test to compare gradually the differences between two treatments together with the sequential Bonferroni Method. Tests between different treatments (C and TE plots) and different soil horizons on one sampling date were carried out using the t-test.

Both TE and C plots were replicated three times and on each plot, gas flux measurements were carried out in three replicates to consider spatial heterogeneity. For each plot, mean fluxes were calculated and for both TE and C plots means of n = 3 ± standard errors including error propagation were calculated. Between two measuring dates, N_2O fluxes were linearly interpolated.

Linear and non-linear regression analyses were used to identify significant correlations between isotopic signatures and N_2O concentrations. The significance of correlations between two parameters from the same treatment and sampling date were tested by using the correlation test after Pearson. N_2O concentrations were log-transformed before executing the analysis to attain a linear relationship between the two according variables.

Values of N_2O fluxes, N_2O concentrations and $\delta^{15}N$ and $\delta^{18}O$ signatures are given as means of n = 3 ± standard error of the mean in the text, if not noted otherwise.

Results

Soil matric potential. Before starting the experimental drought simulation in the summer of 2006, the soil of this site had been exposed to naturally dry conditions (Fig. 1B), that led to a decrease of the soil matric potential in 20 cm depth from ca. -40 hPa (1 June) to ca. -200 hPa at the beginning of the throughfall exclusion of the TE plots (24 June, Fig. 1C). The C plots showed a similar decrease of the soil matric potential at the same time. During the experimentally induced soil drought from late June to early August 2006, the throughfall exclusion of 67 mm (Fig. 1B) resulted in a decrease of the soil matric potential in 20 cm depth from ca. -200 hPa to minimum values of ca. -600 hPa. Since the experimental drought was accompanied by a natural drought in June/July (Fig. 1B), the C plots showed a similar trend, but with significantly higher minimum values of about -400 hPa and with short peaks of higher matric potential values after the sparse rainfall events. Around 10 days before rewetting, soil matric potentials on the TE plots started to increase up to values of -500 hPa. This was accompanied by a stepwise decrease in the mean daily air temperature from 23°C to 12°C (Fig. 1A). During experimental rewetting, the soil matric potential immediately increased and reached initial values within two days. The rewetting was accompanied by natural rain on three days with a total rain amount of 36 mm, resulting in nearly identical behaviour of the C plots (Fig. 1B, C).

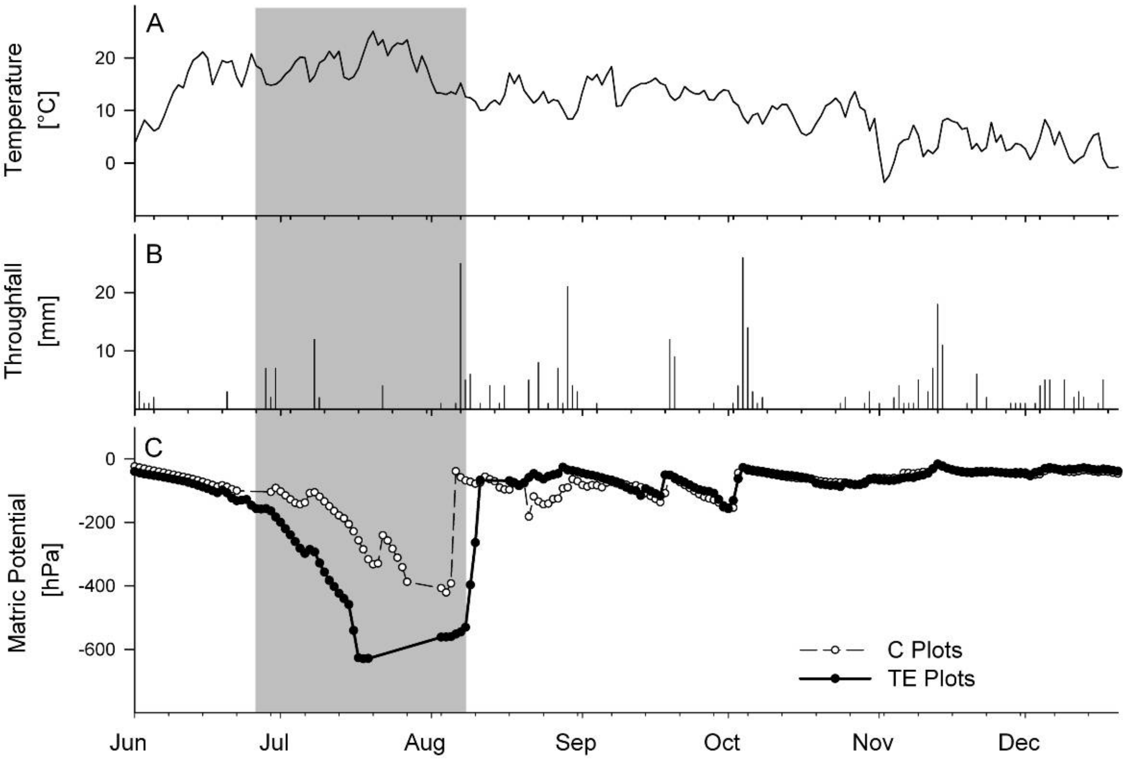


Figure 1. A) Daily mean air temperatures (n=24) at the Norway spruce forest stand recorded at 2 m above the forest floor, B) throughfall measured at the control plots during the drying/rewetting experiment and C) soil matric potential at 20 cm depth below soil surface at the throughfall exclusion (TE) and control (C) plots in 2006. The grey box indicates the throughfall exclusion period.

NH_4^+ and NO_3^- pools. Mean soil NH_4^+ pools (14 to 68 mmol m^{-2}) were generally larger than the NO_3^- pools (3 to 48 mmol m^{-2}; Fig. 2A and B) for both the organic layer and the top mineral soil. Mean soil NH_4^+ as well as NO_3^- pools decreased from the organic layer to the top mineral soil by about 20 to 50 %, respectively (Fig. 2A and B). C and TE plots did not differ with respect to their NH_4^+ and NO_3^- pool sizes for any sampling date. The same was true for the mean stocks of NH_4^+ and NO_3^- between different sampling dates, with one exception: From 25 July to 22 August the NH_4^+ pool in the organic layer decreased significantly at the C and TE plots from 49 mmol m^{-2} or 68 mmol m^{-2} to 15 mmol m^{-2} or 33 mmol m^{-2}, respectively. Overall, neither time nor treatment affected the soil NH_4^+ and NO_3^- pools.

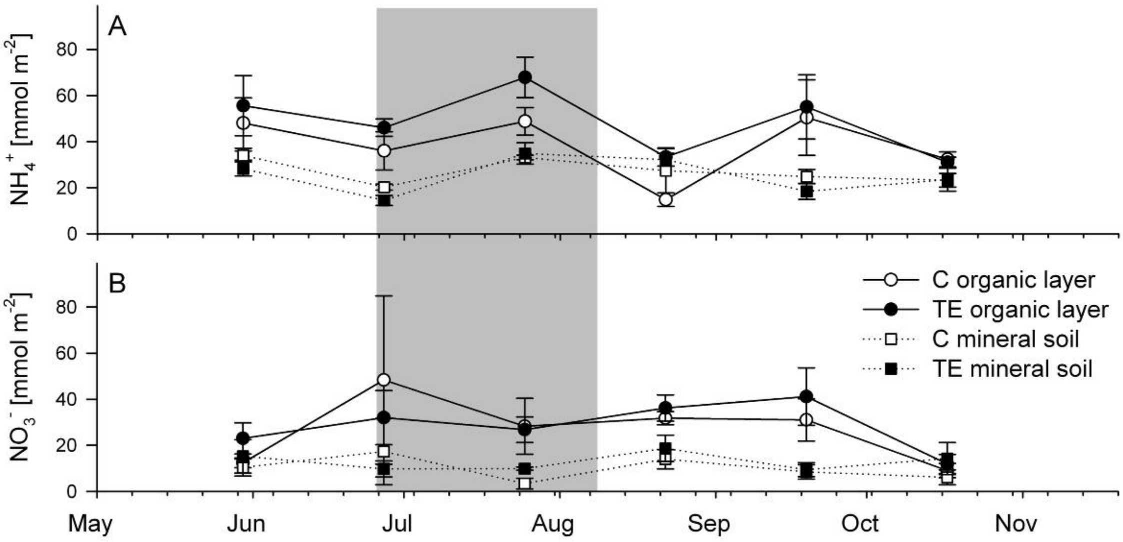


Figure 2. Pools of A) NH_4^+ and B) NO_3^- stored in the organic layer (Oi, Oe and Oa horizons) and mineral soil horizons (EA, Bh and partly Bs horizons) at the throughfall exclusion (TE) and control (C) plots in 2006. The grey box indicates the throughfall exclusion period.

N_2O fluxes and soil N_2O concentration and isotope profiles during drought. Before the drought, both the C and TE plots showed slightly positive N_2O fluxes from the soil to the atmosphere of 0.05 ± 0.04 μmol m^{-2} h^{-1} and 0.02 ± 0.04 μmol m^{-2} h^{-1}, respectively (Fig. 3A). During the drought, a net N_2O flux from the atmosphere to the soil took place. The sink strength of the soil on the TE plots increased with the length of the drought period and was most pronounced in the week before rewetting with fluxes of -0.13 ± 0.01 µmol N_2O m^{-2} h^{-1}. N_2O fluxes on the C plots did not follow the same pattern; negative N_2O fluxes between soil and atmosphere did either increase or decrease from one week to the next and N_2O fluxes became positive at the end of July. The mean N_2O fluxes integrated over the experimental drought period on the C and TE plots were significantly different and amounted to -0.77 ± 0.3 μmol m^{-2} d^{-1} and -1.56 ± 0.3 μmol m^{-2} d^{-1}, respectively (Fig. 3B).

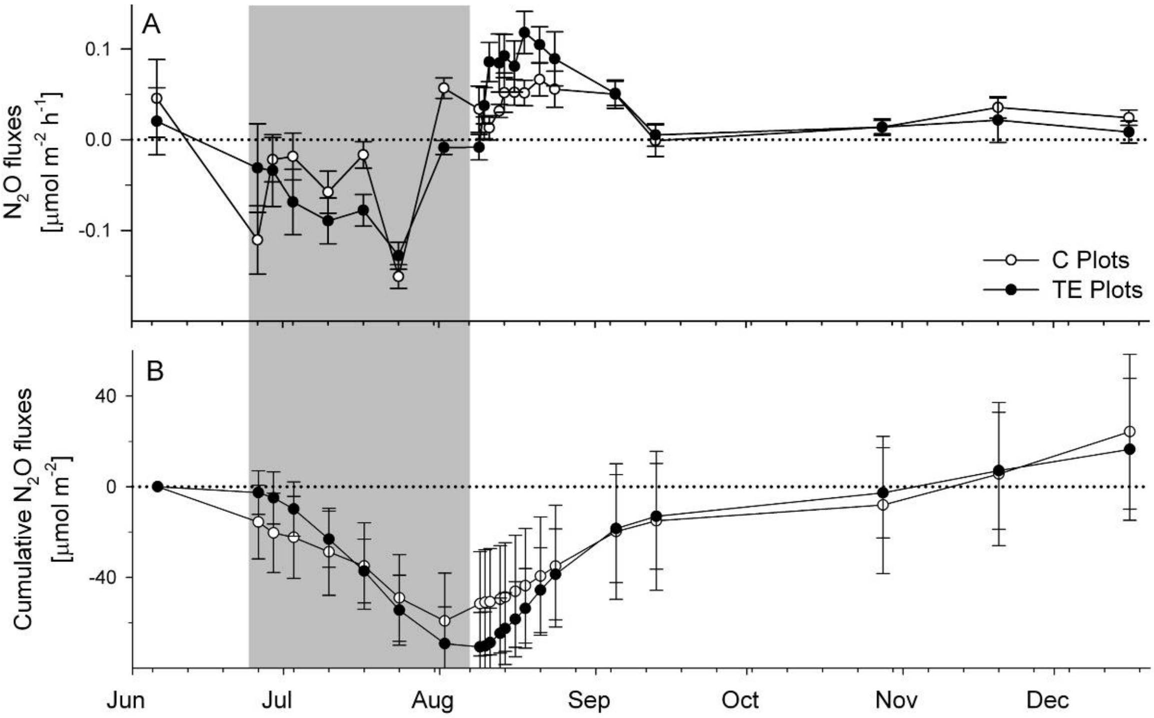


Figure 3. Time course of A) mean N_2O fluxes and B) mean cumulative N_2O flux rates at the throughfall exclusion (TE) and control (C) plots during drying and rewetting of the spruce forest soil in 2006. Error bars indicate the standard error of the mean (n=3). The grey box indicates the experimental drought period.

N_2O analysis along the soil profiles of both the TE plots and the C plots, showed similar behaviour for the time before (12 June) and during throughfall exclusion (10 July): N_2O concentrations were largest in the Cv horizon (at about 70 cm soil depth) and decreased exponentially towards the surface (Fig. 4). On both sampling dates, N_2O concentrations in the uppermost 10 cm (EA horizon) fell below ambient N_2O concentrations (326 ± 12 ppbv on 12 June and 333 ± 9 ppbv on 10 July, respectively) with values of 243 ± 57 ppbv (12 June) and 246 ± 57 ppbv (10 July) on the TE plots and 288 ± 18 ppbv (12 June) and 238 ± 54 ppbv (10 July) on the C plots. N_2O concentrations on both the TE plots and the C plots, increased with depth by one order of magnitude to 4.8 ± 2.5 ppmv and 2.8 ppmv (n = 2), respectively, in the Cv horizon (12 June).

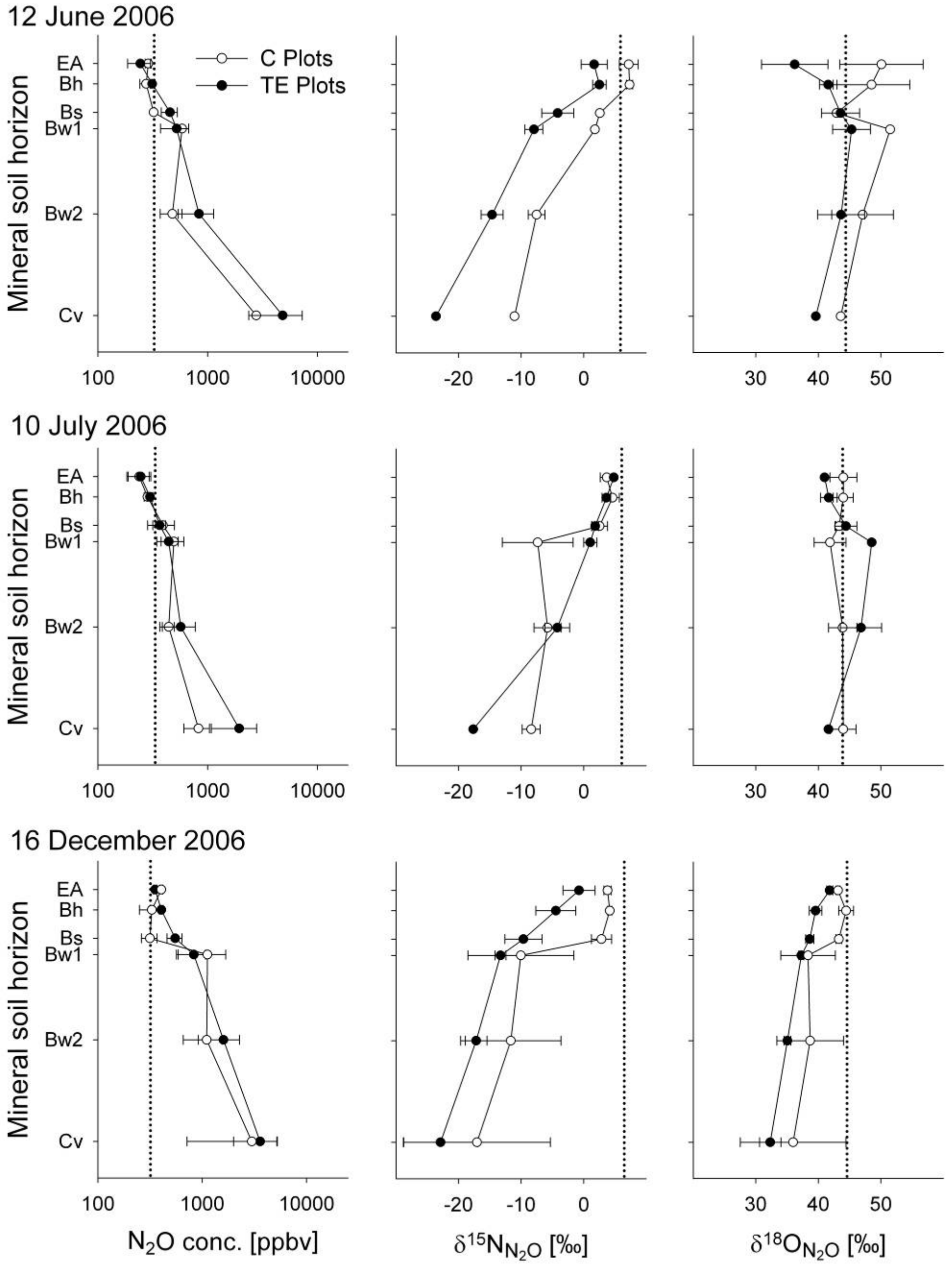


Figure 4. N_2O concentrations, $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in soil air along soil profiles on throughfall exclusion (TE) and control (C) plots before throughfall exclusion (12 June), during throughfall exclusion (10 July) and at the end of the experiment (16 December) in 2006. Error bars indicate the standard error of the mean (n=3). The dotted line reflects the respective values of N_2O in the ambient atmosphere. The space between different soil horizons reflects their mean distance along the vertical profile.

N_2O concentrations did not differ significantly between the two sampling dates in respective depths. However, N_2O concentrations tended to be smaller at the second date (during throughfall exclusion). This was most obvious in deeper soil layers: In the Cv horizon, N_2O concentrations on 10 July (1.9 ± 0.8 ppmv on the TE plots and 824 ± 217 ppbv on the C plots) were almost half of that from four weeks before.

The $\delta^{15}N$ values of the N_2O ranged from -23.6 ‰ (n = 2) to 1.7 ± 2.1 ‰ on the TE plots and from -11.1 ‰ (n = 2) to 7.2 ± 1.5 ‰ on the C plots and were on both sampling dates correlated in a negative logarithmic manner with the N_2O concentrations (the highest N_2O concentrations in the profile coincided with the lowest $\delta^{15}N$ signatures; Fig. 5).

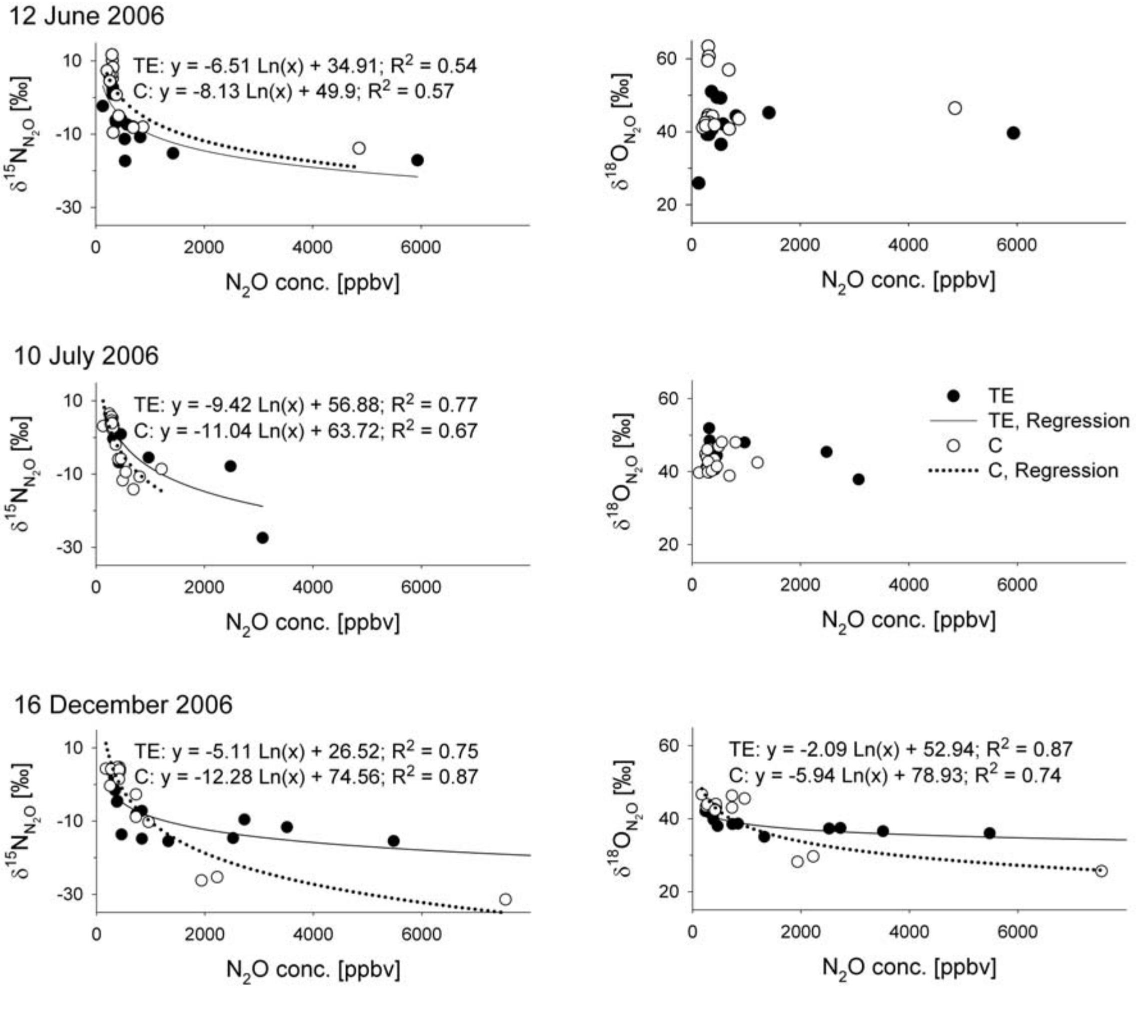
The $\delta^{18}O$ values of the soil N_2O on the TE plots ranged from 36.2 ± 5.3 ‰ to 45.3 ± 3 ‰ and were not correlated with the N_2O concentrations for both sampling dates (Fig. 5). As well, no correlation existed between $\delta^{18}O$ values of N_2O (between 41.9 ± 2.5 ‰ and 50.1 ± 6.7 ‰) and the N_2O concentrations in soil air from the C plots (Fig. 5).

The N_2O in ambient air had $\delta^{15}N$ values of 5.9 ± 0.3 ‰ and $\delta^{18}O$ values of 44.4 ± 0.2 ‰ on 12 June and 6.1 ± 0.1 ‰ and 43.9 ± 0.3 ‰ on 10 July, respectively (Fig. 4).

N_2O fluxes after rewetting. During the experimental rewetting (which was accompanied by a natural rain event, see Fig. 1B), the N_2O flux rates of the TE plots increased immediately up to a significantly ($p < 0.05$) higher level of 0.12 ± 0.01 μmol m^{-2} h^{-1} at the maximum (Fig. 3A). These enhanced N_2O emissions continued for a period of two to three weeks. After this time, the N_2O emissions decreased and reached values slightly above 0 μmol m^{-2} h^{-1}. The N_2O emissions from the C plots showed the same behaviour, with maximum values of 0.07 ± 0.00 μmol m^{-2} h^{-1}.

The integrated mean N_2O fluxes from the C and TE plots over the 127 days subsequent to the throughfall exclusion period were not significantly different from each other with values of 0.51 ± 0.2 μmol m^{-2} d^{-1} and 0.56 ± 0.1 μmol m^{-2} d^{-1}, respectively. After rewetting, it took about 100 days until the cumulative N_2O fluxes compensated for the soil N_2O sink function during the drought period of 46 days (Fig. 3B). Cumulative N_2O emissions of the TE plots over the whole time (193 d) were not significantly different from the total fluxes of N_2O of the controls.

Soil N_2O concentration and isotope profiles at the end of the experiment. Four months after rewetting (16 December), N_2O concentration profiles in the soil followed the same pattern as during drought, with increasing values with depth. The N_2O concentrations in the soil of the TE plots ranged from 354 ± 12 ppbv in the EA horizon to 3.6 ± 1.6 ppmv in the Cv horizon and thus were larger than N_2O concentrations in the atmosphere throughout the soil profile (Fig. 4). $\delta^{15}N$ values of soil N_2O had a similar depth gradient as in summer. N_2O concentrations and $\delta^{15}N$ values were closely correlated (Fig. 5). The same was true for the C plots, with N_2O concentrations ranging from 405 ± 15 ppbv in the EA horizon to 3 ± 2.3 ppmv in the Cv horizon and $\delta^{15}N$ values from 3.8 ± 0.6 ‰ to -17 ± 11.7 ‰. In contrast to the samplings in summer, $\delta^{18}O$ values of N_2O along the soil profiles showed the same pattern as $\delta^{15}N$ values and were highly correlated with N_2O concentrations for both TE and C plots (Fig. 5).


12 June 2006

10 July 2006

16 December 2006

Figure 5. Regressions and correlations for concentrations and $\delta^{15}N$ values and concentrations and $\delta^{18}O$ values of N_2O in soil air collected from six different depths on throughfall exclusion (TE) and control (C) plots before throughfall exclusion (12 June), during throughfall exclusion (10 July) and at the end of the experiment (16 December) in 2006 (n ≤ 18).

The N_2O concentration in ambient air on 16 December (n = 3) was 319 ± 19 ppbv. The N_2O in ambient air had a $\delta^{15}N$ value of 6.5 ± 0.2 ‰ and a $\delta^{18}O$ value of 44.4 ± 0.4 ‰ (Fig. 4).

Discussion

There is increasing evidence from the literature indicating that soils can serve – at least temporarily – as sinks for atmospheric N_2O (Wrage *et al.* 2004, Flechard *et al.* 2005, Chapuis-Lardy *et al.* 2007, Neftel *et al.* 2007). A sink function for N_2O by soils has been recorded on a broad spectrum of soils ranging from tropical to temperate regions and from agricultural to natural soils including forest soils. However, in many of these publications, negative N_2O fluxes were neglected and not discussed or were treated as analytical errors (e.g. Verchot *et al.* 1999, Kamp *et al.* 2000, Erickson *et al.* 2002). Net negative N_2O fluxes reported in the literature vary widely from -0.05 nmol m^{-2} h^{-1} to -17 μmol m^{-2} h^{-1} (Fenn *et al.* 1996, Longoria Ramírez *et al.* 2003, Chapuis-Lardy *et al.* 2007). The net negative N_2O fluxes of our study are within this range.

Quantitatively relevant microbial N_2O consumption is only known to occur during denitrifying processes, i.e. during the reduction of N_2O to N_2. Mostly, N_2O consumption has been reported to occur under conditions of low mineral nitrogen availability and high soil moisture (Chapuis-Lardy *et al.* 2007). These conditions favour microbial N_2O consumption, because NO_3^- is preferred as electron acceptor over N_2O and high soil moisture may support the reduction of N_2O to N_2 due to anaerobic conditions (Granli & Bøckman 1994). There are only very few reports in the literature that point to a soil N_2O sink function under dry conditions (e.g. Donoso *et al.* 1993, Yamulki *et al.* 1995, Klemedtsson *et al.* 1997, Verchot *et al.* 1999, Flechard *et al.* 2005), but underlying reasons have not yet been identified. The data of our N_2O flux measurements clearly indicate for the first time that a Central European Norway spruce forest soil can act as a sink for atmospheric N_2O during summer drought periods. The sink strength for N_2O appears to be related to the soil water status as indicated by higher sink strength on our TE plots compared to C plots. Furthermore, most pronounced negative N_2O fluxes at the C plots coincided with times of lowest throughfall. The finding of a temporary soil N_2O sink function is confirmed by sub-ambient N_2O concentrations in the soil air of surface-near soil horizons during throughfall exclusion and natural drought.

In general, N_2O concentration profiles in our study showed at all times (during and after throughfall exclusion) an exponential increase with increasing soil depth. Interpretation of gas concentration profiles alone is often difficult due to interfering processes: simultaneous occurrence of N_2O production and/or consumption, N_2O diffusion upwards as well as downwards, and N_2O dissolution and degassing in soil water. Irrespective of this complexity, the concentration gradients found in this study point to two conclusions:

(1) A source of N_2O in this Norway spruce forest soil is located in the subsoil. This conclusion is confirmed by laboratory mesocosm experiments from the same forest site, which indicate for topsoil cylinders only very minute N_2O production and response to drying/rewetting (Muhr *et al.* 2008). We expect microbial denitrification to be responsible for the N_2O production in the subsoil for two reasons: (a) Occurrence of anaerobic conditions favouring denitrification is likely in the subsoil and NO_3^- as substrate and DOC as carbon source for denitrification have been shown to occur in this forest even in the groundwater. (b) Activity of nitrifying microorganisms seems to be unlikely in the subsoil of our study area as deflected by minute NH_4^+ availability as substrate for nitrification (Gebauer *et al.* 2000, Callesen *et al.* 2007).

(2) In this soil, N_2O diffuses upwards along a concentration gradient from the subsoil to the soil surface.

However, these conclusions obtained solely from N_2O concentration gradients are obviously not the full story. Together with the exponentially decreasing N_2O concentrations along the soil profile, $\delta^{15}N$ of N_2O increased from negative to positive values close to those found in the atmosphere. Upward diffusion of N_2O alone cannot explain the N_2O isotope gradient, since diffusion is known to discriminate against isotopically heavy N_2O (Pérez 2005). Thus, from diffusion alone, decreasing $\delta^{15}N$ (and $\delta^{18}O$) values in upward moving N_2O should be expected. Our observation of a ^{15}N enrichment in N_2O towards surface-near soil horizons can only be explained by a predominance of N_2O production in the subsoil and upward diffusion together with a stepwise N_2O consumption. Microbial N_2O consumption fractionates against ^{15}N and ^{18}O depending on the proportion of N_2O reduced to N_2 (Barford *et al.* 1999). In general, fractionation factors for N_2O reduction are stronger than for N_2O diffusion (Pérez 2005) leading to a stepwise enrichment in ^{15}N (and ^{18}O) of the N_2O pool escaping from reduction to N_2 (Tilsner *et al.* 2003, Wrage *et al.*

2004). A similar occurrence of N_2O reduction during upward diffusion in soil profiles has already been reported for soils on agricultural land (van Groenigen *et al.* 2005, Rock *et al.* 2007). The fact that the $\delta^{18}O$ values were not correlated with the N_2O concentrations, suggests that the $\delta^{18}O$ values in soil N_2O in addition to microbial processes were additionally affected by non-microbial processes. Interpreting $\delta^{18}O$ values of N_2O is still challenging due to gaps in knowledge about ^{18}O isotope effects (see Pérez 2005, Toyoda *et al.* 2005). Further, physical processes affect the $\delta^{18}O$ values of N_2O. ^{18}O of N_2O precursors is known to exchange with soil water (Tilsner *et al.* 2003, Peréz 2005, Kool *et al.* 2007), thus affecting $\delta^{18}O$ values of N_2O especially when concentration gradients are small (Weeg-Aerssens *et al.* 1987).

The tendency towards decreasing N_2O concentrations in the subsoil air with increasing drought together with fairly constant slopes in N_2O concentration and $\delta^{15}N$ values between subsoil and soil surface throughout the experiment point to the conclusion that the source strength for N_2O in the subsoil was most likely reduced by drought, while simultaneously the sink strength for N_2O along the soil profile remained constant. Apparently, the interaction of both of these factors, reduced N_2O release in the subsoil and continuing N_2O consumption along the soil profile, led to the phenomenon of sub-atmospheric N_2O concentrations in the topsoil and thus, uptake of atmospheric N_2O by the soil under drought conditions.

At our current state of knowledge we can only make suggestions for possible reasons of a reduced N_2O source function of the subsoil during drought. Such reasons might be: (1) A shift downwards of the anaerobic and thus denitrifying zone in the subsoil during drought and thus, leading to a prolonged diffusion and consumption pathway of N_2O to the soil surface. (2) An interruption of downward movement of NO_3^- from topsoil nitrification and/or atmospheric NO_3^- deposition during drought. During experimental throughfall exclusion, about 6.7 mmol NO_3^- m^{-2} have been kept out of the TE plots. Since no significant differences in the topsoil NO_3^- pools on C and TE plots were found throughout the experiment, a reduced NO_3^- input due to prevention of NO_3^- throughfall deposition on our TE plots is unlikely to be the reason for reduced soil air N_2O concentrations on TE plots.

On both sampling dates, N_2O concentrations in the uppermost 10 cm (EA horizon) fell below ambient N_2O concentrations (326 ± 12 ppbv on 12 June and 333 ± 9 ppbv on 10 July, respectively) with values of 243 ± 57 ppbv (12 June) and 246 ± 57 ppbv (10 July) on the TE plots and 288 ± 18 ppbv (12 June) and 238 ± 54 ppbv (10 July) on the C plots. Soil rewetting turned the forest soil from an N_2O sink to a source of atmospheric N_2O

again, but it took twice the time of the preceding drought period to turn the cumulative soil N_2O fluxes from negative (sink) to positive (source) values. Thus, on an annual scale, the N_2O sink function during soil summer drought was considerable.

In the soil profile taken after rewetting in December, N_2O concentrations were significantly correlated with both $\delta^{15}N$ and $\delta^{18}O$ values following a negative logarithmic function. This coincidence leads – in contrast to the summer profiles – to a significant and positive correlation between $\delta^{15}N$ and $\delta^{18}O$ and indicates for the December data microbial formation and consumption processes being the main determinants of the isotopic composition of N_2O (Menyailo & Hungate 2006).

Conclusions

In this study, we observed net negative N_2O fluxes accounting for a total net uptake of 0.35 ± 0.11 mol N_2O ha^{-1} at the non-manipulated C plots during the 46-day measurement period. For the TE plots, net soil N_2O uptake was twice as large (0.72 ± 0.13 mol N_2O ha^{-1}). These N_2O fluxes from the atmosphere into the spruce forest soil during soil drought could result in a considerable sink function of atmospheric N_2O by forest soils when upscaled on a regional or global level. Future investigations have to document the global dimension of this drought-induced N_2O sink function of coniferous forest soils in temperate regions.

Up to now, the only sink for N_2O considered in global models is the destruction of atmospheric N_2O in the stratosphere through photolysis and photooxidation (IPCC 2007). Soils have been identified as the main sources for atmospheric N_2O, however, with high uncertainties in quantity (IPCC 2007). The sum of all known N_2O sources does currently not balance the stratospheric N_2O sink and the tropospheric accumulation of N_2O (Bange 2006). The finding of an underestimated oceanic N_2O source (Bange 2006) additionally indicates an overestimate of another N_2O source or a current underestimate of N_2O sinks. This to our current knowledge unbalanced global N_2O budget underlines the likelihood of a hitherto unconsidered sink function of soils for atmospheric N_2O and emphasises the necessity of better estimates of atmospheric N_2O emission as well as N_2O uptake by soils that can only be achieved by an improved understanding of the underlying processes and supplementary field data. For the future we suggest in addition to conventional N_2O flux measurements further soil N_2O concentration and isotope abundance profile studies as a highly suitable tool to improve our mechanistic understanding of N_2O production and consumption processes under drought/rewetting conditions.

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PART B:

N_2O and NO fluxes between a Norway spruce forest soil and atmosphere as affected by prolonged summer drought

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Abstract

Global change scenarios predict an increasing frequency and duration of summer drought periods in Central Europe especially for higher elevation areas. Our current knowledge about the effects of soil drought on nitrogen trace gas fluxes from temperate forest soils is scarce.

In this study the effects of experimentally induced drought on soil N_2O and NO emissions were investigated in a mature Norway spruce forest in the Fichtelgebirge (Bavaria, Germany) in two consecutive years. Drought was induced by roof constructions over a period of 46 days. The experiment was run in three replicates and three non-manipulated plots served as controls. Additional to the N_2O and NO flux measurements in weekly to monthly intervals soil gas samples from six different soil depths were analysed in time series for N_2O concentration as well as isotope abundances to investigate N_2O dynamics within the soil.

N_2O fluxes from soil to the atmosphere at the experimental plots decreased stepwise during the drought period, and mean cumulative N_2O emissions from the manipulated plots were reduced by 43 % during experimental drought compared to the controls in

2007. N_2O concentration as well as isotope abundance analysis along the soil profiles revealed that a major part of the soil acted as a net sink for N_2O, even during drought. This N_2O sink, together with diminished N_2O production in the organic layers, resulted in stepwise decreased N_2O fluxes during drought, and may even turn this forest soil into a net sink of atmospheric N_2O as observed in the first year of the experiment. Enhanced N_2O fluxes observed after rewetting were not able to compensate for the preceding drought effect.

During the experiment in 2006, with soil matric potentials in 20 cm depth down to -630 hPa, cumulative NO emissions from the throughfall exclusion plots were reduced by 69 % compared to the controls, whereas cumulative NO emissions from the experimental plots in 2007, with minimum soil matric potentials of -210 hPa, were 180 % those of the controls. Following wetting, the soil of the throughfall exclusion plots showed significant higher NO fluxes compared to the control that were responsible for 44 % of the total emission of NO throughout the whole course of the experiment.

NO emissions from this forest soil mostly exceeded N_2O emissions by one order of magnitude or more except during wintertime.

Keywords: summer drought, spruce forest soil, N_2O, NO, $\delta^{15}N$, $\delta^{18}O$

Introduction

The trace gases N_2O and NO have a great relevance for the atmosphere in terms of the greenhouse effect. N_2O is of certain concern, since it is implicated in global climate warming and depletion of the stratospheric ozone layer. NO is indirectly involved in global warming by taking part in reactions leading to the production of tropospheric ozone, a radiatively active greenhouse gas (Hall *et al.* 1996). Additionally, NO contributes, due to wet and dry deposition, significantly to the N-saturation of natural ecosystems (Aber *et al.* 1989), in turn leading to increased emissions of N trace gases (Fenn *et al.* 1996, Davidson & Kingerlee 1997, Pilegaard *et al.* 2006).

Forest soils have been identified to be significant sources for these N trace gases (Davidson & Kingerlee 1997, Gasche & Papen 1999, Pilegaard *et al.* 2006). Closed forests globally cover 3952 million ha or ca. 30 % of the total land area (IPCC 2007). 1001 million ha are in Europe (including all of the Russian Federation; FAO 2006) and may thus have significant importance for the N_2O and NO budgets on global scale.

Soil N_2O and NO emissions mainly originate from microbial nitrification and denitrification, with the latter process also potentially being able to consume N_2O (Granli & Bøckman 1994, Kester *et al.* 1997). Both processes and thus, the quantity of N_2O and NO fluxes between soil and atmosphere, heavily depend on soil physical factors such as soil temperature and water content. Therefore, changes in precipitation and temperature, as predicted within this century (IPCC 2007), are thought to affect emissions of N_2O and NO from soils.

In a preceding drought manipulation study, we showed that drought can turn a Norway spruce forest soil from an N_2O source to a transient sink for atmospheric N_2O (Goldberg & Gebauer 2009; see also Billings 2008). This N_2O sink function was accompanied by sub-ambient N_2O concentrations in the topsoil and the isotope signatures of soil N_2O indicated further reduction of N_2O to N_2 being the process governing the N_2O pool in the top mineral soil. However, in this previous study we were not able to definite clarify the reason for this soil sink function during drought. Since microbial N_2O consumption is only known to occur during anaerobic (denitrifying) processes, it is assumed that high soil moisture may support the reduction of N_2O to N_2 (Granli & Bøckman 1994). Thus, it was our aim to elucidate, why ongoing drought may even enhance the net N_2O sink function of a spruce forest soil. Therefore, we repeated the experiment one year later, continuously analysing N_2O concentration and isotope abundance profiles, which turned out to be a highly suitable tool to identify processes governing the N_2O pool within the soil (van Groenigen *et al.* 2005, Rock *et al.* 2007, Goldberg & Gebauer 2009).

Additionally, we present here NO fluxes that were measured simultaneously to N_2O fluxes. NO fluxes turned out to range around one order of magnitude higher than N_2O fluxes in the investigated spruce forest (Muhr *et al.* 2008, Goldberg *et al.* 2008b). Similar findings were reported by Butterbach-Bahl *et al.* (2002) for a German spruce forest, emphasising the importance of measuring NO in addition to N_2O when assessing primary and secondary greenhouse gas fluxes. We did not analyse NO within the soil profile, since this highly reactive gas to a large amount undergoes chemical conversion before escaping from the soil surface and there is broad evidence that very shallow surface layers are the primary NO production zone (Rudolph *et al.* 1996, Rudolph & Conrad 1996). This is confirmed by findings from mesocosm experiments with undisturbed soil columns from the investigated spruce forest (Muhr *et al.* 2008; Goldberg *et al.* 2008b). Furthermore, isotope abundance analysis on NO is still a challenging matter not suited for routine applications (Lauf & Gebauer 1998, 2001).

Thus, our objectives in this study were (1) to evaluate the effects of experimental summer drought on N_2O and NO fluxes from a Norway spruce forest soil to the atmosphere and to compare these effects in two subsequent years differing in natural drought conditions and (2) to determine processes affecting the N_2O aboveground fluxes within the soil profile and their response to soil drought.

Materials and methods

Experimental site. The experiment was conducted in a mature Norway spruce forest (*Picea abies* (L.) Karst.) in the Fichtelgebirge (northeastern Bavaria), Germany (50°08'N, 11°52'E; 770 m a.s.l.). The average annual air temperature in this region is +5.3°C (1971-2000) and the mean annual precipitation is 1160 mm (Foken 2003). The soil has a sandy loam texture and is classified as a Haplic Podsol according to the FAO soil classification (IUSS Working Group WRB 2006) with a mor-like forest floor of 6 to 10 cm depth comprising Oi, Oe and Oa horizons. The C and N contents of the soil decrease with increasing depth from Oa horizon to Bw horizon from 18 % to 0.4 % and from 1.0 % to <0.05 %, respectively. The pH (H_2O) ranges from 4.0 in the organic layers to 4.5 in the Bw horizon. For a detailed description of the soil see Hentschel *et al.* (2007). The forest floor is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* and *Calamagrostis villosa*.

A storm event on 18 January 2007 severely reduced the density of standing trees in this forest. We, therefore, cannot exclude that our results gained beyond this date may be influenced by the disturbance caused by the storm.

Design of the experiment. The control (C) and the throughfall exclusion plots (TE plots; three of each) were established in summer 2005. In the summer of 2006 and 2007, three 400 m^2 (20 x 20 m) woody roof constructions below the forest canopy and about 2.5 to 3 m above the forest floor were covered with transparent polycarbonate to prevent soil infiltration of natural rainfall. Additionally, a 0.4 m deep trench had been excavated around the perimeter of the treatment plots before the experiment, to reduce the potential for lateral movement of soil water from the surrounding forest into the plots. The throughfall exclusion period lasted 46 days from 24 June to 9 August in both years. After this time the roofs were detached. Subsequent to the throughfall exclusion in 2006, the soil was rewetted for two days with an intensity of 33 mm rain per day with an artificial throughfall solution (see Goldberg & Gebauer 2009). In 2007 we did not rewet the soil, since our objectives were now focussed solely on the investigation of prolonged drought effects.

All measurements reported here were taken at least 5 m from the trench edge to guard against edge effects. Three C plots without roofs and of the same size were not manipulated. The soil matric potential was recorded every ten minutes with TDR-sensors installed in 20 cm soil depth. Air temperatures were recorded hourly at 2 m above ground and precipitation at 30 m above ground.

N_2O and NO flux measurements. N_2O and NO fluxes were measured weekly during the throughfall exclusion period, in 1- to 3-day intervals for two weeks after the throughfall exclusion period and in weekly to monthly intervals during the remaining time. Measurements of N_2O flux rates were carried out using the closed chamber technique in conjunction with a photoacoustic infrared gas analyser (Multigas Monitor 1312, TGA, INNOVA, Denmark, see Yamulki & Jarvis 1999). N_2O concentrations were measured in three chambers (diameter 19.5 cm, height approx. 8 cm) at each plot after 0, 10, 20, 30 and 40 min with a precision of ±15 ppb for a single N_2O concentration measurement. Gas flux rates were calculated from the linear increase or decrease in the gas concentrations in the chamber headspace with headspace volume and time. For a more detailed description, see Goldberg & Gebauer (2009).

NO emissions were measured using the open flow-through chamber technique as described by Yamulki *et al.* (1995). Concentrations of nitric oxides were measured using an NO-NO_x-NO_2-Analyser (AC 31 M Monitor, Environment, Poissy, France). For detail, see Goldberg *et al.* (2008b). Fluxes of NO were calculated from the concentration in outlet air, the flow rate of air through the chamber, and the surface area of the soil column. Because of the high reactivity of NO, the chambers and the lid were covered with Teflon foil inside.

To estimate cumulative N_2O and NO fluxes over the whole measuring period, sum curves were created by multiplying mean emission rates of two consecutive gas flux rates with the corresponding time period and summarising these time weighted means.

Gas sampling in the soil profiles. Six sub-surface soil air tubes were installed horizontally at each plot between ca. 5 to 70 cm soil depth at the border to the soil horizons EA, Bh, Bs, Bw and Cv. In the middle of the Bw horizon an additional soil air tube was installed, because of the thickness of this horizon. These samplers were constructed of 50 cm long polyvinylchloride (PVC) tubes with a 1.6 cm inner diameter and perforations at the front 20 cm. Both ends of the tubes were sealed. Right-angled stainless steel tubes (ID: 1.5 mm) connected the tubes in the soil with the soil surface and were fitted with stopcocks (Luer Lock, Value Plastics, Fort Collins, USA) that were used to extract samples from the tubes. Manual gas sampling was done with glass bottles (100 ml) that were first flushed with N_2 and than evacuated (for further details see Goldberg *et al.* 2008a). The sampling was generally done in biweekly to monthly intervals between January and December 2007 and in weekly intervals during the throughfall exclusion period in the summer of 2007. Samples of ambient air (n = 3) were taken on the respective sampling dates in 50 cm above ground.

Measurement of N_2O isotope ratios and N_2O concentrations. $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of N_2O gas collected from the soil profiles as well as N_2O concentrations were measured using a gas chromatograph-isotope ratio mass spectrometer coupled to a pre-GC concentration interface (PreCon-GC-C-IRMS) (Hewlett-Packard GC 5890 series II, Wilmington, USA; PreCon, Combustion Interface II and gas-IRMS delta V, all Thermo Fisher

Scientific, Bremen, Germany) as described by Brand (1995). For further details see Goldberg *et al.* (2008a). Isotope ratios are presented as δ values:

$$\delta x\ [‰] = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000 \qquad (1)$$

where δx is the δ value of the heavy isotope x (^{15}N or ^{18}O, respectively) and R is the ratio of heavy isotope (atom percent, at %) to light isotope (at %). The international standards are N_2 in ambient air for δ^{15}N (Mariotti, 1983) and standard mean ocean water (Vienna-SMOW) for δ^{18}O.

N_2O concentrations were calculated from the sampled gas volume and the peak area on mass 44 by means of an external calibration (reproducibility ± 4 ppb).

Calculation of N_2O diffusivity and turnover within the soil profiles. Net turnover (R_N) of N_2O (pmol cm^{-3} d^{-1}) in the individual depth layers was calculated from mass balances of diffusive fluxes and changes in storage over time according to Eq. 2 (Goldberg *et al.* 2008a).

$$R_N = \frac{\Delta S_{N_2O}}{\Delta t} + \left[D_A \frac{\Delta c_{N_2O,upper}}{\Delta x} \right]_{upper} \cdot z^{-1} - \left[D_A \frac{\Delta c_{N_2O,lower}}{\Delta x} \right]_{lower} \cdot z^{-1} \qquad (2)$$

The term $\Delta S_{N2O}/\Delta t$ represents the change in storage of N_2O in a layer; the left-hand expression in parenthesis represents the diffusive flux of N_2O at the upper boundary, the right-hand expression at the lower boundary of a layer (D_A: apparent diffusion coefficient in soil, $\Delta c_{N2O}/\Delta x$: concentration gradient at upper or lower end of segment, z: thickness of the layer).

Diffusion coefficients D_A for N_2O in the soil were obtained using the temperature corrected gaseous diffusion coefficient (Pritchard & Currie 1982) and a correction function $\alpha(a) = a^2\ \phi^{-2/3}$ (α: correction factor at air content a, ϕ: soil porosity) (Jin & Jury 1996). Volumetric gas content was derived from total porosity and volumetric water content recorded with TDR soil moisture probes (IMKO, Germany). Since the water content was only recorded on one C and TE plot, but used for turnover calculations on all plots, the inherent uncertainty of these calculations has to be considered.

Determination of NH_4^+ and NO_3^- concentrations in the soil. Concentrations of NH_4^+ and NO_3^- were determined using the *in-situ* coring method after Adams *et al.* (1989). Soil cores (3.1 cm diameter) were collected before and after throughfall exclusion in three replicates from the TE and C plots, respectively, down to 20 cm soil depth and separated into L, O, A and B horizon. Soils were shaken with 1 M KCl for one hour, followed by colorimetric determination of the NH_4^+ and NO_3^- concentrations in the filtered extracts.

Statistical methods. Differences between NH_4^+ and NO_3^- concentrations between C and TE plots and different soil horizons were carried out using the t-test. N_2O and NO fluxes were calculated as means of n = 3 ± standard error including error propagation. Analysis of correlation between $\delta^{15}N$ values and N_2O concentrations, $\delta^{18}O$ values and N_2O concentrations as well as $\delta^{15}N$ and $\delta^{18}O$ values from soil air N_2O were performed by using the correlation test after Pearson. N_2O concentrations were log-transformed before executing the analysis to attain a linear relationship between the two respective variables. Values of N_2O concentrations, $\delta^{15}N$ and $\delta^{18}O$ signature are given in the text as means of n = 3 ± standard error of the mean, if not noted otherwise.

Results

Meteorological conditions and soil matric potentials. The throughfall exclusion period from end of June to early August in 2006 was characterised by warm and dry weather. A mean air temperature of 18.2°C (Fig 1A) compared to a 1994-2007 mean of 15.6°C was recorded, and the amount of precipitation was only 125 mm (Fig. 1B), while reaching 166 mm in the 1994-2007 mean. During the experimental drought period in 2007 the average air temperature was rather low (14.4°C; Fig. 1A) and precipitation of 161 mm during throughfall exclusion (Fig. 1B) did not differ from the 1994-2007 mean.

In the summer of 2006, the naturally dry conditions led to a decrease of the soil matric potential in 20 cm depth from ca. -40 hPa (1 June) to ca. -200 hPa at the beginning of the throughfall exclusion of both the C and the TE plots (Fig. 1C). During the experimentally induced soil drought from late June to early August 2006, the throughfall exclusion resulted in a decrease of the soil matric potential in 20 cm depth from ca. -200 hPa to minimum values of ca. -600 hPa. The C plots showed a similar trend, but with significantly higher minimum values of about -400 hPa and with short peaks of higher matric potential values after the sparse rainfall events. During experimental rewetting, the soil matric potential immediately increased and reached initial values within two days. The rewetting was accompanied by natural rain on three days, resulting in nearly identical behaviour of the C plots (Fig. 1B, C).

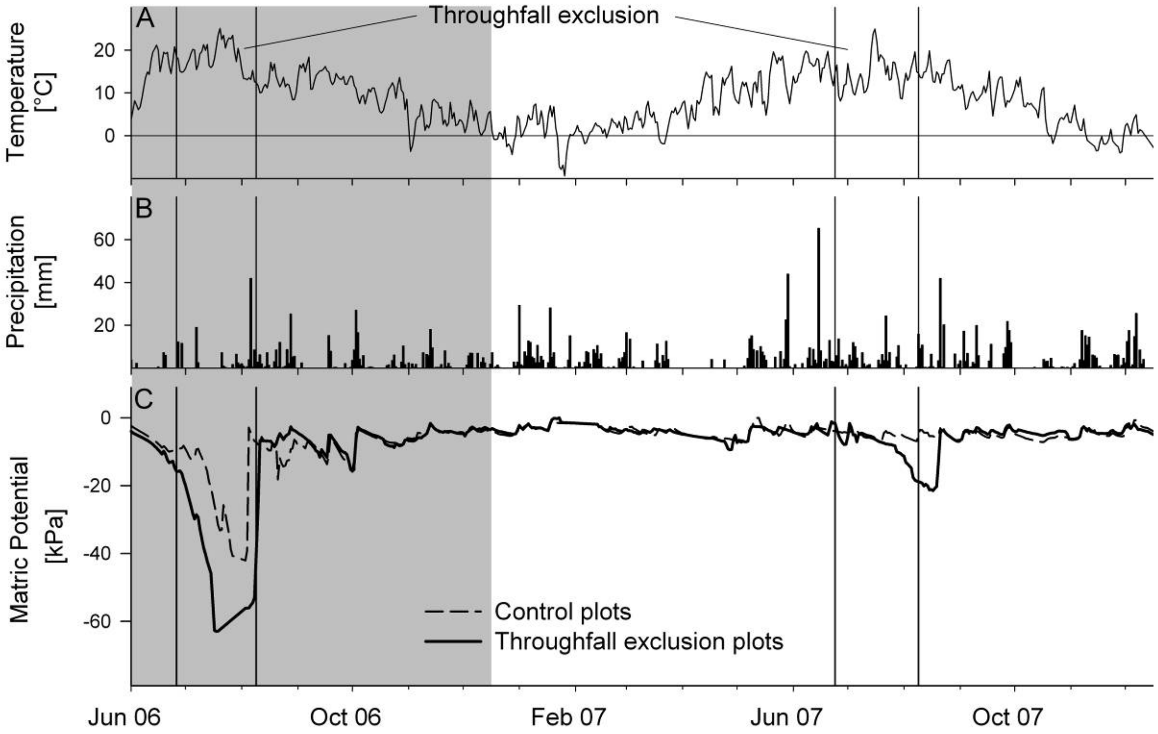

Figure 1. A) Daily mean air temperatures (n=24) at the Norway spruce forest stand recorded at 2 m above the forest floor, B) precipitation and C) soil matric potential at 20 cm depth at the control and throughfall exclusion plots. The lines mark the experimental throughfall exclusion period and the grey box marks already published data (Goldberg & Gebauer 2009).

In 2007, throughfall exclusion resulted in significantly less negative matric potentials of -200 hPa at the end of the experiment. The matric potentials of the throughfall exclusion plots were nearly constant in 20 cm soil depth with values between -50 and -70 hPa from the beginning of the experiment to 31 July (Fig. 1C). Only thereafter the potentials decreased to -190 hPa at the end of the experiment (10 August) and went on to decrease down to -220 hPa on 17 August. Subsequently the matric potentials increased to initial values of -40 hPa on 21 August. The soil matric potentials of the control plots all the time ranged between -30 and -70 hPa.

Soil NH_4^+ and NO_3^- contents. Mean soil NH_4^+ contents (0.4 to 17 µmol g_{dw}^{-1}; Tab. 1) were at all times larger than NO_3^- contents (0.02 to 1.9 µmol g_{dw}^{-1}; Tab. 1) for all measured soil horizons. Mean soil NH_4^+ as well as NO_3^- pools decreased from the organic layer to the top mineral soil. C and TE plots did not differ significantly with respect to their NH_4^+ and NO_3^- concentrations, with one exception: Right after the first rain event following the throughfall exclusion, NO_3^- concentrations in the L layer were significantly higher on the TE plots. NH_4^+ and NO_3^- concentrations did not differ significantly between August 2006 and June 2007.

Table 1. Mean NH_4^+ and NO_3^- concentrations (n=3 ± standard error of the mean) in µmol g_{dw}^{-1} in the L, O, A and B horizon of control and throughfall exclusion plots after the throughfall exclusion in 2006 (14 August), and before (12 June) and after the throughfall exclusion (13 August) in 2007.

Sampling		Control plots				Throughfall exclusion plots			
date		L	O	A	B	L	O	A	B
14-Aug-06	NH_4^+	7.86±2.97	4.39±0.42	1.79±0.31	0.42±0.11	10.16±0.12	7.01±0.22	1.56±0.03	0.59±0.05
	NO_3^-	0.10±0.02	0.38±0.10	0.04±0.01	0.01±0.00	0.20±0.03	0.25±0.06	0.02±0.01	0.02±0.01
12-Jun-07	NH_4^+	9.36±1.76	2.42±0.78	1.03±0.50	0.46±0.23	12.84±5.25	3.38±0.79	1.40±0.18	0.56±0.15
	NO_3^-	0.20±0.10	0.13±0.04	0.10±0.03	0.06±0.00	0.14±0.04	0.13±0.06	0.06±0.08	0.05±0.03
13-Aug-07	NH_4^+	9.22±0.88	3.43±1.17	0.85±0.69	0.63±0.28	17.12±2.75	8.83±0.63	1.80±0.18	0.81±0.24
	NO_3^-	0.03±0.01	0.11±0.09	0.05±0.04	0.03±0.01	1.85±0.61	0.12±0.06	0.06±0.01	0.02±0.01

N_2O fluxes. In 2007 net N_2O fluxes were always positive (i.e. the soil served as a source for N_2O) with the only exceptions in April and November with mean values between -0.01 µmol m^{-2} h^{-1} and -0.1 µmol m^{-2} h^{-1} (Fig. 2A). In the month before the experimental drought period, N_2O fluxes from the TE plots ranged between 0.1 and 0.2 µmol m^{-2} h^{-1}. In contrast to the experiment in 2006, net negative fluxes did not occur during throughfall exclusion in 2007, but N_2O fluxes on the TE plots stepwise decreased from 0.18 ± 0.05 µmol m^{-2} h^{-1} to 0.02 ± 0.01 µmol m^{-2} h^{-1}. N_2O fluxes remained at this low level until 17 August. Together with moderate rainfall events (Fig. 1 B) and increasing matric potentials (Fig. 1C),

N_2O fluxes from the TE plots increased up to 0.1 ± 0.02 µmol m^{-2} h^{-1} on 20 August. The N_2O fluxes on the C plots were significantly enhanced compared to the TE plots on most measurement dates during the throughfall exclusion period with values between 0.15 ± 0.04 µmol m^{-2} h^{-1} and 0.25 ± 0.06 µmol m^{-2} h^{-1}. Even after the throughfall exclusion period N_2O fluxes on the C plots exceeded those of the TE plots.

The mean N_2O fluxes on the TE plots integrated over the experimental drought period in 2007 amounted to 57 % of those of the C plots (Fig. 2B). Also after the end of the experiment, cumulative N_2O fluxes increased faster on C than on TE plots. Cumulative N_2O emissions in 2007 exceeded those of the year before by far.

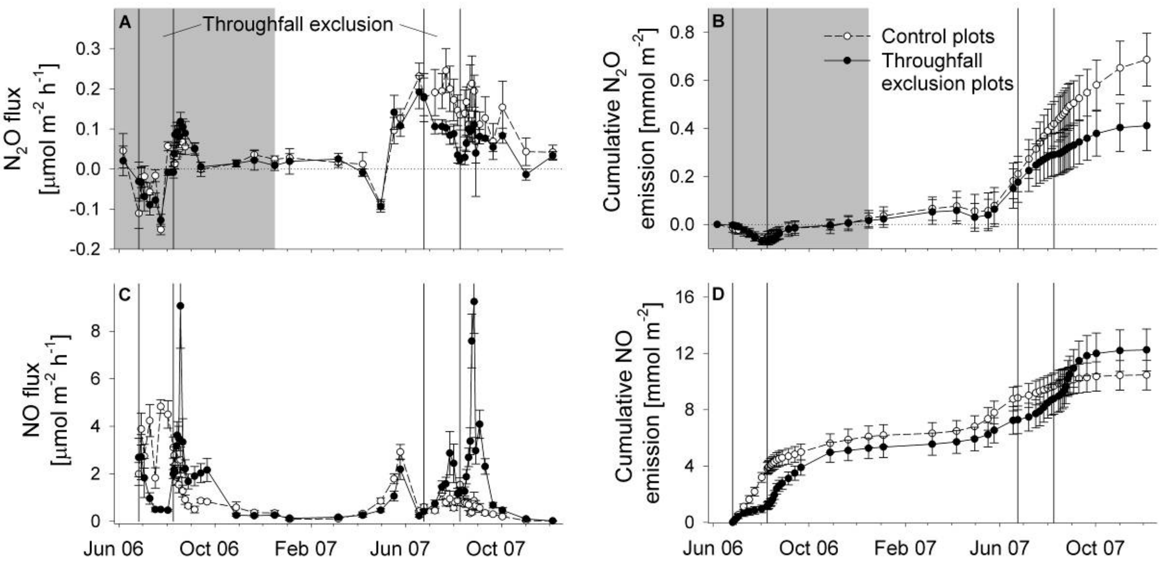

Figure 2. Time course of A) daily N_2O fluxes, B) cumulative N_2O emissions, C) daily NO fluxes and D) cumulative NO emissions on the throughfall exclusion and control plots. Error bars indicate the standard error of the mean (n=3). The lines mark the experimental throughfall exclusion period and the grey boxes mark already published data (Goldberg & Gebauer 2009).

N_2O concentration and isotope profiles and net N_2O turnover rates. Throughout the entire study period, mean N_2O concentrations in the soil profile exponentially increased from concentrations close to ambient air (between 9.7 and 10.7 nmol l^{-1}) near the soil surface to 18 - 417 nmol l^{-1} at a depth of 60 to 70 cm in the Cv horizon (Fig. 3).

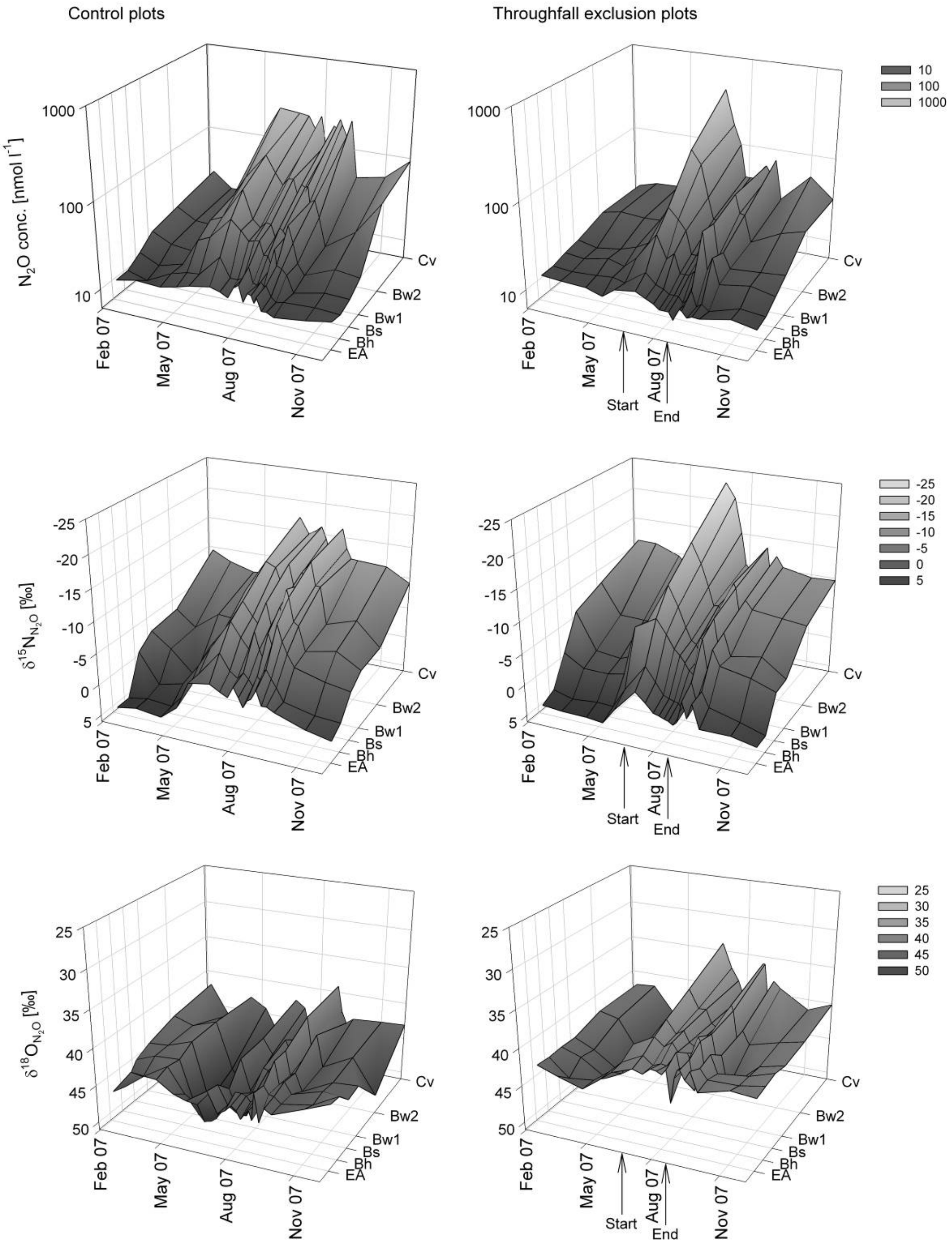

Figure 3. Mean N_2O concentrations (top), $\delta^{15}N$ (middle) and $\delta^{18}O$ values (bottom) of N_2O in soil air (n=3) along soil profiles on control (left) and throughfall exclusion plots (right). The arrows indicate start and end of the throughfall exclusion period.

Both N_2O concentrations and isotope signatures revealed a clear annual pattern with highest concentrations and most pronounced ^{15}N and ^{18}O depletion of N_2O in summer. This annual pattern was most obvious in the subsoil.

Throughfall exclusion had a clear effect on N_2O concentrations in the Cv horizon: N_2O concentrations continued to decrease from 417 ± 183 nmol l^{-1} to 41 ± 17 nmol l^{-1} from beginning (24 June) to the end of the experimental drought period (9 August). Such a decrease was observed for all soil horizons, but less distinct towards the topsoil. In the EA horizon the decrease in N_2O concentrations from 14 ± 2 nmol l^{-1} to 10 ± 1 nmol l^{-1} was least pronounced.

After the end of the throughfall exclusion N_2O concentrations went on to decrease in the Cv horizon until 20 August (27 ± 7 nmol l^{-1}) and started to increase from 24 August to 10 September with mean values of 58 nmol l^{-1} and 74 nmol l^{-1}, respectively. In the EA horizon N_2O concentration already started to increase again on 20 August.

The N_2O concentrations in the different soil horizons of the control plots remained mostly constant during the same time with values between 100 ± 71 nmol l^{-1} and 217 ± 87 nmol l^{-1} in the Cv and between 12 ± 5 nmol l^{-1} and 19 ± 5 nmol l^{-1} in the EA horizon.

Turnover calculations revealed the subsoil to be a weak net N_2O source throughout the year of 2007 (data not shown except for the throughfall exclusion period), with highest net production rates in summer. During throughfall exclusion this source strength continuously decreased from 160 pmol cm^{-3} d^{-1} to 20 pmol cm^{-3} d^{-1} (Fig. 4). The topsoil turned out to be a net N_2O sink throughout the year, except during winter time (data not shown). This sink strength (integrated over EA and Bh horizon) was reduced during throughfall exclusion from -260 pmol cm^{-3} d^{-1} to -42 pmol cm^{-3} d^{-1}. If integrated over all mineral horizons down to 70 cm depth, net N_2O turnover rates were negative throughout the throughfall exclusion period, even though this sink strength decreased with time.

The $\delta^{15}N$ values were on most sampling dates correlated with the N_2O concentrations in a negative logarithmic manner (the highest N_2O concentrations in the profile coincided with the lowest $\delta^{15}N$ signatures; Fig. 3). During throughfall exclusion, $\delta^{15}N$ values increased from -21 ‰ to -7 ‰ in the Cv horizon and from -1 ‰ to +3 ‰ in the EA horizon. The horizons in-between showed similar shifts, with highest changes towards the subsoil.

The $\delta^{18}O$ values of the soil N_2O showed a much narrower range compared to $\delta^{15}N$ (Fig. 3) and thus were not correlated with the N_2O concentrations for most sampling dates. Just in summer, with largest ranges, $\delta^{18}O$ values were linearly correlated with $\delta^{15}N$ and in a negative logarithmic manner with the N_2O concentrations in soil air. On the TE plots, however, these correlations disappeared two weeks after starting the throughfall exclusion.

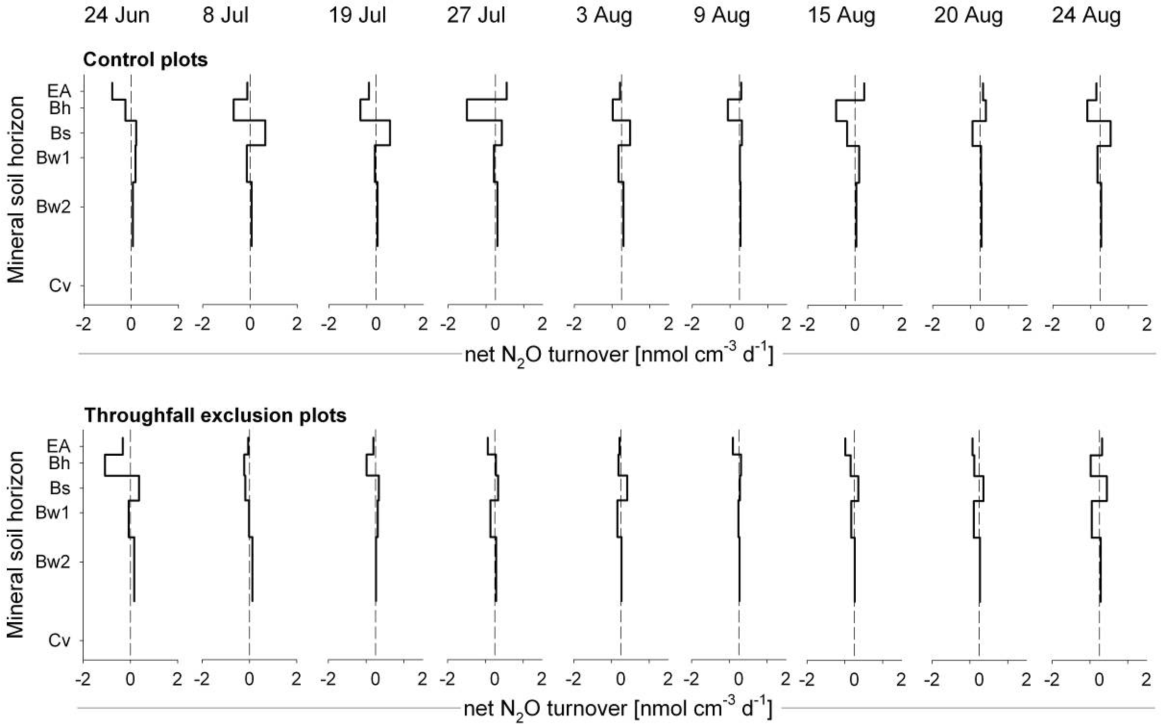

Figure 4. Mean net N_2O turnover rates of N_2O in soil air along soil profiles on control (top) and throughfall exclusion plots (bottom) during the throughfall exclusion period (24 June to 9 August) and thereafter. The space between different soil horizons reflects their mean distance along the vertical profile.

NO fluxes. Daily NO fluxes showed a seasonal pattern with lowest values in winter and highest ones in summer. NO fluxes during the summer were strongly affected by the drought experiment (Fig. 2C).

In 2006 NO emissions on the TE plots continuously decreased from 2.7 ± 0.8 μmol m^{-2} h^{-1} to 0.5 ± 0.04 μmol m^{-2} h^{-1} during throughfall exclusion, following the pattern of the soil matric potential. Immediately after rewetting, NO fluxes increased with a mean maximum value of 9.1 ± 1.8 μmol m^{-2} h^{-1} on 18 August, and mean NO emissions were 2.7 times higher than those on the C plots during the month after rewetting. On the C plots, NO fluxes ranged between 1.8 ± 0.4 μmol m^{-2} h^{-1} and 4.5 ± 0.6 μmol m^{-2} h^{-1} during the throughfall exclusion period. With major rain events during August the NO fluxes decreased from 2.8 ± 0.4 μmol m^{-2} h^{-1} to 0.5 ± 0.1 μmol m^{-2} h^{-1}.

In 2007, mean NO fluxes at the TE plots stepwise increased from 0.4 ± 0.1 μmol m^{-2} h^{-1} to 2.9 ± 1.0 μmol m^{-2} h^{-1} from the beginning of the throughfall exclusion to 27 July. After 27 July, together with decreasing soil matric potentials in 20 cm depth, mean NO fluxes successively decreased to 1.2 ± 0.2 μmol m^{-2} h^{-1} on 9 August. After the first rain event following the throughfall exclusion period, mean NO fluxes increased up to a maximum of 9.2 ± 1.3 μmol m^{-2} h^{-1} on 27 August and were on average 6.5 times higher than NO emissions from the C plots during the month following wetting. NO emissions from the C plots ranged between 0.5 ± 0.1 μmol m^{-2} h^{-1} to 1.5 ± 0.3 μmol m^{-2} h^{-1} during the throughfall exclusion period and between 0.3 ± 0.1 μmol m^{-2} h^{-1} and 0.9 ± 0.4 μmol m^{-2} h^{-1} during the subsequent month. From October 2007 to the end of the measurement period, mean NO fluxes of both C and TE plots did not differ and ranged between 0.0 μmol m^{-2} h^{-1} and 0.6 μmol m^{-2} h^{-1} as in the year before.

In spite of differences in NO fluxes due to throughfall exclusion and rewetting, total cumulative NO emissions from the C and TE plots did not significantly differ with 10.5 ± 1.1 mmol m^{-2} and 12.3 ± 1.5 mmol m^{-2}, respectively (Fig. 2D). Whereas the rewetting of dry soil had similar effects in both years, soil drought differently affected NO emissions. During severe drought in 2006, cumulative NO emissions from the TE plots were reduced by about 70 % compared to the C plots, whereas cumulative NO emissions from the TE plots in 2007 were 180 % those of the C plots. Following rewetting, the soil of the TE plots showed significant higher NO fluxes compared to the control over a period of 39 days in 2006 and 36 days in 2007 with cumulative fluxes of 2.5 and 2.9 μmol m^{-2}, respectively, during this time. These periods of 75 days in total, were responsible for 44 % of the total NO emission from the TE plots throughout the whole course of the experiment (597 days).

Discussion

N_2O fluxes and processes governing the N_2O pool within the soil. The throughfall exclusion in 2007 had strong effects on N_2O fluxes from soil to atmosphere at our study site, resulting in reduced N_2O emissions. The fact, that we did not observe a net soil N_2O

sink function in 2007, as found during the drought period in 2006, is most likely related to the different meteorological conditions in the two consecutive years (see below).

Throughout the year of 2007, soil N_2O concentration profiles showed an exponential increase with increasing soil depth, as in the year before, suggesting the subsoil to be an N_2O source in this Norway spruce forest. This was also true for the throughfall exclusion period, but drastically decreasing N_2O concentrations in the subsoil together with increasing $\delta^{15}N$ and $\delta^{18}O$ values indicated decreasing N_2O production with ongoing drought. However, N_2O turnover calculations revealed that reduced N_2O production in the subsoil during summer drought has low potential to influence N_2O fluxes between the soil surface and the atmosphere.

N_2O isotope abundance analysis revealed N_2O consumption, i.e. reduction of N_2O to N_2 during denitrifying processes, taking place throughout the soil profile, since $\delta^{15}N$ values (and $\delta^{18}O$ in most cases; see below) increased with decreasing soil depth. N_2O consumption fractionates against ^{15}N and ^{18}O (Barford *et al.* 1999), and thus more N_2O enriched in ^{15}N and ^{18}O is left during reduction of N_2O to N_2 (Tilsner *et al.* 2003, Wrage *et al.* 2004). In contrast, isotope fractionation by diffusion would have led to decreasing $\delta^{15}N$ (and $\delta^{18}O$) values in N_2O towards the topsoil (Pérez 2005). An N_2O isotope depth gradient as dominated by diffusion was hitherto only found in this forest soil during periods of severe frost suppressing any further microbial N_2O consumption in the topsoil (S.D. Goldberg *et al.*, unpublished data).

During throughfall exclusion N_2O consumption was still the dominating process along the whole soil profile until the border to the organic layers, although stepwise decreasing with ongoing drought, as concluded for three reasons:

(1) The $\delta^{15}N$ values of N_2O further on increased throughout the throughfall exclusion period from the site of N_2O production (in subsoil) to the topsoil, indicating that N_2O consumption was still the governing process. However, the $\delta^{15}N$ gradient became steeper, especially in the topsoil. This successive decreasing range of $\delta^{15}N$ values along the soil profile suggests that N_2O consumption was stepwise diminished.

(2) From the correlation between $\delta^{15}N$ and $\delta^{18}O$ of N_2O in the soil before and during the first two weeks of experimental drought, it can be concluded that further reduction of N_2O to N_2 was the process mainly governing the N_2O pool within the soil. Production processes do not result in correlation between both parameters, since 1) during N_2O

production *via* denitrification with regard to ^{18}O both intermolecular and intramolecular isotope effects with opposite $\delta^{18}O$ shifts can occur. That means preferential reduction of the lighter molecules yields a negative shift, whereas preferential elimination of ^{16}O over ^{18}O during the NO_3^- reduction to N_2O yields a positive shift (Toyoda *et al.* 2005). 2) N_2O production *via* nitrification would also not result in a correlation between both parameters since the N in N_2O originates from NH_4^+, while the O has multiple sources, like H_2O and atmospheric oxygen (Sutka *et al.* 1998, Pérez *et al.* 2000). In contrast to these processes, during reduction of N_2O to N_2, the $\delta^{18}O$ shift is similar to that of $\delta^{15}N$, i.e. positive, resulting in covariance between both parameters (Menyailo & Hungate 2006). Thus, the disappearing correlation between $\delta^{15}N$ and $\delta^{18}O$ of N_2O during throughfall exclusion indicates a decline of N_2O consumption within the soil.

(3) The turnover calculations additionally demonstrate diminished N_2O consumption, especially in the topsoil. However, integrated net turnover rates over the whole soil profile were negative throughout the throughfall exclusion, even if stepwise declining.

Decreasing N_2O consumption during drought is a plausible result, since this is an anaerobic process that is hindered by decreasing soil water content (Granli & Bøckman 1994). Thus, we conclude that the observed positive net N_2O fluxes were due to net N_2O production in the organic layers. This obvious N_2O source was as well diminished during drought – even more than N_2O consumption in the mineral soil. This conclusion is confirmed by laboratory mesocosm experiments from the same forest site, which indicated for topsoil cylinders nearly stopped net N_2O production at the end of a drought period lasting for six weeks (Muhr *et al.* 2008). Many studies on different soil types reported on decreasing net N_2O fluxes during drought (Yamulki *et al.* 1995, Davidson *et al.* 2004, van Haren *et al.* 2005, del Prado *et al.* 2006). Drought favours soil aeration and thus makes conditions unfavourable for N_2O production by denitrification (Castaldi 2000) or nitrifier denitrification. Furthermore, drought limits the overall activity of soil microorganisms, reducing the amount of N cycled in the ecosystem (Kieft *et al.* 1987).

In contrast to the experiment in the previous year, in 2007 we never observed subambient N_2O concentrations in the topsoil despite ongoing N_2O consumption, even though the sink strength of the soil achieved by turnover calculations was similar (data not shown). Thus, N_2O production in the topsoil must have compensated this N_2O sink in the 2007 experiment. The difference in N_2O topsoil production between both years was mostly due to different soil moisture. Measurements revealed that the water-filled pore space (WFPS) in 6 cm depth of one TE plot (soil water content was only recorded in one TE plot) at the end of the experiment in 2007 was twice as high as in 2006 (28 % *versus* 13 % WFPS; see also Fig. 5). In 2006, the experiment even started with a WFPS as high as at the end of the experiment in 2007.

Enhanced substrate availability in the experiment in 2007 due to the preceding storm event in spring could have also been a potential reason for enhanced N_2O production in the organic layers. However, since NH_4^+ and NO_3^- concentrations did not differ between both years in the organic layers, this reason is rather unlikely.

After rewetting, N_2O consumption stepwise increased to net rates as before the drought experiment. The reaction of N_2O production in the organic layers was faster, as indicated by increasing N_2O fluxes immediately after the first rain event. This release of N_2O after rewetting was probably due to fast microbial substrate utilisation, as observed in earlier studies. Prolonged drought periods generally lead to an accumulation of organic material (Seneviratne & Wild 1985, van Gestel *et al.* 1991) and both carbon and nitrogen mineralisation rates are commonly enhanced for a few days after rewetting of dry soil (Birch 1958, Bloem *et al.* 1992, Cui & Caldwell 1997, Franzluebbers *et al.* 2000). The N_2O production is stimulated by the increased availability of nitrogen, as in our study deflected by the enlarged NO_3^- concentrations on the TE plots, and in the case of denitrification additional by easily available organic carbon (Mummey *et al.* 1994, Włodarczyk 2000).

NO fluxes. The annual emissions of NO observed in this study are within the range reported for other European forest soils (Butterbach-Bahl *et al.* 1997, Meixner *et al.* 1997, Pilegaard *et al.* 2006, and references therein). NO emissions were much higher than N_2O emissions, at least in summer, as already reported for temperate forest soils (Butterbach-Bahl *et al.* 2002, Goldberg *et al.* 2008b, Muhr *et al.* 2008).

Whereas wetting of the soil after experimental drought in both years, with different rainfall intensities, resulted in equal net fluxes, the NO fluxes reacted different during the drought periods of the two consecutive years.

While N_2O fluxes in general increase at higher water contents due to higher denitrifying microbial activity (Wolf & Russow 2000), the relation between NO fluxes and water content is more complex. At high soil moisture, gas diffusivity is limited and the aerobic microbial activity decreases with further wetting of the soil. Additionally, increasing residence time due to increasing soil moisture and slower diffusion facilitates the conversion of the highly reactive NO. In contrast, at low soil moisture the microbial activity decreases with decreasing soil moisture due to limited substrate diffusion (Skopp *et al.* 1990, Pilegaard *et al.* 2006). Data from the literature indicate maximum NO emissions at intermediate soil moisture, however, with a wide range between 20 % and 85 % WFPS (Pileaard *et al.* 1999, van Dijk & Meixner 2001, van Dijk & Duyzer 1999).

Indeed, also in our study NO fluxes increased with decreasing WFPS in the organic layers, as observed in one TE plot (Fig. 5), and reached a maximum at around 33 % WFPS. At lower WFPS, as in the whole experiment in 2006, the NO fluxes strongly decreased again. This explains the different course of NO emissions during the throughfall exclusion periods in the two consecutive years. However, with regard to the C plots such a relation could not be observed (not shown).

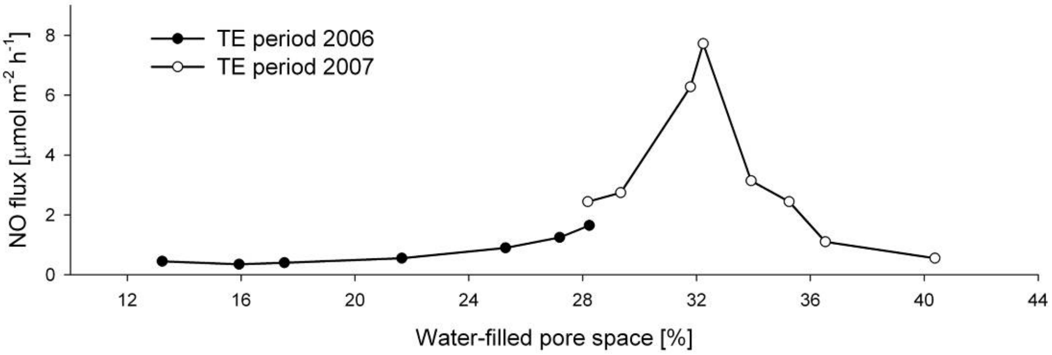

Figure 5. Mean daily NO fluxes in one throughfall exclusion in dependence on the water-filled pore space in the organic layers (6 cm depth) in this plot during both experimental periods.

In this study, we observed decreasing NO emissions during natural rain events, but a large NO emission peak during and after rainfall following prolonged summer drought. The former findings are in agreement with Gasche & Papen (1999), who reported on decreasing NO emissions from forest soils after (heavy) rain events. This is most probably due to conversion of NO when the soil becomes wetter and diffusion becomes slower. In contrast, addition of water to very dry soil mostly results, as in our study, in NO peaks (Yamulki *et al.* 1995, Scholes *et al.* 1997, Ludwig *et al.* 2001). These emission peaks seem to be due to nutrient accumulation during soil drought and dormant, drought-stressed microorganisms that immediately utilise these nutrients after rewetting (see above; Ludwig *et al.* 2001). As reported for other soils, this 'pulse', which significantly exceeded the NO fluxes on all other days, was short-lived, lasting for a few days only (Yamulki *et al.* 1995, Scholes *et al.* 1997, Ludwig *et al.* 2001). However, NO fluxes after these short peaks were still enhanced compared to the controls for one month after rewetting, revealing a considerable impact on the annual NO budget.

Conclusions

Our results based on N_2O flux measurements in combination with N_2O concentration and isotope abundance gradients along soil profiles emphasise the outstanding role of microbial N_2O consumption in the mineral horizons of this forest soil. This process has mostly not been considered so far. In combination with a stepwise reduced N_2O production in the organic soil layer during summer drought, a potential for turning the soil from an N_2O source to a temporary N_2O sink arises from this mineral soil N_2O sink function. The occurrence of a soil net N_2O sink function obviously depends on the length and severity of the soil drought period leading to high between-year variations.

Furthermore, our results show that severe drought leads to decreasing NO emissions, whereas moderate drought may increase NO emissions from forest soils. Rainfall after long-lasting drought periods in any case leads to increasing NO emissions with considerable impact on the annual NO budget.

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CHAPTER 4

Forest soil N_2O emissions as affected by freezing/thawing

N_2O emission in a Norway spruce forest due to soil frost – Concentration and isotope profiles shed a new light on an old story

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Abstract

In mountain regions of Central Europe an increase of soil frost periods is predicted for this century due to reduced snow fall. To investigate the effects of freezing and thawing on soil N_2O fluxes in a mature Norway spruce forest in the mountainous Fichtelgebirge, Germany, the natural snow cover on three experimental plots was removed to induce soil frost. Three plots with natural snow cover served as controls. Soil N_2O fluxes were recorded in biweekly to monthly intervals during the frost and subsequent thawing period of the below-average cold winter in 2005/06 and for reasons of comparison in the above-average warm winter in 2006/07. In addition, N_2O concentrations and isotope signatures in soil air were measured along soil profiles in six different depths (from 6 to 70 cm).

The soil of the snow removal plots was frozen down to 15 cm depth from January to April 2006 while the soil of control plots remained unfrozen under snow cover. Both soil freezing and thawing resulted in almost 10-fold enhanced N_2O fluxes on snow removal plots contributing 84 % to annual N_2O emissions. In the subsequent winter without soil frost no effects were observed.

Vertical gradients of N_2O concentrations together with isotope abundance suggest that the subsoil of all plots was a probably weak, but continuous N_2O source throughout the year. Isotope signatures and N_2O concentration gradients in the soil profile indicate that microbial N_2O production and reduction of N_2O to N_2 did not or just marginally occur in frozen soil layers of the snow removal plots. Consequently, elevated N_2O fluxes in the late winter were attributed to the release of accumulated N_2O originating from the subsoil. At unfrozen soil, however, N_2O emissions were reduced due to a shift of the N_2O production-consumption ratio towards more consumption in the topsoil of both the control and snow removal plots. These findings contradict the general assumption that N_2O production in the organic layer is responsible for bursts of N_2O during soil frost.

Keywords: N_2O; N_2O consumption; soil frost; soil thawing; stable isotopes; ^{15}N; ^{18}O

Introduction

The trace gas N_2O considerably contributes to the greenhouse effect and is involved in the destruction of atmospheric ozone. Emissions from terrestrial soils are assumed to be the largest source of atmospheric N_2O (IPCC 2007). N_2O is produced in soils mainly as a by-product of microbial nitrogen transformations – primarily nitrification and denitrification. The amount of N_2O emission during the intermediate steps in these processes is controlled by a wide range of soil parameters. Most pronounced are soil temperature, soil water content and substrate availability (Granli & Bøckman 1994, Smith *et al.* 2003). Due to nitrogen fertilisation, specifically soils from intensively managed agricultural grassland systems are considered as high N_2O emitters (e.g. Smith 1997, Velthof *et al.* 2000). In general, the highest microbial activities are observed in temperate regions during the warm summer growing season (Sommerfeld *et al.* 1993). However, high N_2O emissions have repeatedly also been found at low soil temperatures during the winter season (Röver *et al.* 1998, Teepe *et al.* 2000, Yashiro *et al.* 2006), and even the largest seasonal emissions of N_2O have been observed specifically during freezing and thawing events (Papen & Butterbach-Bahl 1999, Teepe *et al.* 2000, Teepe & Ludwig 2004, Groffman *et al.* 2006). The few field studies currently available for forests considering N_2O emissions during soil frost periods (Papen & Butterbach-Bahl 1999, Teepe *et al.* 2000, Groffman *et al.* 2006) indicate that during freeze/thaw events N_2O emission levels can be as high as in agricultural systems. Nonetheless, the mechanisms involved in these N_2O emission bursts still remain a matter of debate.

With regard to forest soils it is generally assumed that N_2O production and/or consumption occurs predominantly in the organic layer (e.g. Menyailo & Huwe 1999, Pihlatie *et al.* 2007). Accordingly, N_2O peaks during soil frost have mostly been attributed to substrate accumulation in small water films resulting in enhanced microbiological activity in the unfrozen soil water (Papen & Butterbach-Bahl 1999, Teepe *et al.* 2000) and N_2O emission bursts during thawing periods have been ascribed to enhanced microbial N_2O production in the topsoil due to increased substrate and easily decomposable carbon availability (Papen & Butterbach-Bahl 1999, Neilsen *et al.* 2001, Teepe *et al.* 2000). The quantitative importance of forest soil freezing and thawing for annual stand-level N_2O budgets is highly uncertain. Nonetheless, estimates from some field investigations indicate that such events may contribute up to 70 % to the annual N_2O emissions (Papen & Butterbach-Bahl 1999, Teepe *et al.* 2000). In contrast to these findings from field studies, in the vast majority of laboratory freeze/thaw experiments with forest soils similar high N_2O emission peaks never could be observed (Priemé & Christensen 2001, Teepe & Ludwig 2004, Goldberg *et al.* 2008b).

These contradictory findings in field and laboratory investigations have been attributed to 1) other effects of freezing being responsible for a stimulation of C and N cycling under field conditions, e.g. disruption of soil structure (Neilsen *et al.* 2001), 2) artifacts, i.e. experimental designs in laboratory experiments that do not reflect natural conditions (Henry 2007) or 3) apparently minor contribution of the organic layer to the total soil N_2O emission after freezing (Teepe & Ludwig 2004, Goldberg *et al.* 2008b).

The objective of this field study was to induce dry-cold winter climate conditions and to examine N_2O fluxes during and after soil frost in comparison to unfrozen control conditions. Specifically, we wanted to obtain more information about reasons for the phenomenon of N_2O bursts during soil freezing and thawing by using a new tool: The investigation of temporal variations in N_2O concentration and isotope signature along soil profiles.

Materials and methods

Site description. The experiment was carried out in a mature Norway spruce forest (*Picea abies*) in the Fichtelgebirge (northeastern Bavaria), Germany, at the Coulissenhieb II research site (50°8'N, 11°52'E) in an elevation of 770 m a.s.l. This site belongs to the Lehstenbach catchment (size: 4.5 km^2), which is almost completely covered by Norway spruce forest. The experimental site is exposed to WNW with a slope of about 3 %. Measurements from 1988 to 2003 at a groundwater well in 200 m distance from our experimental forest site revealed DOC, NO_3^- and NH_4^+ concentrations in the groundwater to be in the range of 0.5 to 2 mg l^{-1}, 0.1 to 0.2 mmol l^{-1} and 0.5 to 4 μmol l^{-1}, respectively (G. Lischeid, personal comm.). NO_3^- leaching from nitrification in the topsoil as well as from atmospheric deposition is known to contribute in this area considerably to the nitrate found in the groundwater (Durka *et al.* 1994). The DOC and NO_3^- leaching to the subsoil is a prerequisite for potentially occurring microbial denitrification in the subsoil of this site.

The mean annual temperature in this region is +5.3°C and the average annual precipitation is 1160 mm of which approximately 20 to 30 % are snow or mixed precipitation (Foken 2003). The soil is a Haplic Podsol with a 6-10 cm thick humus layer with distinct Oi, Oe and Oa horizons. The pH (H_2O) follows a rather small vertical gradient from 4.0 in the organic layers to 4.5 in the Bv/Cv horizon. For more detailed description of the soil see Hentschel *et al.* (2008). The understorey vegetation is dominated by *Calamagrostis villosa* (Chaix ex VIII), *Deschampsia flexuosa* (L.), *Vaccinium myrtillus* (L.) and *Oxalis acetosella* (L.).

Experimental design. Three control (C) and three snow removal (SR) plots, each of an area of 20 m x 20 m, were established in the summer of 2005. All plots were equipped with identical basal instrumentations for measurement of soil temperature, soil matric potential and soil solution chemistry. To induce soil frost snow was manually removed at the snow removal plots between the end of December 2005 and the beginning of February 2006. To avoid damage to the forest floor due to snow removal plastic nets (mesh width 1 cm) were used to cover the soil. The C plots were not manipulated and a substantial snow cover of 50-80 cm developed at these plots from January 2006 to the end of March 2006. The amount of snow removed from the SR plots was equal to 147 mm of water (Fig. 1A).

Measurements of N_2O fluxes. On each of the six plots, three stainless steel collars, 8 cm in height and with an inner diameter of 19.5 cm, were installed permanently for N_2O flux measurements. During the snow cover period the chambers on the C plots were inserted 4 cm into the snowpack just before measurement. Concentrations of N_2O, CO_2 and H_2O in the headspace were determined using a closed chamber technique in conjunction with a photoacoustic infrared gas analyser (Multigas Monitor 1312, INNOVA, Denmark, see Yamulki and Jarvis 1999). To exclude influences of changing CO_2 and H_2O concentrations on N_2O concentration measurements (see Yamulki and Jarvis 1999), the air was pumped through a CO_2 trap filled with soda lime pellets (Merck KGaA, Darmstadt, Deutschland) and a water trap filled with Drierite (98 % $CaSO_4$, 2 % $CoCl_2$, 8 mesh, W.A. Hammond Drierite Co. LTD, Xenia, USA) before passing the Multigas Monitor. N_2O concentrations were measured with a precision of ±15 ppb for a single N_2O concentration measurement.

N_2O fluxes on each of the six plots were monitored in biweekly to monthly intervals from November 2005 until February 2007. At each measurement date on two plots, one SR and one C plot, N_2O fluxes were simultaneously monitored in three replicates each as described by Goldberg & Gebauer (2009). For each chamber N_2O concentrations in the headspace were analysed 5 times over the total period of one hour. Gas flux rates were calculated from the linear increase or decrease in the gas concentrations in the chamber headspace with headspace volume and time. Sum curves were created by multiplying mean emission rates of two consecutive gas flux rates with the corresponding time period and summarising these time weighted means.

Gas sampling along soil profiles. N_2O gas samples along soil profiles from each plot were taken by use of six sub-surface soil air tubes. The 50 cm long plastic tubes with a 1.6 cm inner diameter were installed horizontally between 5 to 70 cm soil depth at the transition to the soil horizons EA, Bh, Bs, Bw, Cv. In the middle of the Bw horizon an additional soil air tube was installed, because of the thickness of this horizon. Mean installation depths of the gas tubes were 4-6, 10-15, 19-22, 24-30, 40-45 and 65-70 cm, varying from plot to plot because of differences in the thickness of soil horizons. Stainless steel tubes (ID: 1.5 mm) were connected at right angles with the soil air sampling tubes and led to the soil surface. For a more detailed description of the soil air sampling devices see Goldberg *et al.* (2008a). Soil air was sampled during induced soil frost (7 March 2006) and three months after the end of the soil frost period (10 July 2006) as well as in the subsequent mild winter without any soil frost periods (16 January 2007). Samples of ambient air (n = 3) were also taken on the respective sampling dates.

Measurement of N_2O isotope ratios and N_2O concentrations. $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of N_2O gas were measured using a pre-concentration device coupled with a gas chromatograph-isotope ratio mass spectrometer (PreCon-GC-C-IRMS) (Hewlett-Packard GC 5890 series II, Wilmington, USA; Combustion Interface II and gas-IRMS delta S, both Finnigan MAT, Bremen, Germany) as described by Brand (1995). The internal reproducibility of the measurement system is typically ± 0.15 ‰ for N and ± 0.30 ‰ for O. Isotope ratios are presented as δ-values, which are defined as:

$$\delta x\,[‰] = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \cdot 1000 \qquad (1)$$

where δx is the δ-value of the heavy isotope x (^{15}N or ^{18}O, respectively) and R is the ratio of heavy isotope (atom percent, at %) to light isotope (at %).

N_2O concentrations were calculated from the sampled gas volume and the peak area on mass 44 with the help of a calibration curve. The reproducibility of N_2O quantification based on this method is ± 4 ppb. For further details on this method see Goldberg *et al.* (2008a).

Data analysis. Each treatment was replicated three times and on each plot, gas flux measurements were carried out with three chambers to consider spatial heterogeneity. For each plot, mean fluxes were calculated by treating the chambers on the same plot as pseudo-replicates and the different plots as true replicates. Thus, for both treatments (SR and C plots) means of n = 3 ± standard errors were calculated including error propagation. For the calculation of cumulative N_2O emissions, N_2O fluxes between two consecutive measuring dates were linearly interpolated.

The significance of correlations between isotope signatures and N_2O concentrations in the soil profiles from the same treatment and sampling date were tested by using the correlation test after Pearson. N_2O concentrations were log-transformed before executing the analysis to attain a linear relationship between the two according variables.

Results

Soil matric potential, air and soil temperature. Before beginning of the snow removal period in the winter of 2005/06 no major differences in matric potentials of both C and SR plots were to be found in a depth of 20 cm beneath soil surface (Fig. 1B). During soil freezing and thawing (mid January to May 2006), the soil matric potentials of C and SR plots in 20 cm soil depth differed significantly due to decreased matric potentials on the SR plots. Almost constant matric potentials between 0 and -7 kPa were measured on the C plots, whereas the soil matric potential of the SR plots rapidly decreased to -20 kPa at the end of January 2006 and stepwise increased to the level of the controls again from March to May. In contrast, the soil matric potential in 40 and 90 cm soil depth did not show any significant differences between both treatments throughout the whole year (data not shown).

Compared with a 10-year average of -1.5°C the mean winter temperatures (December-March) in 2005/06 were below-average cold (-3.8°C), whereas the following, snow-free winter (2006/07) was above-average mild (1.2°C) (Fig. 2A). Thus, climate conditions in the winter period 2005/06 were almost ideal for the experiment. Due to snow removal below-zero temperatures were attained down to 15 cm soil depth. In January and February the daily mean soil temperature at 5 cm depth dropped to -5°C and remained <0°C until 26 March (Fig. 2A). At 15 cm soil depth, temperatures ranged between -1°C and -0.1°C from 24 January to 29 March. At 25 cm soil depth, temperatures did not differ between C and SR plots at any time (data not shown). The soil of the C plots remained unfrozen at all depths throughout the entire measurement period.

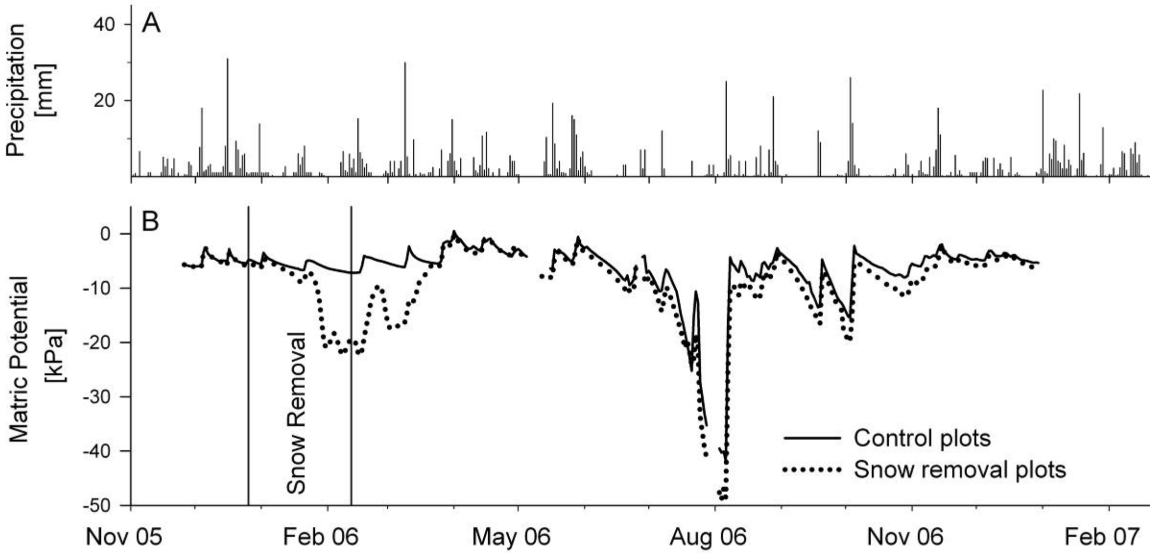

Figure 1. A) Precipitation and B) time course of the daily mean soil matric potential at 20 cm soil depth at the control and snow removal plots.

N_2O fluxes. Prior to the snow removal treatment and at the beginning of the soil freezing period, N_2O fluxes between soil and atmosphere of both C and SR plots were similar and ranged between 0.0 and 0.1 μmol m^{-2} h^{-1} (Fig. 2B). From end of February until end of April 2006 soil N_2O emissions on SR plots were considerably enhanced compared with the C plots. This period of enhanced soil N_2O emissions included a major part of the soil frost period as well as the soil thawing period. During this time mean N_2O fluxes of the SR plots ranged between 1.2 ± 0.5 μmol m^{-2} h^{-1} and 3.5 ± 0.9 μmol m^{-2} h^{-1}. Maximum N_2O fluxes occurred on 6 March 2006, after an extreme cold week with daily mean temperatures <-5°C and on 30 March 2006, three days after the air temperature showed a first maximum of 8°C. Thereafter, N_2O emission rates decreased with increasing air and soil temperature, but were still five times higher than that of the C plots three weeks after the beginning of soil thawing. N_2O emissions on the SR plots reached the level of the C plots in mid of May 2006. Mean N_2O fluxes of the SR plots were 10 to 20 fold higher compared to those of the C plots, but C plots also showed highest emission rates during the late winter and spring period.

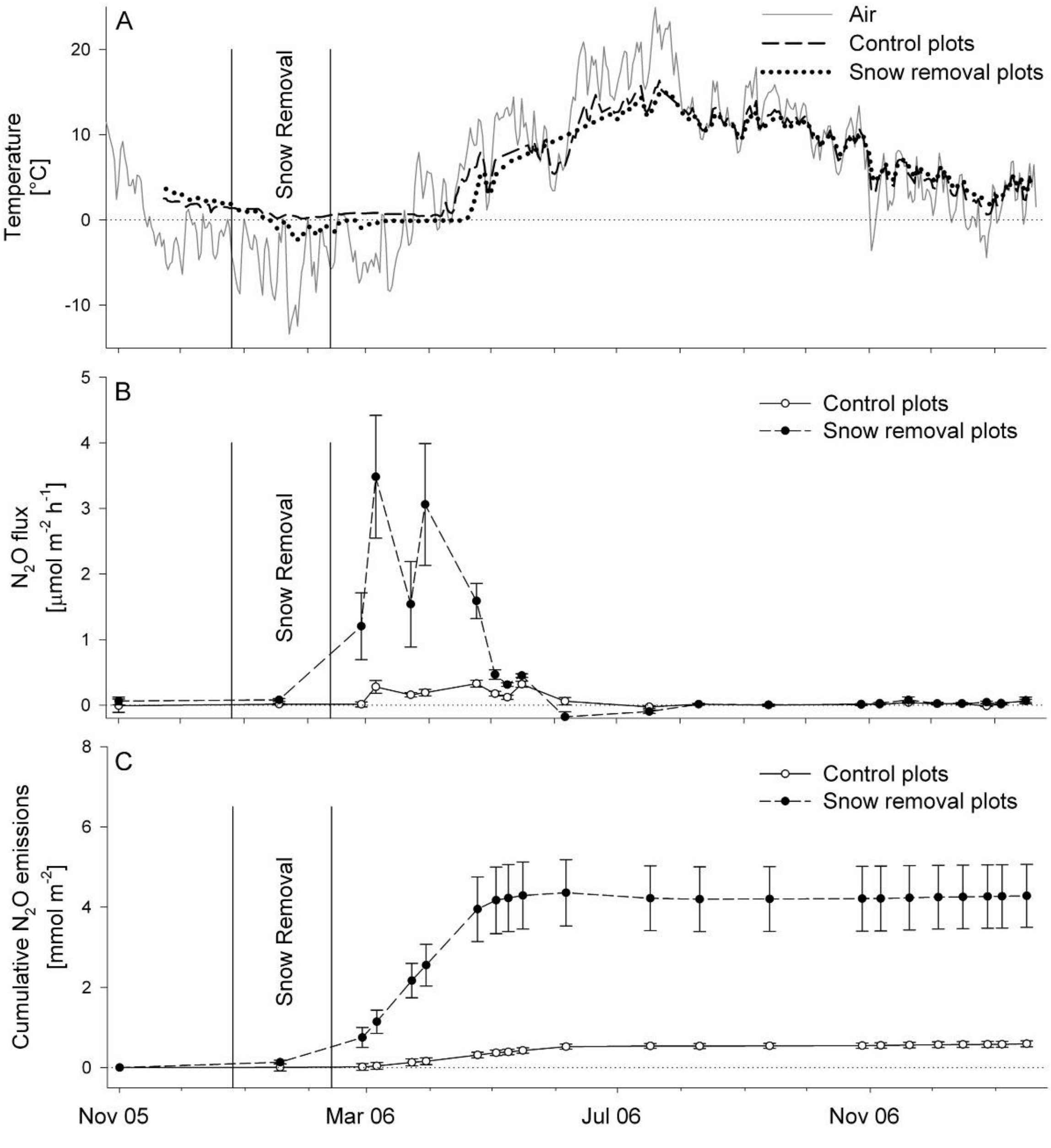

Figure 2. Time course of A) daily mean soil temperature at 5 cm depth and air temperature, B) mean N_2O fluxes, and C) mean cumulative N_2O flux rates. Error bars indicate the standard error of the mean (n=3).

From June 2006 to the end of the investigation period in January 2007, N_2O fluxes between SR and C plots did not differ significantly and were always <0.1 μmol m^{-2} h^{-1}.

On an annual scale the frost and thaw period of 78 days (from 27 February to 16 May 2006) contributed 84 % to the N_2O emissions (3.5 mmol m^{-2}). Even on the snow covered C plots – that were not subjected to soil freezing and thawing – enhanced N_2O emissions during this period contributed 75 % (0.4 mmol m^{-2}) to the total annual N_2O emissions of these plots. In total, 4.3 mmol N_2O m^{-2} were emitted from the SR plots throughout the whole course of the experiment (14 months), which significantly exceeded the emissions from the C plots (0.6 mmol N_2O m^{-2}).

N_2O concentration and isotope profiles. Throughout the experiment N_2O concentrations exponentially increased with increasing soil depth (Fig. 3). In the uppermost mineral soil horizon (EA) of both C and SR plots N_2O concentrations were mostly close to ambient air N_2O concentrations (310 to 380 ppbv). In the lowermost horizon of the investigated soil profiles N_2O concentrations ranged between 850 and 3400 ppbv. The only exceptions from this general trend were the N_2O concentration profiles of the SR plots during soil frost on 7 March 2006: On this date N_2O concentrations were drastically enhanced in all soil horizons with values ranging from 26 ± 13 ppmv to 50 ± 11 ppmv along the general concentration gradient from the EA to the Cv horizon. The N_2O concentrations on the SR plots increased in a logarithmic manner with soil depth, however, the relative differences between two consecutive soil horizons were significantly lower than on the C plots. Quotients of N_2O concentrations in two consecutive soil horizons ranged between 1.0 to 4.8 in the C plots and between 1.0 and 1.2 in the SR plots. In the summer of 2006 and the winter of 2006/07, with soil temperatures at any depth remaining above 0°C, N_2O concentrations as well as concentration gradients of the C and SR plots did not differ significantly and were similar to those found for the C plots in the winter of 2005/06.

In general, the highest soil air N_2O concentrations in the profile coincided with lowest $\delta^{15}N$ signatures and *vice versa* (Fig. 3) revealing a negative logarithmic correlation. Thus, subsoil N_2O was more depleted in $\delta^{15}N$ than that of the topsoil. The gradient in $\delta^{15}N$ was similar for both C and SR plots, with a mean difference in $\delta^{15}N$ between EA and Cv horizon ranging between 12 ‰ and 17 ‰.

In contrast to this general trend, during soil frost on 7 March 2006 $\delta^{15}N$ values of soil air N_2O on the SR plots slightly increased from the unfrozen subsoil to the frozen topsoil and N_2O concentrations were in a positive logarithmic manner correlated with the $\delta^{15}N$

signatures. On this date, mean δ^{15}N values of -8.4 ± 9 ‰ were observed in the Cv horizon, stepwise decreasing to -16.4 ± 9 ‰ in the EA horizon, with the largest mean depletion of 5.3 ‰ occurring from the Bh to the EA horizon.

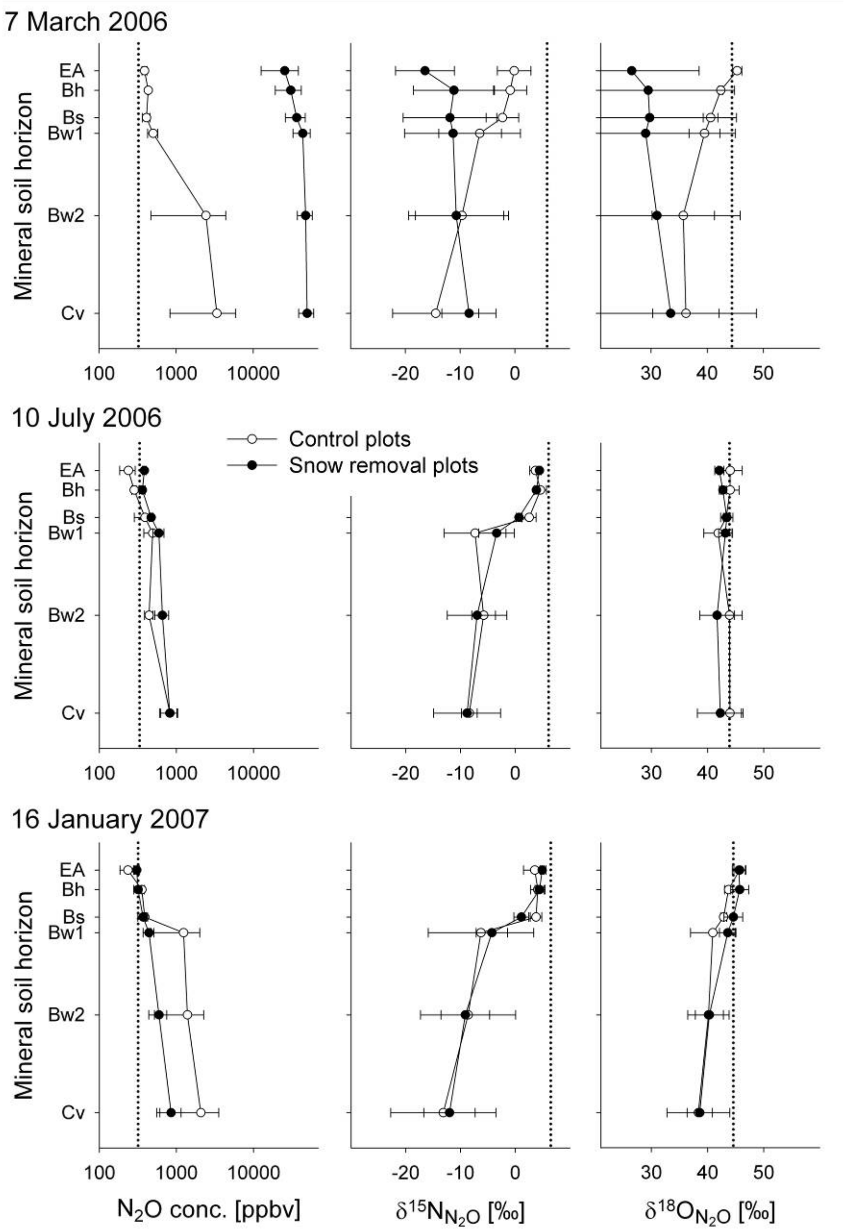

Figure 3. N_2O concentrations, δ^{15}N and δ^{18}O values of N_2O in soil air along soil profiles during soil frost (9 March) and after the experiment (10 July) in 2006, and in the mild winter (16 January) in 2007. Error bars indicate the standard error of the mean (n=3). The dotted line reflects the respective values of N_2O in the ambient atmosphere. The space between single horizons reflects their mean distance in the field.

$\delta^{18}O$ values mostly followed the same pattern as the $\delta^{15}N$ signatures (Fig. 3) and were in most cases significant in a negative logarithmic manner correlated with N_2O concentrations. This trend holds true for the N_2O depth profiles investigated on C and SR plots on 10 July 2006 and 16 January 2007 as well as for the C plots on 7 March 2006. However, as for $\delta^{15}N$ signatures this relationship to N_2O concentrations over soil depth was positive logarithmic on the SR plots on 7 March 2006. Again, the largest mean depletion in $\delta^{18}O$ (2.9 ‰) was found in N_2O diffusing from the Bh to the EA horizon on the SR plots on this date. $\delta^{18}O$ and $\delta^{15}N$ values were always strongly correlated, with only one exception in summer. On 10 July 2006 the $\delta^{18}O$ gradient of soil air N_2O along the soil profile was extremely slight. Gradients in $\delta^{18}O$ were always smaller than for $\delta^{15}N$, with differences between the lower- and uppermost soil horizon ranging between 0 ‰ and 9 ‰.

Over the complete experimental period $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in ambient air varied in narrow ranges between 4.9 ‰ and 6.2 ‰ or between 43.1 ‰ and 45.9 ‰, respectively (Fig. 3).

Discussion

N_2O emissions from our mountainous Central European Norway spruce forest soil were significantly enhanced on plots with soil frost induced by snow removal. This observation holds true for both the soil frost and the subsequent thaw period. The magnitude of these bursts in soil N_2O emission with maximum values of 3.5 μmol m^{-2} h^{-1} contributes considerably to annual N_2O emission budgets and is within the range of those found by Papen & Butterbach-Bahl (1999), Teepe *et al.* (2000) and Groffman *et al.* (2006) who observed maximum N_2O emissions of approximately 14, 4 and 2 μmol m^{-2} h^{-1}, respectively, for other temperate forest stands.

Explanations for N_2O emission peaks occurring during soil frost are often contradictory in the literature. With regard to agricultural soils, explanations range from N_2O production in unfrozen subsoil and the escape of N_2O (e.g. Burton and Beauchamp 1994, Kaiser *et al.* 1998) to microbial activity together with N_2O production in unfrozen compartments at the soil surface (e.g. Goodroad and Keeney 1984, Teepe *et al.* 2001). For temperate forest soils, however, it is assumed that a large portion of N_2O is produced in the organic layer during frost. Papen & Butterbach-Bahl (1999) and Teepe *et al.* (2000) suggested that huge N_2O releases resulted from high microbial N turnover rates in unfrozen water films at high concentrations of easily degradable substrates. However, results from laboratory experiments investigating specifically topsoils of similar forest soil types, in some cases even from the same study site, showed much smaller N_2O bursts as observed in field studies during soil frost events (Neilsen *et al.* 2001, Teepe & Ludwig 2004, Goldberg *et al.* 2008b). Hence, the humus layer is apparently not the major source of frost-related N_2O bursts from temperate forest soils.

The N_2O concentration profiles of both the C and SR plots indicate that N_2O was continuously produced in the subsoil, at 70 cm soil depth or deeper, throughout the year with generally low net production rates of 10 to 200 pmol cm^{-3} d^{-1}, as derived from turnover estimates (see Goldberg *et al.* 2008a). At any time – except for the soil frost period – largest N_2O concentrations were accompanied by most depleted $\delta^{15}N$ and $\delta^{18}O$ signatures in the subsoil. The depleted $\delta^{15}N$ and $\delta^{18}O$ signatures confirm N_2O production in the subsoil (Goldberg & Gebauer 2009). The concentration gradient implies upward diffusion of N_2O, a transport process linked with isotopic discrimination against ^{15}N and ^{18}O (ε_{15N} = -4.4 ‰, ε_{18O} = -8.6 ‰, with ε being the enrichment factor, i.e. isotope enrichment of a reaction product relative to that of the substrate; detected by Pérez *et al.* [2000] for a tropical forest soil). Thus, decreasing $\delta^{15}N$ and $\delta^{18}O$ values during upward transported N_2O would have been expected, if diffusion was the dominating process for the soil N_2O pool. The fact, that N_2O became isotopically enriched in unfrozen topsoil throughout the year, denotes the occurrence of dominating N_2O consumption (reduction of N_2O to N_2) over new N_2O production. N_2O consumption takes place *via* denitrification and fractionates against ^{15}N and ^{18}O, leading to a stepwise enrichment in ^{15}N and ^{18}O of the remaining N_2O pool. Accompanying fractionation against ^{15}N and ^{18}O depends on the proportion of N_2O reduced to N_2 (Barford *et al.* 1999), but in general exceeds the fractionation by diffusion (Pérez *et al.* 2000). Similar N_2O profiles with N_2O production in subsoil and upward diffusion together with stepwise consumption of N_2O have already been observed in different soils (Peréz *et al.* 2000, van Groenigen *et al.* 2005, Rock *et al.* 2007, Goldberg *et al.* 2008a, Goldberg & Gebauer 2009).

In contrast to this general trend, the N_2O profile affected by soil frost followed a completely different pattern. Under conditions of frozen topsoil, N_2O concentrations in the soil atmosphere were by one to two orders of magnitude higher compared to unfrozen control plots. We assert the higher N_2O concentrations in the soil atmosphere under conditions of a frozen topsoil to be due to the following reasons: 1) Continuing N_2O production in the (unfrozen) subsoil as indicated by 15-fold higher subsoil N_2O concentrations compared to those of the C plots on 7 March 2006. 2) A decreased N_2O consumption during upward movement of subsoil-derived N_2O in the topsoil, being most pronounced in the EA horizon as indicated by largest ^{15}N and ^{18}O depletion in N_2O diffusing from the Bh to the EA horizon. This drop of N_2O consumption in the topsoil may be due to a lowered activity of the enzyme N_2O reductase, as observed for soil temperatures around and below 0°C (Holtan-Hartwig *et al.* 2002). And 3) hindered N_2O exchange between topsoil and atmosphere due to frozen soil water as diffusion barrier in the topsoil as pointed out by smaller detected N_2O fluxes between soil and atmosphere as to be expected from N_2O concentrations in the top mineral soil.

Our results give evidence for the importance of microbial processes in the subsoil on N_2O production, and thus, topsoil fluxes in this forest. Furthermore, the results contradict previous assumptions that an increase in source availability and therewith in microbial activity in the organic layer are chiefly responsible for bursts of N_2O during soil frost periods.

In this study, the observed N_2O emissions during soil frost were of similar importance for the annual N_2O budget as N_2O emissions in the subsequent thawing period. Peak emissions during forest soil thawing are up to now explained by stimulated microbial activity due to an enhanced supply of nutrients, which is caused by die back of microbial biomass and/or disruption of aggregates during soil frost (Papen and Butterbach-Bahl 1999, Neilsen *et al.* 2001, Teepe *et al.* 2000).

We conclude from our results that the largest amount of N_2O released from our spruce forest during soil thawing is due to a slow release of subsoil N_2O along the concentration gradient and most probably a delayed activation of N_2O reductase in the topsoil after soil frost due to low soil temperatures. Enhanced nutrient supply due to soil freezing can be excluded as reason for the enhanced N_2O emissions, as reflected by concentration measurements of dissolved organic carbon and N solutes carried out throughout the experiment (Hentschel *et al.* 2009). This is also confirmed by Muhr *et al.* (2009), who did not find enhanced CO_2 fluxes during soil thawing.

Conclusions

Our results indicate that N_2O fluxes during cold winters with soil frost in Central European Norway spruce forests are of huge importance on an annual scale. Even on control plots without soil frost highest annual N_2O emissions were found throughout the period of snow cover as observed by others, too (Sommerfeld *et al.* 1993, Maljanen *et al.* 2003, Yashiro *et al.* 2006). However, N_2O emission maxima found during periods of snow cover were at least one order of magnitude lower compared to those observed during both, soil freezing and thawing. This study supports the until now scarce findings of a huge relevance of soil frost and thaw periods on N_2O losses from temperate forest soils to the atmosphere and emphasises that such winter fluxes have to be taken into account in global N_2O models. Our findings of subsoil production of N_2O together with reduced N_2O consumption in the topsoil during soil frost contradict previous mechanistic explanations of soil frost effects on N_2O emission. This emphasises the necessity to investigate N_2O dynamics along soil profiles in various temperate forest ecosystem types and to identify parameters inducing a subsoil N_2O production in order to improve our mechanistic understanding of N_2O freeze/thaw fluxes from temperate forest soils. Combining N_2O concentration with isotope abundance analysis appears to be a powerful tool in this context. Furthermore, our results give a hint for the reason of different findings in laboratory mesocosm experiments and field investigations on N_2O emissions associated with frost/thaw events in forest soils: Laboratory investigations focussed mostly on topsoil processes, and therefore, excluded potential subsoil N_2O production.

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CHAPTER 5

Fen soil N_2O and NO emissions as affected by water table

Impact of experimental drying and rewetting on N_2O and NO turnover and emissions from a temperate acidic fen

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Abstract

The impact of experimentally intensified summer drought and precipitation on N_2O and NO turnover and fluxes was investigated in a minerotrophic fen over a 2-year period. Drought was induced for 6 and 10 weeks by means of roofs and drainage and decreased water table levels by 10 cm to 30 cm compared to control plots. Both N_2O and NO emission hardly reacted to a water table decrease of about 10 cm in one plot. However, N_2O (530 %) and NO emissions (270 %) increased during both drought periods in the plot with the highest (30 cm) water table reduction. N_2O emissions were positively related to NO_3^- concentrations in 5 cm depth, whereas NO emissions were not. Rewetting reduced the NO emissions to background levels (0.05 - 0.15 μmol m^{-2} h^{-1}), but heavily enhanced N_2O emission (18 - 36 μmol m^{-2} h^{-1}) in the strongly dried plots for some days. These peaks contributed up to 40 % to the cumulative N_2O fluxes on the drought plots and were caused by rapid N_2O production *via* denitrification according to isotope abundance data. According to N_2O concentrations and isotope abundance analysis N_2O was mostly produced at depths between 30 and 50 cm. During water table reduction net N_2O production in 10 cm depth steadily increased in the most effectively dried plot from 2 pmol cm^{-3} d^{-1} up to 44 pmol cm^{-3} d^{-1}. Rewetting immediately increased net N_2O production in the topsoil of the drought plots, showing rates of 18 pmol cm^{-3} d^{-1} to 174 pmol cm^{-3} d^{-1}. This study demonstrates that drought and rewetting can temporarily increase N_2O emission to levels that have to date only been reported from nutrient rich and degraded fens that have been drained for agricultural purposes.

Keywords: summer drought; fen; N_2O; NO; $\delta^{15}N$; $\delta^{18}O$

Introduction

Soils contribute considerably to the greenhouse effect due to emissions of climate-relevant trace gases such as CO_2, N_2O and NO. Although the absolute quantities of emitted N_2O are up to three orders of magnitude less compared to emissions of CO_2, N_2O is of concern, since its global warming potential is nearly 320 times that of CO_2 when calculated for a 100 years time frame (Rodhe 1990). NO is involved in global warming by taking part in reactions leading to the production of tropospheric ozone, a radiatively active greenhouse gas (Hall *et al.* 1996). Additionally, deposition of NO contributes to the N-saturation of natural ecosystems (Aber *et al.* 1989) in turn leading to concomitant emission of N trace gases (Fenn *et al.* 1996, Davidson & Kingerlee 1997, Papen & Butterbach-Bahl 1999, Pilegaard et al 2006). N_2O and NO are produced in soils as facultative by-products of nitrification and intermediate products of denitrification, which can also consume NO and N_2O (Granli & Bøckman 1994, Kester *et al.* 1997).

Waterlogged fens are usually considered as marginal sources of N_2O and NO, owing to low rates of nitrification and, subsequently, denitrification (Martikainen *et al.* 1993, Lång *et al.* 1995, Regina *et al.* 1996, Augustin *et al.* 1998). However, N_2O emissions drastically increase with drainage of minerotrophic peat soils (Martikainen *et al.* 1993, Augustin *et al.* 1998, Merbach *et al.* 2001) due to increased N mineralisation and nitrification (Lång *et al.* 1993, Updegraff *et al.* 1995). Little is yet known about NO emissions from wetlands (Davidson 1991, Davidson & Kingerlee 1997), but drainage may result in increased NO emissions from wetlands as well (Lång *et al.* 1995). Water table levels of fens may not only be lowered due to anthropogenic drainage but also by intensified summer droughts, as predicted for most of Central Europe due to climatic change (IPCC 2007). The effects of the induced short-term water table fluctuations on N trace gas emissions are yet not well understood. Thus, more information is needed regarding the production of nitrogen oxides during such periods and their effect on annual balances for European fens under global climate change conditions.

It is obvious that flux measurements of the respective gases between soil surface and atmosphere do not suffice to trace back the underlying biogenic processes in the soil. Measurement of gas concentration profiles in the soil together with stable isotope abundance analysis turned out to be a suitable tool to overcome these limitations for N_2O (Pérez 2005, van Groenigen *et al.* 2005, Rock *et al.* 2007, Goldberg *et al.* 2008) and thus, may contribute to an improved mechanistic understanding of greenhouse gas budgets on an ecosystem level. Sampling techniques, as applied for the chemically rather inert gas N_2O, are not applicable for the highly reactive NO, with a short lifetime of about 1.5 d. Due to the difficulties associated with this gas species isotope abundance analysis on NO is still a challenging matter and not suited for routine applications (Lauf & Gebauer 1998, Lauf & Gebauer 2001). Nonetheless, there is broad evidence that very shallow surface layers are primarily the production zone for NO emitted from soils (Rudolph & Conrad 1996, Rudolph *et al.* 1996, Ludwig *et al.* 2001).

We hypothesised that intensified drought would increase mineralisation activity in the soils due to aeration, and thus also initiate nitrification, secondarily denitrification, and as a consequence also the production of nitrogen oxides. Increased N turnover could result in increased losses of N trace gases. Rapid rewetting after drought would then be followed by a short mineralisation pulse as previously observed, leading also to concomitant effluxes of NO and N_2O.

To test these hypotheses, we (1) quantified *in-situ* the effects of water table drawdown and subsequent rewetting by irrigation on the exchange of the trace gases N_2O and NO between soil and atmosphere in treatment plots of a temperate minerotrophic fen in comparison to untreated control plots subjected to natural weather conditions and (2) identified and localised the underlying biogenic N_2O production and consumption processes in the soil profile. For this second objective we particularly focussed on changes in concentration and isotopic composition (^{15}N and ^{18}O) of N_2O dissolved in the soil water and in the soil atmosphere.

Materials and methods

Study site. The experiment was conducted at a minerotrophic fen site (Schlöppnerbrunnen, fen area: 0.8 ha) situated in northeastern Bavaria in the Fichtelgebirge, Germany (50°07’ N, 11°52’ E; 700 m a.s.l.). The mean annual air temperature at the site is +5.3°C (1971-2000) and the mean annual precipitation is 1160 mm (Foken 2003). The soil is a Fibric Histosol on granite bedrock and the ground vegetation consists mostly of *Agrostis sp.*, *Nardus stricta*, *Molinia coerulea*, *Sphagnum cf. fallax*, *Brachythecium rivulare*, *Atrichum undulatum*, *Eriophorum vaginatum*, *Polytrichum commune, Galium hercynicum* and *Carex rostrata*. Peat accumulation ranges from 40-70 cm. A moisture gradient characterises the experimental site, thus the three control and treatment plots may not be seen as ‘true replicates’. In the north-western part, the fen is wet throughout the year and the vegetation is dominated by *Carex rostrata* and *Sphagnum fallax*. Towards the south-eastern part, the site becomes dryer and is only periodically waterlogged. The vegetation in this part mainly comprises *Nardus stricta*, *Agrostis sp.*, *Molinia coerulea*, *Eriophorum vaginatum*, *Brachythecium rivulare* and *Polytrichum commune*. For a detailed description of soil characteristics see Goldberg *et al.* (2008). An overview on the investigation site and the experimental plots is given in Fig. 1. The groundwater flow direction through the peat is from the north to the south (Paul *et al.* 2006).

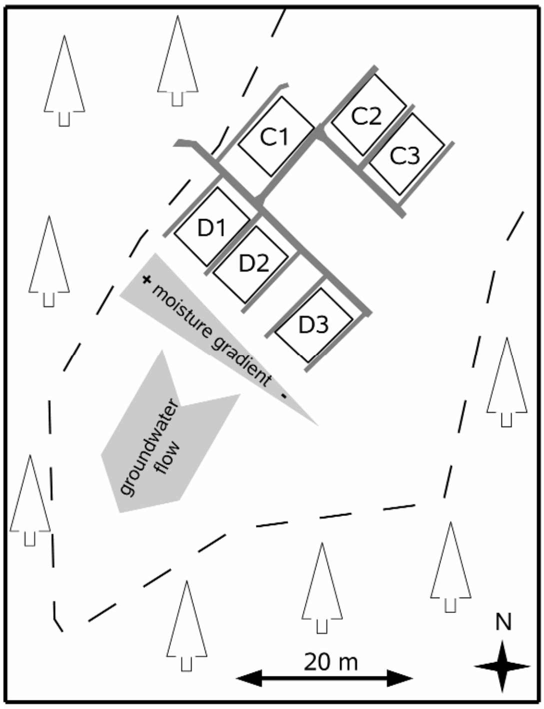

Figure 1. Overview of the study site with control (C) and drought (D) plots.

Experimental design. Three untreated control (C) plots and three drought (D) plots, each with an area of 7.2 m x 5 m, were established in the summer of 2005. The D plots were located downstream of the C plots in terms of groundwater flow direction. Each plot was equipped with soil temperature sensors in 5 to 60 cm soil depth, three collars for gas flux measurements to account for spatial heterogeneity, and one gas sampling device for profile measurements. Piezometers next to the gas sampling devices allowed for monitoring the water table levels. Air temperature and precipitation were measured at 2 m height nearby. On the D plots drought was experimentally induced using a roof and by extracting groundwater from drainage tiles. The roofs had large open side walls to minimise temperature and wind speed effects. Soil temperatures did not differ in the uppermost soil layers of C and D plots. Drainage tiles were installed at 1 m depth at two ends of the experimental plots, perpendicular to the groundwater flow direction. During the experiment in 2006 and partly in 2007, the tile drains were emptied manually every second or third day by a submersed pump. During the 2007 manipulation experiment an automated pumping system was used for the second half of the experiment.

In the summer of 2006 the water table level was experimentally lowered for 6 weeks from 14 August to 26 September, and in 2007 for 10 weeks from 10 May to 19 July. At the end of the drought experiments, the D plots were rewetted by 110 mm artificial rainwater in 2006 and 182 mm in 2007 with an irrigation intensity of 10 mm h^{-1} and 11 mm h^{-1}, respectively. In 2006, rewetting was completed within one day, whereas in 2007 111 mm of the artificial rainwater were applied on 19 July and 71 mm on 23 July. The artificial rainwater consisted of deionised water, and contained (μmol l^{-1}): Na^+ 39, K^+ 30, NH_4^+ 34, NO_3^- 34, SO_4^{2-} 12, resembling its natural analogue. Irrigation raised the water table to the level of the control plots (0-5 cm below surface; see Fig. 2).

NH_4^+ and NO_3^- concentration measurements. Soil solution was sampled using Rhizon® samplers (pore size ~0.16 µm, length 10 cm, diam. 3 mm, Eijkelkamp Agrisearch, The Netherlands). A peat core of 10 by 10 cm and 50 cm length was extruded and two samplers per depth were placed at 5, 10, 20, 30, 40 and 50 cm below the soil surface. The pit was subsequently refilled with the previously extruded peat core. Soil solution was sampled on 6 dates in 2006 between 14 August and 10 October and weekly from 30 April 2007 to 14 September 2007. NH_4^+ concentrations were analysed on a spectrophotometer (Varian Cary 1E, Varian Inc. USA) at 690 nm (Searle 1984), NO_3^- concentrations were analysed using ion chromatography with chemical suppression and conductivity detector (Metrohm modular IC system, Anion Dual 3 Column, Metrohm, Switzerland).

N_2O and NO flux measurements. N_2O and NO fluxes were measured weekly during the drought period, in 1- to 3-day intervals for two weeks after the drought period and biweekly to bimonthly during the remaining time. N_2O flux rates were determined using the closed chamber technique in conjunction with a photoacoustic infrared gas analyser (Multigas Monitor 1312, INNOVA, Denmark) (Yamulki & Jarvis 1999). N_2O concentrations were measured in three chambers (diameter 19.5 cm, height approx. 20 cm) at each plot after 0, 10, 20, 30 and 40 min with a precision of ±15 ppb for a single N_2O concentration measurement. For a more detailed description, see Goldberg & Gebauer (2009). Gas flux rates were calculated from the linear increase or decrease in the gas concentrations in the chamber headspace, the individual headspace volume, and sampling intervals.

NO emissions were measured using the open flow-through chamber technique as described by Yamulki *et al.* (1995). Charcoal and Purafil ($KMnO_4/Al_2O_3$) filtered air was pumped through the chamber at a constant rate of 150 l h^{-1}. Purafil removes NO_x from the air stream; charcoal is used to remove ambient O_3, thus eliminating reactions between ambient O_3 and NO within the chamber. Concentrations of nitric oxides were measured using an NO-NO_x-NO_2-Analyser (AC 31 M Monitor, Environment, Poissy, France) with a precision of 1 ppb. Fluxes of NO were calculated from the concentration in outlet air, the flow rate of air through the chamber, and the surface area of the chamber. Because of the high reactivity of NO, the chambers and the lid were covered with Teflon foil inside and Teflon-tubes were used to connect the chambers with the analyser.

Gas fluxes between consecutive sampling dates were linearly interpolated. To estimate cumulative N_2O and NO fluxes over the entire measuring period, sum curves were created by multiplying mean emission rates of two consecutive gas flux rates with the corresponding time period and summing these time weighted means.

Gas sampling along soil profiles. On each plot a passive diffusion gas sampler containing 6 sampling cylinders was installed to sample soil gas at 10, 20, 30, 40, 50 and 60 cm depth. Each PVC cylinder (ID: 7 cm, AD: 7.9 cm; 10 cm height) had a total sampling volume of 35.34 ml using 5 m of silicon tubing (ID: 3 mm; AD: 5 mm). Sampling was performed from the soil surface using gas impermeable polyurethane (PUR) tubing (ID: 1.8 mm; AD: 3 mm) fitted with stopcocks (Luer Lock, Value Plastics, Fort Collins, USA). To this end, glass bottles of 100 ml were first flushed with N_2 and then evacuated using a membrane vacuum pump and checking the vacuum (TensioCheck TC 03S, Tensio-Technik, Geisenheim, Germany). Subsequently, gas samples were taken from the various soil depths, replacing the extracted volume with ambient air. Samples of ambient air were collected (n = 3) on the respective sampling dates at 50 cm above the soil surface. For a more detailed description, see Goldberg *et al.* (2008). Soil gas samples were taken from April 2007 to April 2008.

Measurement and calculation of N_2O isotope ratios, concentrations and turnover rates. $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of N_2O gas as well as N_2O concentrations were measured using a gas chromatograph-isotope ratio mass spectrometer coupled to a pre-GC concentration interface (PreCon-GC-C-IRMS) (Hewlett-Packard GC 5890 series II, Wilmington, USA; PreCon, Combustion Interface II and gas-IRMS delta V, all Thermo Fisher Scientific, Bremen, Germany) as described by Brand (1995). Isotope ratios are presented as δ-values relative to air nitrogen for $\delta^{15}N$ (Mariotti 1983) and standard mean ocean water (Vienna-SMOW) for $\delta^{18}O$ as the standard:

$$\delta x\ [‰] = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \cdot 1000 \tag{1}$$

where δx is the δ value of the heavy isotope x (^{15}N or ^{18}O, respectively) and R is the ratio of heavy isotope (atom percent, at %) to light isotope (at %).

N_2O concentrations were calculated from the sampled gas volume and the peak area on mass 44 by means of an external calibration (reproducibility ± 4 ppb).

When soils were water-saturated, dissolved nitrous oxide (N_2O) concentrations in the soil solution were obtained using solubility data of Weiss & Price (1980) recalculated for measured *in-situ* temperature and salinity (data not shown). Passive diffusion samplers as used in this study also allow for analysing soil gas under unsaturated conditions (Kammann *et al.* 2001).

Since the gas vessels contained a mixture of sample air and ambient air, N_2O concentrations as well as δ values had to be corrected for N_2O in the ambient air. N_2O concentrations were corrected by subtracting the fraction of the ambient air from the measured N_2O concentration of a sample. Values of δ were corrected according to:

$$\delta x_{\text{soil } N_2O}\ [‰] = \frac{\delta x_{\text{measured}} \cdot c_{\text{measured}} - \delta x_{\text{ambient air}} \cdot c_{\text{ambient air}}}{c_{\text{measured}} - c_{\text{ambient air}}} \tag{2}$$

where δx is the δ value of the heavy isotope x, c is the N_2O concentration and the indices “soil N_2O”, “measured” and “ambient air” indicate soil N_2O sampled from various depths, total N_2O in the gas vessel and N_2O in ambient air, respectively (Tilsner *et al.* 2003b).

Net turnover R_N of N_2O (pmol cm^{-3} d^{-1}) in the individual depth layers in the fen was calculated from mass balances of diffusive fluxes and changes in storage over time according to Eq. (3).

$$R_N = \frac{\Delta S_{N_2O}}{\Delta t} + \left[D_A \frac{\Delta c_{N_2O,upper}}{\Delta x} \right]_{upper} \cdot z^{-1} - \left[D_A \frac{\Delta c_{N_2O,lower}}{\Delta x} \right]_{lower} \cdot z^{-1} \qquad (3)$$

The term $\Delta S_{N2O}/\Delta t$ represents the change in storage of N_2O in a layer; the left-hand expression in parenthesis represents the diffusive flux of N_2O at the upper boundary, the right-hand expression at the lower boundary of a layer (D_A: apparent diffusion coefficient in soil, $\Delta c_{N2O}/\Delta x$: concentration gradient at upper or lower end of segment, z: thickness of the layer).

The diffusion coefficients D_A for N_2O in the pore-water of the fen were calculated for *in-situ* temperature following Wilke & Chang (1955), and were corrected for porosity using $D = D_0\ \phi^2$ (Lerman 1988). Diffusion coefficients D_A for N_2O in the unsaturated soil were obtained using the temperature corrected gaseous diffusion coefficient (Pritchard & Currie 1982) and a correction function $\alpha(a) = a^2\ \phi^{-2/3}$ (α: correction factor at air content a, ϕ: soil porosity) (Jin & Jury 1996). Volumetric gas content in the unsaturated soil was derived from total porosity (oven drying of 100 cm^3 subcores) and volumetric water contents (VWCs). From a laboratory mesocosm study, a linear relationship of VWC and the respective distance from the water table with an r^2 of > 0.9 had been derived previously (unpublished data).

Statistical methods. Daily NO and N_2O fluxes and total gas fluxes on C and D plots were tested for differences by the Mann-Whitney U-Test. Analysis of correlation between $\delta^{15}N$ values and N_2O concentrations, $\delta^{18}O$ values and N_2O concentrations as well as $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in the fen soil were performed by using the correlation test after Pearson. N_2O concentrations were log-transformed before executing the analysis to attain a linear relationship between the two respective variables. Tests of significance were conducted at the 0.05 probability level.

All presented values of NO and N_2O fluxes, N_2O concentrations and $\delta^{15}N$ and $\delta^{18}O$ signature represent mean values of three replicates and standard errors are given, if not otherwise stated.

Results

Meteorological conditions and water table. The experimental drought period in 2006 was characterised by warm and dry weather; a mean air temperature of 13.6°C compared to a 1995-2006 mean of 12.4°C was recorded, and the amount of precipitation was only 103 mm, while reaching 126 mm in the 1995-2006 mean. In 2007 this period was also above-average warm (14.5°C compared to a 1995-2007 mean of 13.2°C), but precipitation during the 10 weeks of the experiment (343 mm) exceeded the long-term annual mean of 237 mm by far, leading to very wet conditions and impeding a deep water table drawdown (Fig. 2).

As a consequence of the prevailing weather, the water table level in the fen was low at the beginning of the drought experiment in 2006 and varied between 20 and 30 cm depth on all plots. Prior to the manipulation experiment in 2007, the water table level was much closer to the surface due to a preceding rainstorm. Furthermore, inflowing groundwater refilled the drainage tiles quickly and thus it took about three weeks until the drainage system worked effectively (Fig. 2).

Not surprisingly, lowering of the groundwater level was most pronounced near the tile drains, and substantially less in the center of the experimental plots. At the gas sampling and measuring devices, the water table level in the D plots could in both years successfully be held at 10 to 20 cm below the levels of the C plots for a period of 30 or 40 days, respectively. Among the experimental plots, the soil moisture gradient as expressed in the vegetation pattern (see methods section) was reflected by C1 and D1 being the wettest, and C3 and D3 being the driest plots. The water table in D1 was only lowered by about 10 cm compared to C1, while in D3 the water level was up to 30 cm lower than in C3. Irrigation with artificial rainwater raised the groundwater level of the D plots to the same or even above the level of the C plots within one or two days.

NH_4^+ and NO_3^- concentrations. Near-surface NH_4^+ concentrations did not differ between plots and sampling dates and ranged from 5 to 20 µmol l^{-1} in 2006 (Fig. 3). In 2007 NH_4^+ concentrations in 5 and 10 cm depth were mostly below 50 µmol l^{-1} in all plots, with the exception of the C1 plot, where up to ~80 µmol l^{-1} were measured in August. Also in the other plots, there was a tendency of increasing NH_4^+ concentrations over time, regardless of the treatment. Only for C1 and D1, NH_4^+ concentrations seemed to be somewhat lower in the drought plots.

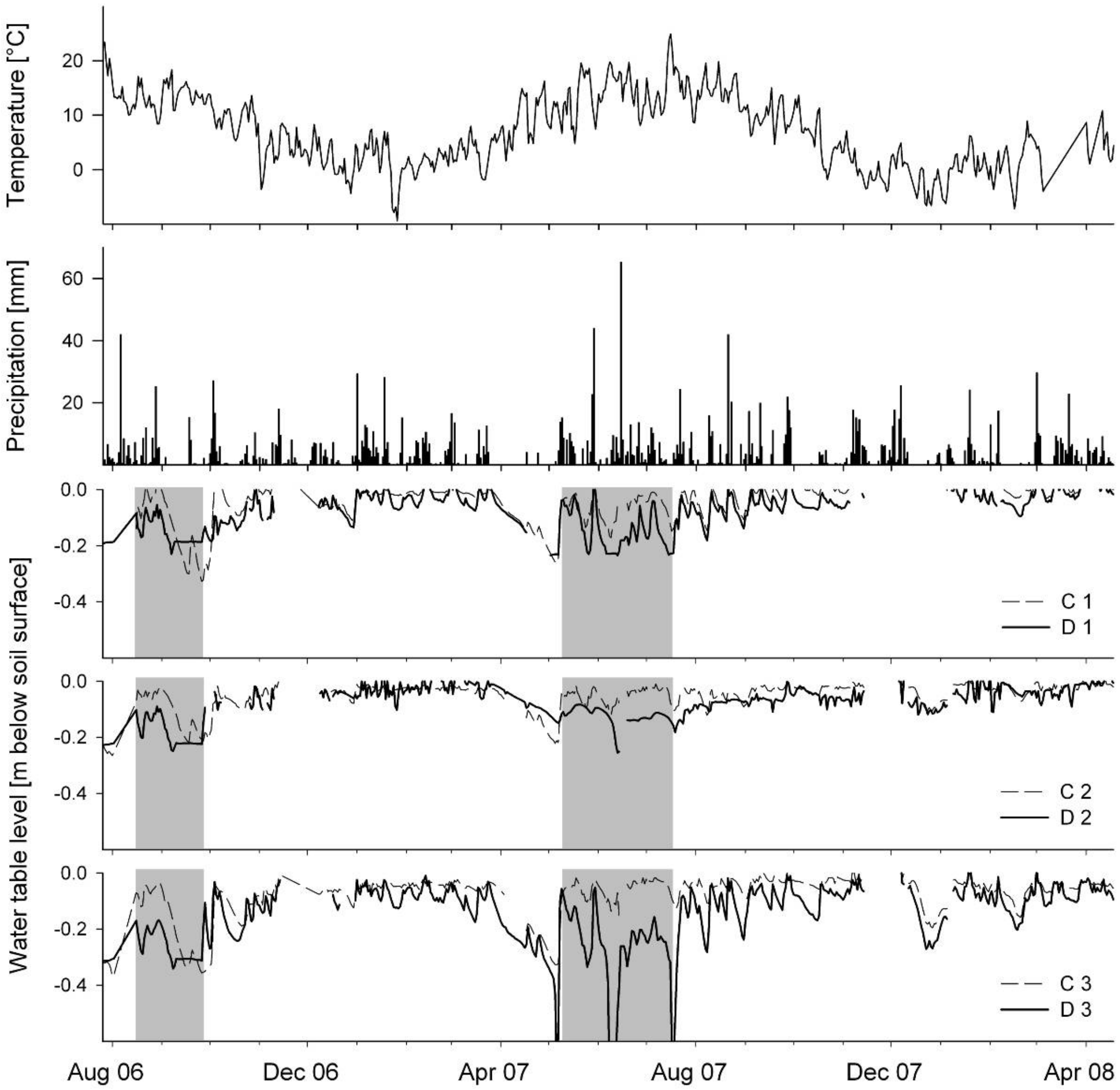

Figure 2. Daily mean air temperatures (n=24), precipitation and water table levels in the control (C) and drought (D) plots. The grey boxes indicate the drought periods.

NO_3^- concentrations of >10 µmol l^{-1} were only occasionally detected in 5 and 10 cm depth (Fig. 3) and nitrate was not detectable below. In 2006, NO_3^- concentrations were almost always below 5 µmol l^{-1}. Only at the end of the drought period NO_3^- concentrations reached 20 µmol l^{-1} in D1. In 2007 concentrations tended to be higher in C3 and D3. NO_3^- concentrations mostly did not differ between corresponding C and D plots at the respective depths. The only exception was D3: NO_3^- concentrations in 5 cm depth in this plot were mostly elevated compared to C3. Before the beginning of the drought experiment NO_3^- concentrations in D3 reached values as high as 130 μmol l^{-1}.

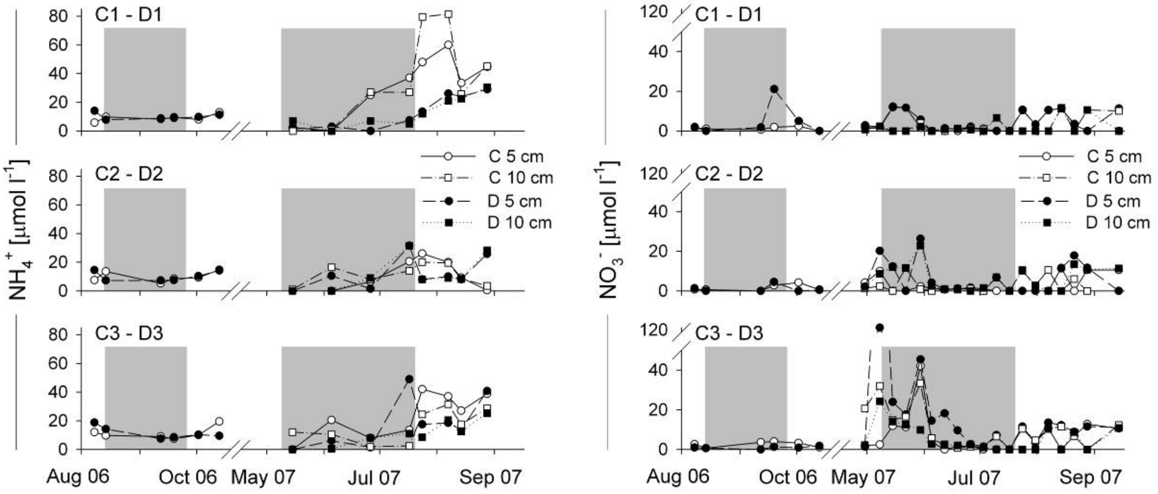

Figure 3. Concentrations of NH_4^+ and NO_3^- at 5 cm and 10 cm depth in the control (C) and drought (D) plots. The grey boxes indicate the experimental drought periods.

N_2O fluxes. Cumulative N_2O emissions over the experimental period of 537 days from all C plots were similar with mean values between 1.6 and 4.0 mmol m^{-2} (Fig. 4). Coinciding with the observed weak trend in nitrate concentrations, there was also a tendency of increasing cumulative N_2O emissions from D1 to D2 and D3, reaching mean values of 2.9, 7.4 and 26.3 mmol m^{-2}, respectively, over the measurement period. However, these differences were not statistically significant due to the high spatial variability of the N_2O fluxes on small scales in the D2 and especially in the D3 plot. Regarding our treatment, the D1 plot did neither respond to the water table manipulation nor to irrigation in terms of N_2O fluxes. The plot D2 did not show any differences in N_2O emissions compared to C2 during the water table manipulation either, but N_2O was rapidly emitted during rewetting. The N_2O emission pulse began the day after rewetting in both 2006 and 2007 and lasted for about 15 days. Maximum fluxes reached 17.8 ± 11 μmol m^{-2} h^{-1} and 24.4 ± 23 μmol m^{-2} h^{-1}, respectively. These emission peaks resulted in slightly higher cumulative N_2O emissions from D2 compared to C2. Cumulative N_2O emissions from D3 clearly exceeded those from C3 during both drought periods and after rewetting, with emission peaking at 36.0 ± 26 µmol m^{-2} h^{-1} and 34.2 ± 31 µmol m^{-2} h^{-1}, respectively.

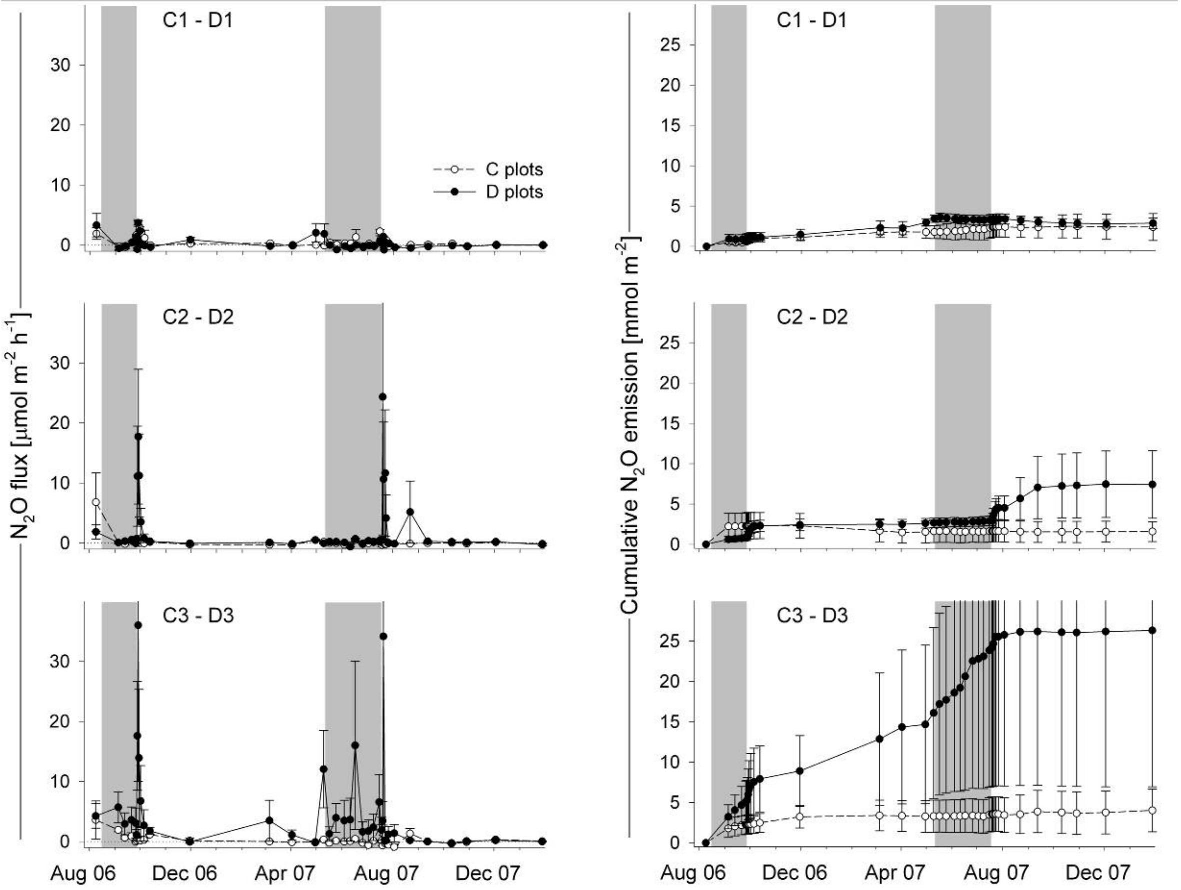

Figure 4. Time course of mean N_2O fluxes (left) and mean cumulative N_2O flux rates (right) at the control (C) and drought (D) plots. Error bars indicate the standard error of the mean (n=3). The grey boxes indicate the experimental drought period. The drought plots were rewetted following the drought with 110 mm artificial rain in 2006 and 182 mm in 2007.

NO fluxes. Daily NO fluxes were always low (0 to 1 µmol m^{-2} h^{-1}) but slightly elevated during the summer months (Fig. 5). NO emissions from both C and D plots were higher in the fairly dry year of 2006 than in the rather wet year of 2007. Among the three C plots, mean cumulative NO fluxes did not differ significantly over the measurement period from August 2006 to January 2008 with values between 0.6 and 1.0 mmol m^{-2}. In contrast, on the drought plots NO emissions increased from D1 (0.6 mmol m^{-2}) and D2 (to 0.9 mmol m^{-2}) to D3 (1.4 mmol m^{-2}).

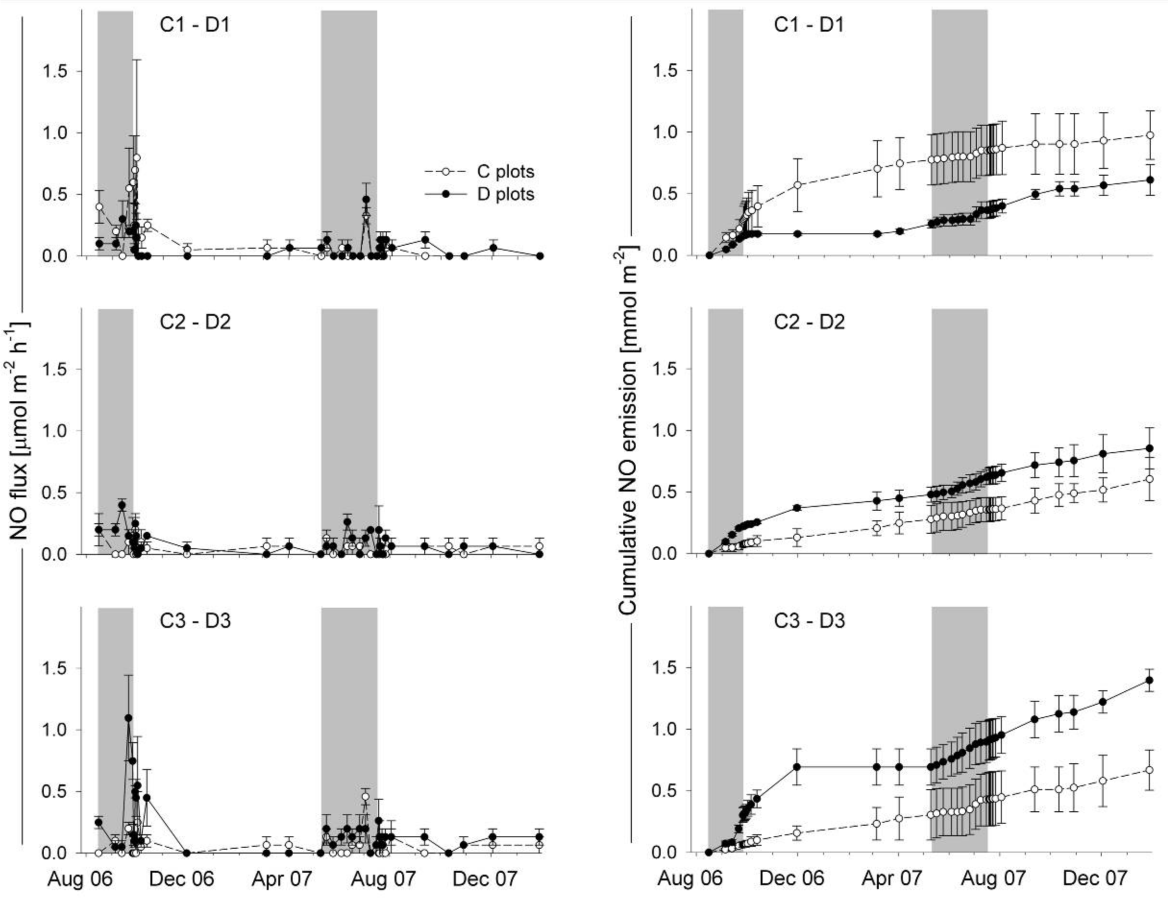

Figure 5. Time course of mean NO fluxes (left) and mean cumulative NO flux rates (right) at the control (C) and drought (D) plots. Error bars indicate the standard error of the mean (n=3). The grey boxes indicate the experimental drought period. The drought plots were rewetted following the drought with 110 mm artificial rain in 2006 and 182 mm in 2007.

Cumulative NO emissions from D1, D2 and D3 during both drought periods (112 days) amounted to 44 %, 43 % and 37 %, respectively, of the total NO emissions, whereas emissions from the C2 and C3 plots during these periods amounted to only 14 % and 17 %, respectively. C1, however, showed relatively large NO emissions during the drought period in 2006 although not being treated, and cumulative emissions during the experimental drought periods did thus contribute 60 % to the total emissions. Rewetting decreased NO fluxes on all D plots immediately by up to 80 %. NO fluxes were in general by about one order of magnitude lower than N_2O emissions.

N_2O concentration and isotope profiles and net N_2O turnover rates. Mean dissolved N_2O concentrations varied by more than three orders of magnitude from 1 nmol l^{-1} to 8.3 μmol l^{-1} on each of the treatment and C plots. Highest concentrations occurred during the fall and winter months at intermediate depths of 30 to 50 cm (Fig. 6). In the unsaturated zone, N_2O concentrations in soil air in general varied by more than two orders of magnitude between 3 nmol l^{-1} and 390 nmol l^{-1}. Just at the beginning of the drought experiment, N_2O concentrations in soil air in D3 (10 cm depth) reached 1.7 μmol l^{-1}. Mean ambient air N_2O concentrations were fairly constant, adjusting between 10 and 11 nmol l^{-1}.

$\delta^{15}N$ values of soil N_2O in the fen varied between -36 ‰ and +10 ‰ (Fig. 6), while values of $\delta^{15}N$ of N_2O in ambient air were in a narrow range between +4 ‰ and +5 ‰. With only few exceptions, the most negative $\delta^{15}N$ values coincided with the highest N_2O concentrations and *vice versa*, thus revealing a negative logarithmic relationship.

The ^{18}O signatures of soil N_2O ranged from -4 ‰ to +68 ‰ (Fig. 6), while values of $\delta^{18}O$ of ambient air N_2O varied only between +39 ‰ and +45 ‰. The $\delta^{18}O$ values of N_2O in the fen soil were also on most dates correlated in a negative logarithmic manner with the soil N_2O concentrations and linearly correlated with the $\delta^{15}N$ values of soil N_2O.

Until the end of the drought period in 2007, net N_2O turnover was around 0 pmol cm^{-3} d^{-1} in the soils (Fig. 7). This was accompanied by little variation of $\delta^{15}N$ and $\delta^{18}O$ values within the soil profiles. The only exception was the topsoil of D3: Prior to the experimental manipulation, coinciding with a very low water table of below 40 cm depth, a net N_2O production of 50 to 70 pmol cm^{-3} d^{-1} was calculated for this plot. However, following a rainstorm and decreasing water table levels in June, the topsoil of D3 became a net sink for N_2O with a net consumption rate of -14 pmol cm^{-3} d^{-1}. An obvious effect of the experimental drought on the net N_2O production only occurred on D3, where net N_2O production in 10 cm depth steadily increased from mid of June to the end of the manipulation in July, from 2 pmol cm^{-3} d^{-1} up to 44 pmol cm^{-3} d^{-1}. This trend was accompanied by a stepwise decrease in $\delta^{15}N$ values from -3 ‰ to -12 ‰.

Rewetting immediately, but only temporarily, increased net N_2O production in the topsoil of D2 and D3, and rates reached 18 pmol cm^{-3} d^{-1} and 174 pmol cm^{-3} d^{-1}, respectively.

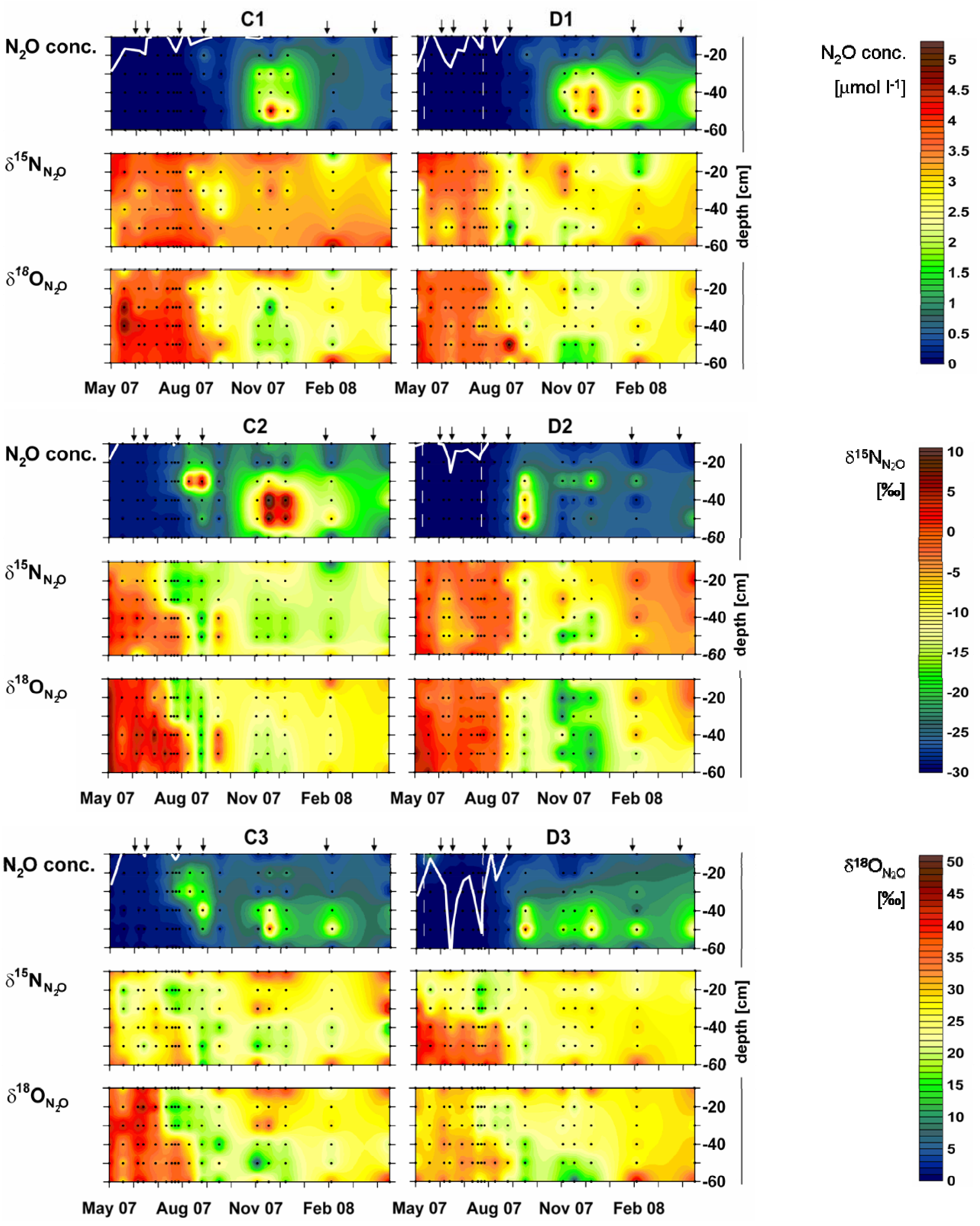

Figure 6. N_2O concentrations, $\delta^{15}N$ and $\delta^{18}O$ values of dissolved N_2O in soil water or N_2O in soil air, respectively, along soil profiles on control (C) and drought (D) plots. Beginning and end of the drought phase in 2007 are indicated by white, dashed lines. Arrows indicate major rain events (> 20 mm) and the white, solid line denotes the approximate water table. Black dots indicate soil gas sampling.

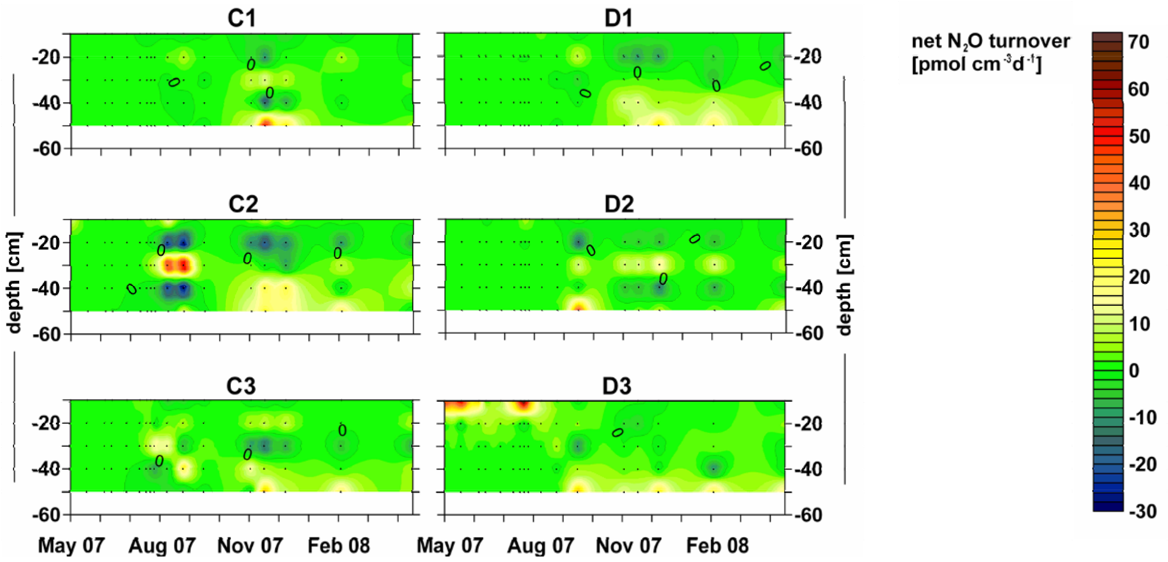

Figure 7. N_2O turnover rates along soil profiles on control (C) and drought (D) plots. Black dots indicate soil gas sampling.

This was accompanied by an immediate decline of $\delta^{15}N$ values right after rewetting by 6 ‰ to 11 ‰. After the drought period, the water table level was generally close to the soil surface and thus all sampling devices were within fully saturated soil.

In September, net N_2O production was low in all C plots and in D 1 (-3 pmol cm^{-3} d^{-1} to 3 pmol cm^{-3} d^{-1}), while in the D2 and D3 plots net N_2O production was significantly elevated in 50 cm depth compared to prior dates, with values of 44 and 58 pmol cm^{-3} d^{-1}.

From October 2007 to the spring of 2008 net N_2O production was similar on all C and D plots, with maximum rates in 50 cm soil depth (8 pmol cm^{-3} d^{-1} to 69 pmol cm^{-3} d^{-1}). N_2O loss, i.e. further reduction of N_2O to N_2, was always most pronounced at 20 cm and 40 cm depth with maximum rates of -58 pmol cm^{-3} d^{-1}.

Discussion

N_2O and NO fluxes. Long-term drainage is known to increase N_2O fluxes from Central European fens (Augustin *et al.* 1998, Merbach *et al.* 2001) but it is poorly known to what extent short-term lowering of water tables affects N_2O emissions from these environments. Freeman *et al.* (1993) detected enhanced N_2O emissions from Welsh peat monoliths 12 weeks after starting the manipulation of the water table position and Regina *et al.* (1999) observed an increase in N_2O emissions from boreal peat monoliths not until 14 weeks after lowering the water table. These observations have been taken as evidence for the potential of fens to increase N_2O production and emission due to short, dry periods (Regina *et al.* 1999).

In light of our results this judgement may have to be revised to some extent, as N_2O and NO emissions reacted much faster than reported by Freeman *et al.* (1993) and Regina *et al.* (1999). Even more important, the reported N_2O fluxes during drying were below 2 μmol m^{-2} h^{-1} in both studies and thus small compared to emission peaks of 16 μmol m^{-2} h^{-1} in our study. Such differences may be related to the long-term N deposition at our site, which has been estimated at 20.1 kg N ha^{-1} a^{-1}, with 13.5 kg N ha^{-1} a^{-1} resulting from wet and 6.6 kg N ha^{-1} a^{-1} from dry deposition (Schulze 2000). Whereas dry and wet N deposition has been reported to severely affect the N status of temperate ecosystems, i.e. leading to N-saturation (Aber *et al.* 1989), this appears to be less pronounced in the boreal zone (Tamm 1991). For the boreal zone total N depositions of below 2 kg N ha^{-1} a^{-1} are recorded (Schulze 2000). It may thus be speculated that temperate fens with high N content or input from the surrounding watershed have a high potential for nitrogen oxides emissions due to water table fluctuations. Durka *et al.* (1994) showed that NO_3^- leaching from nitrification in the topsoil of the surrounding forests as well as from atmospheric deposition contributes considerably to the NO_3^- found in the groundwater in our investigated area. This NO_3^- originating from the N-saturated catchment may well be denitrified in the fen.

Water table drawdown increased N_2O and NO emissions within four to five weeks – at least in the driest plot D3. Even during natural drought, at the end of the experiment in 2006 and before starting the drought manipulation in 2007, NO and/or N_2O emissions were increased in some collars. N_2O emissions were generally largest when NO_3^- concentrations were elevated in 5 cm depth; corresponding with high N_2O net production rates in 10 cm depth.

Temporal water table drawdown in two consecutive years did affect N_2O and NO emissions, depending on the height of the reduction: Both N_2O and NO emissions did not increase in the rather wet D1 plot, and only slightly in the D2 plot, when compared to C1 and C2. Against in D3, with the largest reduction of water table height, a notable increase in N_2O (530 %) and NO emissions (270 %) during both drought periods was observed compared to the corresponding C3 plot. The increase in N_2O and NO fluxes could, however, not be explained by the water table levels only. Presumably a complex interaction of water table height, nutritional status, available substrates, and also preceding soil moisture conditions affected N_2O and NO production, consumption, and emission. Highest N_2O and NO fluxes occurred only during drought periods but did not necessarily happen. At high water table levels, N_2O and NO fluxes were generally low, though.

Average flux rates of N_2O from the C plots (0.3 to 0.8 kg N_2O-N ha^{-1} a^{-1}) coincided with rates reported earlier in studies from undisturbed minerotrophic fen mires with high groundwater level (0.04 to 1.2 kg N_2O-N ha^{-1} a^{-1}, (Martikainen *et al.* 1993, Martikainen *et al.* 1995, Merbach *et al.* 2001). The increased N_2O flux observed upon water table drawdown was in agreement with findings from mires that were drained for forestry or agriculture by ditching several years before (Martikainen *et al.* 1993, Augustin *et al.* 1998, Merbach *et al.* 2001). Mean N_2O flux rates in D3 reached up to 5 kg N_2O-N ha^{-1} a^{-1}, if averaged over the total measurement period, and thus exceeded rates reported for undisturbed fens (Martikainen *et al.* 1993, Martikainen *et al.* 1995, Merbach *et al.* 2001). They almost reached rates as reported for a drained, slightly degraded German fen rich in N (6 to 28 kg N_2O-N ha^{-1} a^{-1}) (Augustin *et al.* 1998, Merbach *et al.* 2001). Our finding documents for the first time that a short term manipulation of water table levels in near natural fens may produce temporarily a similar N_2O emission as observed in fens subjected to a constant drainage and anthropogenic interferences.

Low production or even uptake of N_2O in waterlogged fens are a result of a lack of oxygen, which constraints nitrification, and ultimately also denitrification due to the ensuing lack of NO_3^- (Blackmer & Bremner 1976, Robertson & Tiedje 1987, Regina *et al.* 1996). In line with this reasoning, aeration following water table drawdown increases mineralisation and nitrification of organic N from within the peat matrix (Williams & Wheatley 1988), which then shifts denitrification deeper into the soils or within anaerobic microsites (Sexstone *et al.* 1985). The partly elevated nitrate concentrations in C3 and D3 compared to the C1 and D1 plots were presumably also related to the observed moisture gradient, with nitrate being most likely to occur in the drier C3 and D3 plots. This would also explain a high potential denitrifying activity in these plots, causing higher N-losses in form of N_2O.

Rewetting caused a short-lived, but strong increase in soil N_2O emission in D2 and D3 that lasted about 15 days in both years. Based on the entire experimental period (537 days) these periods contributed 40 % and 20 %, respectively, to the total N_2O emissions from D2 and D3. Up to now such N_2O emission peaks after rewetting of drained fens have not been reported. Contrarily, in the study of Regina *et al.* (1999), the authors found an immediate reduction of N_2O fluxes from peat mesocosms after rewetting. The short-lived nature and significance of the emissions on the annual scale underline the importance of short term water table fluctuations and illustrate the difficulties encountered with calculating N trace gas budgets of peatlands. Flux estimates neglecting water table fluctuations could severely underestimate N_2O emissions.

The observed N_2O peaks after rewetting were accompanied by enhanced NH_4^+ concentrations in D2 and D3 in 5 cm depth and large net N_2O production rates in 10 cm depth. Increased NH_4^+ concentrations could arise from the NH_4^+ input *via* the artificial rainwater. Nevertheless, mineralisation pulses after rewetting events have often been described in terms of increased CO_2 production (Blodau & Moore 2003, Fierer & Schimel 2003). A concomitant release of N compounds from the organic matrix is thus likely. Compared to the dynamics of NH_4^+ concentrations, low NO_3^- concentrations in the topsoil also directly after rewetting were most probably caused by rapid denitrification.

With regard to NO, we are not aware of studies that have quantified fluxes from wetlands during water table manipulation experiments. We assume that water table drawdown enhances NO fluxes due to the following reasons: 1) In water-saturated soils NO can be reduced before escaping to the atmosphere (Davidson 1991). 2) Increase in nitrification activity after draining might have a great importance for the NO production (Lång *et al.* 1995). Indeed, NO emissions were enhanced during experimental drought, with the exception of the D1 plot. NO fluxes were by about one order of magnitude lower compared to N_2O emissions, as to be expected in moist soils (Tilsner *et al.* 2003a, Yamulki *et al.* 1995) and neither related to NH_4^+ nor NO_3^- concentrations. Our results thus suggest that nutrient rich, minerotrophic peatlands have only a low potential for increased NO emission even after water table drawdown.

N_2O concentrations, isotope signatures and turnover along soil profiles. N_2O concentrations in the pore water of this fen are within the range reported for different aqueous systems (Heincke & Kaupenjohann 1999, Hiscock *et al.* 2003, Deurer *et al.* 2008). The experimental manipulation apparently did not have a decisive impact on their temporal dynamics, which was generally characterised by increasing levels during winter. N_2O emissions have been found to increase when the vegetation is removed as plants may successfully compete with denitrifiers for nitrate (Nykänen *et al.* 1995, Rückauf *et al.* 2004, Silvan *et al.* 2005). The elevated N_2O concentrations during winter time could be caused by higher availability of N_2O precursors. Furthermore, a lowered activity of the enzyme N_2O reductase, as observed for soil temperatures around and below 0°C (Holtan-Hartwig *et al.* 2002), may have caused a drop in N_2O consumption. Such an effect could explain the higher emission of N_2O during summer despite lower concentrations in the soil. The increased solubility of N_2O at colder temperatures could not explain this observation, because a temperature decrease of 10°C would only increase solubility by a factor of ~1.5 (Weiss & Price 1980). Fluxes and pools of N_2O in such types of fens thus seem to be decoupled to some extent.

Despite NO_3^- being mostly limited to the topsoil, net N_2O production in near-surface soil was only calculated for D3 during most pronounced water table reduction and during rewetting in the topsoil of D2 and D3. Highest calculated net N_2O production occurred at 30, 40 and 50 cm soil depth, with a shift from 30 cm in the summer to 50 cm in the winter. This shift may be related to reduced microbial activity in winter and thus deeper penetration of rain water. Similar rain water infiltration depths have been found to occur at this site using oxygen isotope techniques (C. Weyer and G. Lischeid, pers. comm.).

We assume microbial denitrification to be responsible for N_2O production in the waterlogged fen profile, since anaerobic conditions make microbial nitrification rather unlikely (Davidson 1991). Moreover, denitrification was presumably also the dominating process in the unsaturated zones, since highest net N_2O production occurred when NO_3^- concentrations were enlarged and anaerobic microniches are very likely to occur in near saturated peat (Knorr *et al.* 2008). Niedermeier and Robinson (2007) could show that air may permeate into a similar peat soil only at water tensions as high as 25 cm, thus reducing conditions could still have persisted 25 cm above the water table level.

Additional information about the processes leading to N_2O emissions can be gained from spatio-temporal changes in the natural abundance of nitrogen isotopes. The robust correlation between $\delta^{15}N$ and $\delta^{18}O$ of N_2O in the fen soil throughout the year suggests that at any time N_2O was to a large extent reduced to N_2 (Toyoda *et al.* 2005, Menyailo & Hungate 2006). This is in agreement with findings that N_2 emissions from waterlogged soils strongly contribute to total N loss and that N_2O emissions may be of minor importance (Well *et al.* 2001, Mander *et al.* 2003, Rückauf *et al.* 2004, Velty *et al.* 2007). Indeed, a prompt reduction of N_2O may well be expected in a peat matrix in which ferric iron, sulphate, and CO_2 reduction predominate in most of the peat matrix (Paul *et al.* 2006).

The simultaneous depletion in ^{15}N and ^{18}O of N_2O in 10 cm depth of D3 during drought suggests increasing net N_2O production, i.e. increasing N_2O/N_2 ratios during denitrification, since further reduction of N_2O to N_2 enriches the remaining N_2O in both ^{15}N and ^{18}O (Pérez 2005, Barford *et al.* 1999). The continued decrease of $\delta^{15}N$ values in 10 cm depth right after rewetting indicated another pulse in N_2O production and emission that was unrelated to physical displacement of soil N_2O. This phenomenon was observed on all treatment plots, but was weakest in D1. Rainfall, marked by solid arrows in Fig. 5, also entailed falling $\delta^{15}N$ and $\delta^{18}O$ values on the C plots, although this did not translate in high fluxes due to saturated conditions that did not allow such high N_2O fluxes as observed in the D plots. Natural rainfall events may induce mobilisation of NO_3^- from the upper parts of the fen above the groundwater level, e.g. from the hummocks, and may additionally induce N_2O production due to direct deposition of NO_3^-. An input of NO_3^- from the surrounding forest seems unlikely (G. Lischeid, unpubl. data), but cannot fully be excluded, minding the direct response of N_2O production to natural rain events across all plots. Regarding absolute changes in the ^{15}N and ^{18}O isotopic composition of N_2O, a seasonal pattern dominated, thus coinciding with absolute N_2O concentrations. During the winter, $\delta^{15}N$ and $\delta^{18}O$ of soil N_2O did not change much any more.

We cannot conclusively explain why net N_2O production was highest at 30 to 50 cm soil depth in absence of detectable concentrations of nitrate that only once reached 30 μmol l^{-1}. Redox potentials in these depths suggest that available NO_3^- immediately is further reduced and is hence not detectable in the porewater. Greater input of NO_3^- into these depths may eventually be linked to effects of preferential flow or small scale heterogeneity in redox conditions. Nevertheless, high N_2O concentrations together with most negative $\delta^{15}N$ and $\delta^{18}O$ values in these depths support an occurrence of denitrification there.

Conclusions

The presented results demonstrate that the initiation of more intense fluctuations of water table levels in a temperate minerotrophic fen as predicted for Central Europe by climate change scenarios strongly alters the budget of the greenhouse gas N_2O. Drought appears to substantially increase N_2O emissions from minerotrophic fens to levels as already described for their long-term drainage. The effect of drought was exacerbated by additional emission related to the intense rainfall that we induced experimentally and that had a significant impact on the annual N_2O loss. The additionally emitted N_2O was also newly produced, as the depletion in ^{15}N and ^{18}O showed. Our findings thus underline the importance of short term water table fluctuations and the need for highly resolved measurements in calculating N trace gas budgets of peatlands. Models neglecting water table fluctuations could severely underestimate N_2O emissions. Our results additionally revealed a high spatial variability of N_2O fluxes in soil subjected to more intense drought and rewetting. Further studies are needed to identify the reasons for this heterogeneity.

The contrast between partly low N_2O fluxes despite high concentrations in the subsoil showed that fluxes and pools of N_2O in the subsoil are not clearly linked. Highest production rates were also calculated for intermediate soil depths, while nitrate was only more abundant at shallow depths. However, a positive relation between N_2O concentrations in the topsoil and N_2O surface fluxes suggests that the N_2O pool in the uppermost soil, albeit small and difficult to assess, is relevant for the N_2O fluxes between soil and atmosphere. This point needs further investigation, though.

Regarding NO fluxes, our results support earlier findings that this highly reactive gas is either only marginally produced by microbial nitrification and/or denitrification in the fen soil or undergoes chemical conversion before escaping to the soil surface. The drought periods induced by us still resulted in significantly increased net NO emission. Up to now it has been assumed that peatlands are rather insignificant sources of NO. Our results suggest that neglecting NO fluxes related to drought likely results in an underestimation of global NO emissions from soils.

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APPENDIX

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List of further publications

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Hiermit erkläre ich, dass diese Arbeit von mir an keiner anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde, sowie, dass ich noch kein Promotionsverfahren endgültig nicht bestanden habe. Ferner erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die darin angegebenen Quellen und Hilfsmittel benutzt habe.

Stefanie D. Goldberg