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Sources and sinks of nitrous oxide in drained and rewetted temperate peatlands

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Zusammenfassung

Um den Beitrag der Moore zum Klimawandel zu senken, wurden insbesondere in den letzten Jahrzehnten immer mehr Moorstandorte wiedervernässt. Hierdurch bleiben die großen Mengen an Kohlenstoff, die hier gespeichert sind, besser erhalten und werden nicht durch Mikroorganismen zu dem Treibhausgas Kohlendioxid abgebaut. Welchen Einfluss die Wiedervernässung auf die Produktion von Lachgas (N_2O), einem weiteren wichtigen Treibhausgas, hat, ist noch nicht gut erforscht. Insbesondere bei den eher nährstoffreicheren Niedermooren könnten Lachgasemissionen erhöht sein, wobei die anaeroben Bedingungen aber auch zu einer weiteren Reduktion von N_2O zu N_2 führen könnten. In dieser Arbeit soll daher der Einfluss der Wiedervernässung auf Niedermoorstandorten bezüglich der N_2O-Emissionen untersucht werden.

Hierfür wurden sowohl entwässerte als auch bereits wiedervernässte Flächen dreier Niedermoortypen (Küstenüberflutungsmoor, Durchströmungsmoor, Erlenwald) untersucht. Zudem sollten die mikrobiellen Produktionswege von N_2O sowie deren Beiträge auf den unterschiedlichen Moortypen analysiert werden. Vor diesem Hintergrund wurden folgende Fragen untersucht: a) Wie hoch sind die N_2O-Flüsse der Standorte, schwanken diese über einen längeren Messzeitraum und von welchen Faktoren werden die N_2O-Emissionen maßgeblich beeinflusst? Unter welchen Bedingungen können Hotspots entstehen? b) Welche mikrobiellen Produktionswege weisen die höchsten Beiträge zur Produktion von N_2O auf? c) Wie können isotopische Tracer, welche während der Inkubationsversuche zur Ermittlung der Quellen von N_2O im Labor genutzt werden, direkt im Feld ausgebracht werden, ohne die natürliche Struktur des Bodens zu zerstören?

Um diese Fragen zu beantworten, wurden von 2017 bis 2020 Langzeitmessungen von N_2O mittels manueller Haubentechnologie durchgeführt. Zudem wurden Bodenproben entnommen und in einem Inkubationsversuch mit der Doppelisotopenmethode im Labor analysiert. Des Weiteren wurden Ende 2018 Versuche zur Ausbringung von Tracern auf einem Boden in Neuseeland durchgeführt.

Insgesamt waren die Emissionen von N_2O auf allen Niedermoorstandorten über den gesamten Messzeitraum sehr gering, sodass N_2O-Emissionen auf den untersuchten Standorten keine große Rolle zu spielen scheinen. Ein Standort könnte sogar über ein gesamtes Messjahr eine Senke für N_2O gewesen sein. Die Emissionen schwankten allerdings

über den gesamten Zeitraum stark, und Hotspots bzw. Hot Moments schienen die Emissionen stärker zu beeinflussen als die Wiedervernässung, Wasserstandsschwankungen, Bodenfeuchte oder Substratverfügbarkeit. Des Weiteren konnte für alle Flächen und selbst unter vergleichsweise trockenen Bedingungen festgestellt werden, dass zwischen 5 und 50% des N_2O zu N_2 reduziert werden konnte.

Die Auswertung des Inkubationsversuches zeigte, dass sowohl Nitrifikations- als auch Denitrifikationsprozesse auf allen Standorten zur Produktion von N_2O beitrugen. Dabei stellte sich heraus, dass in den untersuchten Böden zeitgleich Nitrifikations- sowie Denitrifikationsprozesse abliefen. Denitrifikation spielte dabei eine große Rolle, allerdings konnte für zwei der sechs Standorte festgestellt werden, dass etwa die Hälfte des produzierten N_2O von Nitrifizierern stammte. Hohe N_2O-Emissionen konnten insbesondere für die Bodenproben festgestellt werden, welche aufgrund eines sehrhohen Wassergehaltes vor Versuchsbeginn getrocknet werden mussten. Demzufolge scheinen kurzfristige Schwankungen im Wassergehalt stärkere Auswirkungen auf die N_2O-Emissionen zu besitzen als andere Faktoren.

Bezüglich der homogenen Ausbringung der Tracer im Feld ließ sich feststellen, dass dies mit den in dieser Arbeit genutzten Methoden (Injektion, Gießkanne und Sprühflasche) nicht oder nur schwer möglich war. Die besten Ergebnisse konnten mit der Sprühflasche erreicht werden, da hier im Vergleich zu den anderen Methoden der meiste Tracer wiedergefunden werden konnte. Die Applikation per Gießkanne führte aufgrund des schnellen Auftragens zu einem erhöhten Abfließen auf der Oberfläche, sodass der Tracer über die Versuchsfläche hinaus verteilt wurde. Die Kanülen der Injektionsspritzen wurden durch eindringende Bodenpartikel direkt verstopft, so dass keine Ausbringung möglich war. Makroporen, welche beispielsweise durch Wurmgänge oder Wurzeln im Boden entstanden, führten zu einer inhomogenen Verteilung des Tracers, da dieser hier bevorzugt abfloss und somit auch unterhalb des untersuchten Bereiches gefunden werden konnte. Für eine weitere Suche nach einer verbesserten Methode wurde empfohlen, mit eher trockenem Boden und größeren Flüssigkeitsmengen zu arbeiten und eine Methode zu finden, bei der der Tracer möglichst langsam in den Boden eingebracht wird.

Insgesamt ließ sich feststellen, dass die Lachgasemissionen auf den von uns untersuchten Standorten eine eher untergeordnete Rolle zu spielen scheinen. Diese hingen nicht wie

erwartet vom generellen Wasserstand ab, sondern stiegen insbesondere durch kurzfristige Schwankungen in der Feuchtigkeit. Demzufolge sollten Wasserstandsschwankungen auf wiedervernässten Standorten möglichst vermieden werden. Zudem konnte sowohl auf entwässerten als auch auf wiedervernässten Standorten eine teils erhebliche Reduktion von N_2O zu N_2 beobachtet werden. Wovon die Höhe der N_2O-Reduktion abhing, konnte im Rahmen dieser Arbeit nicht geklärt werden. Weitere Forschung zu diesen Themen ist dringend geboten, um die Wiedervernässungstrategien weiterhin zu optimieren und ein optimales Wassermanagement in Mooren zu ermöglichen.

Abstract

In order to reduce the contribution of peatlands to climate change, increasing numbers of peatland sites have been rewetted, particularly in recent decades. As a result, large amounts of carbon stored here are preserved more efficiently and are not degraded by microorganisms into the greenhouse gas carbon dioxide. The influence of rewetting on the production of nitrous oxide (N_2O), another important greenhouse gas, has not yet been well researched. In particular, N_2O emissions could be increased in more nutrient-rich fens, although anaerobic conditions could also lead to further reduction of N_2O to N_2. This study therefore aims to analyze the influence of rewetting on fen sites with regard to N_2O emissions.

For this purpose, both drained and already rewetted sites of three fen types (coastal fen, percolation fen and alder forest) were studied. In addition, the microbial production pathways of N_2O and their contributions from the different fen types were analyzed. In this context, the following questions were investigated: a) How large are N_2O emissions from fens sites, are they fluctuating over a longer measurement period/ the seasons and which factors significantly influence N_2O emissions? Under which conditions can hotspots and hot moments occur? b) Which microbial production pathways show the largest contributions to the production of N_2O? c) How can isotope tracers, which are used during incubation experiments to determine the sources of N_2O in the laboratory, be applied directly to the soil in the field without destroying the natural structure of the soil?

To answer these questions, long-term measurements of N_2O were performed from 2017 until 2020 using manual chamber technology. Soil samples were taken and analyzed in an incubation experiment using the dual isotope method in the laboratory. Furthermore, isotopic tracer application experiments were carried out on soil in New Zealand in 2018.

Overall, N_2O emissions were small at all sites over the entire measurement period, indicating that N_2O emissions do not appear to play an important role at the studied sites. One of the sites might even have been a sink for N_2O over an entire measurement year. N_2O emissions fluctuated strongly over the entire measurement period, and hotspots or hot moments appeared to have a stronger influence on N_2O emissions than rewetting, water level fluctuations, soil moisture or substrate availability. Furthermore, for all sites and even under

relatively dry conditions, it was found that between 5 and 50% of the N_2O could be reduced to N_2.

The interpretation of the results of the incubation experiment showed that both nitrification and denitrification processes contributed to the production of N_2O at all sites. It was observed that nitrification and denitrification processes took place simultaneously in the studied soils. Denitrification played a major role, but on two of the six sites, it was found that half of the N_2O produced originated from nitrifiers. Large N_2O emissions were found in particular for soils samples that had to be dried before the start of the incubation experiment due to an extremely high water content. As a result, short-term fluctuations in water content appear to have a greater impact on N_2O emissions than other factors.

With regard to the homogeneous application of tracers in the field, it was observed that with the methods used in this study (injection, watering can and sprayer), a homogeneous application was hard to achieve or almost not possible. The best results were achieved with the sprayer, as most of the tracer could be recovered here compared to the other methods. Application by watering can led to increased run-off on the surface due to rapid application and therefore a higher hydraulic head, causing the tracer to be distributed beyond the plot. The cannulas of the syringes were directly clogged when penetrating the soil, so that no application of tracer was possible. Nevertheless, macropores, caused by wurm burrows or roots in the soil, led to an inhomogeneous distribution of the tracer, as it preferentially flowed through these burrows and could therefore also be found underneath the tested plot. For a further improvement of the method, it was recommended to work with rather dry soil and larger amounts of the tracer. Besides, a method should be developed in which the tracer is applied to the soil as slowly as possible.

Overall, it was found that N_2O emissions appear to play a rather smaller role at the sites we studied. The emissions did not depend on the general water table level as we expected, but increased in particular due to short-term fluctuations in moisture. Consequently, water level fluctuations should be avoided as far as possible on rewetted sites. In addition, a reduction of N_2O to N_2 was observed both on drained and rewetted sites. The extent of N_2O reduction could not be clarified in this study. Further research on these topics is urgently needed in order to further optimize rewetting strategies and enable optimal water management in peatlands.

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1. Introduction

The earth has always been affected by climate change, i.e. long-term changes in temperatures and weather patterns. These changes can be caused by natural factors, for example fluctuations in solar activity or volcanism or the composition of the earth's atmosphere (Nikolov and Petrov, 2014). About 4 billion years ago, the composition of the atmosphere was characterized by degassing from soil and volcanic eruptions. The most important products were water vapor and carbon dioxide, which made up 90% of the atmosphere, along with smaller amounts of methane, nitrogen, hydrogen sulfide and others. In the atmosphere of the primordial earth, there was no oxygen available. About 2.5 billion years ago, the first cyanobacteria were able to perform photosynthesis, which became increasingly important and strongly influenced the composition of the atmosphere (Farquhar et al., 2011).

Temperatures all over the world have already risen sharply in recent decades (Intergovernmental Panel on Climate Change, 2021). If emissions continue at their current rate, global average warming of 3.2 °C is projected by 2100 (Intergovernmental Panel on Climate Change, 2023). To prevent this, a climate target was decided in 2015 with a maximum temperature increase of 1.5 °C (Hoegh-Guldberg et al., 2022), calculated from the beginning of the industrialization to 2100. Past warming of about 1 °C is expected to persist for centuries to potentially millennia, causing long-term changes in the climate system such as rising sea levels and loss of biodiversity (Hoegh-Guldberg et al., 2022).

Since the 19th century, climate change has been mainly due to human activities: since the industrialization, the composition of the atmosphere has been increasingly influenced by the anthropogenic release of greenhouse gases. These are, in particular, carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). The concentration of these greenhouse gases has risen sharply since the beginning of the industrialization (Fig. 1). The global surface temperature increased by around 1.1 °C in 2011 – 2020 compared to 1850 – 1900 (Intergovernmental Panel on Climate Change, 2023).

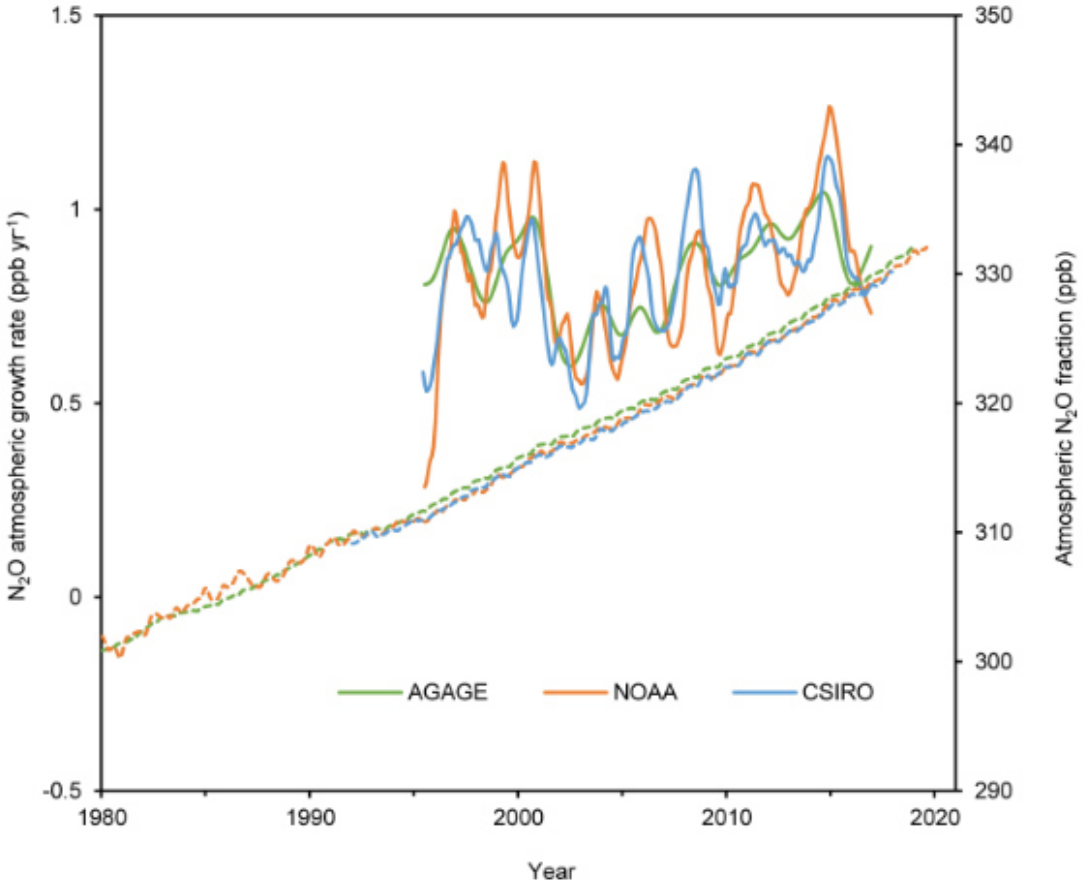

Fig. 1: Increase in concentrations of N_2O (ppb) from 1980 until 2020 from different networks (AGAGE = Advanced Global Atmospheric Gases Experiment, NOAA = National Oceanic and Atmospheric Administration, CSIRO = Commonwealth Scientific and Industrial Research Organisation) (Tian et al., 2020).

1.1 Nitrous oxide in the environment

N_2O or more common "laughing gas" is a greenhouse gas with a high global warming potential of 273 times higher than that of CO_2 (Neubauer and Megonigal, 2015). Although N_2O and CH_4 have rather small concentrations in the atmosphere, due to their high global warming potential, they contribute 26 % to global warming (Imer et al., 2013).

N_2O is colorless and smells lightly treacly (Ussiri and Lal, 2013). The overall concentration in the atmosphere is about 332 ppb (Intergovernmental Panel on Climate Change, 2023). N_2O has a rather long lifetime in the atmosphere of 120 years. In contrast, CH_4 for example, only has a lifetime of five years (Intergovernmental Panel on Climate Change, 2015). Since 1750, there has been a huge increase in concentrations in the atmosphere. From 1750 to 2017, the concentration in the atmosphere increased from 270 ppb in the pre-industrial era to 330

ppb, resulting in an increase of 22% since industrialization (Jia et al., 2019). N_2O concentrations have increased with 0.73 ± 0.03 ppb/yr over the last 30 years (Intergovernmental Panel on Climate Change, 2014).

N_2O plays an important role in photochemical reactions in the troposphere and stratosphere (Minami, 1997). There, greenhouse gases absorb and remit infrared thermal radiation. Consequently, the radiation of the earth is prevented from dissipating to outer space (Ussiri and Lal, 2013). Photolysis decomposes N_2O into molecular nitrogen and oxygen. This oxygen can react with another N_2O molecule to nitric oxide (NO) as a free radical. NO is able to react with ozone molecules and destroys the ozone layer (Crutzen, 1970).

Thus, N_2O not only acts as a greenhouse gas in the atmosphere, it is also significantly involved in the depletion of the ozone layer (Ravishankara et al., 2009). To enable effective mitigation strategies for N_2O emissions, the production processes as well as the individual factors that influence these processes must be better understood. Factors driving N_2O production and reduction and therefore also N_2O emissions include water table level, availability of nitrogen (N) and oxygen, pH and soil temperature (Loick et al., 2017). The diverse processes react differently to these factors.

The main natural sources of N_2O are oceans and natural soils. On the anthropogenic side, the main part is produced in agricultural soils (Syakila and Kroeze, 2011). However, there are many chemical and microbial sources producing N_2O in the soil, which are challenging to distinguish as they can take place simultaneously (Wrage-Mönnig et al., 2018; Heil et al., 2015; Stein, 2019).

The increase in N_2O emissions is mainly caused by agricultural soil management, use of N fertilizer, fossil fuel burning and other industrial processes (Ussiri and Lal, 2013). N_2O from soils can be produced in direct and indirect ways. The basis for the production of N_2O in soils is reactive N. In contrast, atmospheric N_2 is very inert, unlike N in soils (Galloway et al., 2008). Reactive nitrogen inputs from organic and mineral fertilizers and atmospheric N deposition as well as reactive nitrogen in soils from plant residues and biological N fixation provide the basis of direct emissions. Indirect N_2O emissions are caused if reactive nitrogen compounds such as nitrate (NO_3^-) or ammonium (NH_4^+) are released to surrounding systems and form the basis for N_2O production there.

1.2 Microbial production pathways of N_2O in soils

N_2O is formed from reactive nitrogen compounds via different microbial production pathways in the soil: nitrification, bacterial or fungal denitrification or nitrifier denitrification are considered to be among the main ones (Butterbach-Bahl et al., 2013; Wrage-Mönnig et al., 2018).

The reactive N enters the soil by various pathways like atmospheric deposition as well as plant residues, the use of fertilizer and biological fixation of N_2. Subsequently, the organic N is converted to NH_4^+ by microorganisms. This pathway allows the nitrogen to become available to other microorganisms and plants. At the same time, NH_4^+ represents the substrate for the process of nitrification.

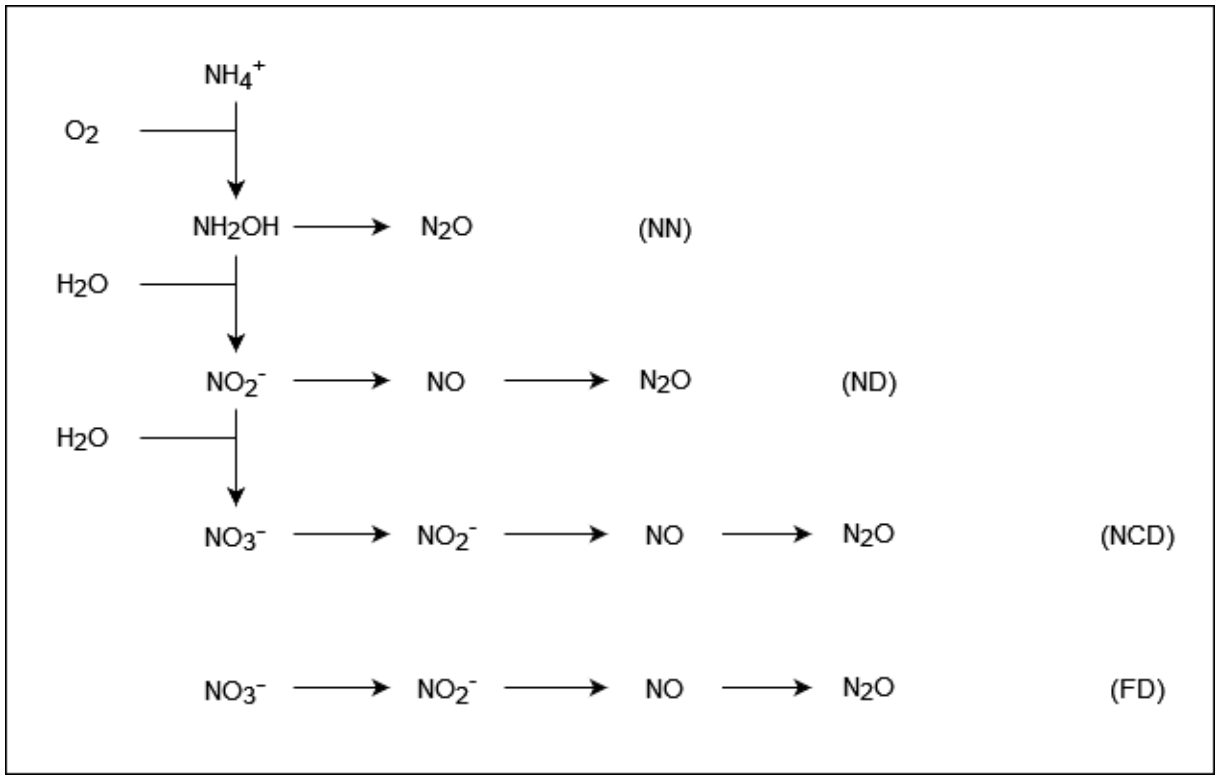

Fig. 2: Major pathways of N_2O production: nitrifier nitrification (NN), nitrifier denitrification (ND), fertilizer denitrification (ND) and nitrification-coupled denitrification (NCD) after Kool et al. (2011).

Nitrification is carried out by autotrophic bacteria, the so-called ammonia oxidizers and nitrite oxidizers. NH_3 is the initial product, which is oxidized to nitrite via the intermediate product hydroxylamine by ammonia oxidizers. During oxidation to hydroxylamine, N_2O can be released into the atmosphere (Fig. 2) as a by-product (Hooper and Terry, 1979).

Furthermore, ammonia oxidizers are also able to produce N_2O from NO_2^- (Jung et al., 2014; Stieglmeier et al., 2014). The further oxidation of nitrite to nitrate is then performed by nitrite oxidizers (Paul and Clark, 1996). In addition, there is also complete ammonia oxidation, which is also known as comammox. The term comammox refers to a pathway that can metabolize ammonia by nitrification into nitrite and then nitrate (Costa et al., 2006). In the past, it was assumed that nitrification is always a two-stage process and that no bacteria were capable of performing both processes. In 2015, two *Nitrospira* species were discovered to carry genes for the enzymes of both oxidation processes (van Kessel et al., 2015). The autotrophic nitrifiers use nitrification to generate energy for the fixation of carbon.

In contrast, heterotrophic bacteria like denitrifiers use carbon as a source for their energy (Castignetti, 1990; Robertson and Kuenen, 1990). As already mentioned, denitrification represents another important pathway of N_2O production. Denitrification is carried out by heterotrophic bacteria that use the nitrate present as an electron acceptor under anaerobic conditions in the soil. In this process, nitrate is reduced via NO_2^- and nitric oxide to N_2O (Fig. 2), which appears as an intermediate in this pathway (Zumft, 1997). If the conditions sufficient for a further reduction to N_2 do not exist, the last step in this pathway may be missing. In this case, the N_2O can be released into the atmosphere before it has been completely reduced to N_2. Denitrifying bacteria are a diverse group of microorganisms. Most of them are not able to perform complete denitrification. In addition, also fungi and archaea are capable of performing denitrification as part of the nitrogen cycle (Zumft, 1997).

In addition to the two mentioned pathways, there are a number of other processes involved in the production of N_2O: coupled nitrification-denitrification, fungal denitrification, co-denitrification and many more. All these processes are performed by a number of different organisms in the soil. In particular, there is one pathway that has gained increasing attention in recent years: nitrifier denitrification (Wrage et al., 2001). In this process, autotrophic ammonia oxidizers (AOB) generate N_2O through a reduction of NO_2^- before nitrate is built (Fig. 2). Some experimental studies indicate that this process may contribute significantly to the production of N_2O (Wrage et al., 2004; Venterea, 2007). Besides, ammonia oxidizing archaea (AOA) can also produce N_2O from NO_2^- (Jung et al., 2014; Stieglmeier et al., 2014).

In addition to the different initial products of nitrification and denitrification, the conditions under which these processes take place also differ. During nitrification, oxygen is essential,

whereas denitrification takes place mostly under anaerobic conditions (Paul and Clark, 1996). Furthermore, both processes prefer a high pH value in the soil of 7 – 8, but react differently to low pH (Paul and Clark, 1996): for example, nitrification and bacterial denitrification are inhibited under low pH (Herold et al., 2012), as the N_2O reductase of bacteria is sensitive to low pH (Knowles, 1982). In contrast to this, it is assumed that fungal denitrification lacks the N_2O reductase, resulting in potentially more N_2O production under low pH (Shoun and Tanimoto, 1991).

One method for distinguishing between the different sources of N_2O is the use of isotopic methods. The approach of isotopic methods is based either on the natural abundances of N and O or on tracer labeling. The two main production pathways nitrification and denitrification can be differentiated by different isotopic labeling of ^{15}N in ammonium and nitrate (Webster and Hopkins, 1996), as these are the initial substrates of the different production pathways (ammonium in nitrification, nitrate in denitrification). However, the nitrogen used in nitrifier denitrification also originates from ammonium, meaning that it is subsequently not possible to distinguish between nitrification and nitrifier denitrification by this method. For this reason, Wrage et al. (2005) suggested that isotopically labeled oxygen (^{18}O) could be used as well, enabling a distinction between the N_2O originating from nitrification and the N_2O originating from nitrifier denitrification. This approach allows to determine the pathways that produced N_2O by isotopically labeling the O atom. The isotopic approaches can also be combined with modeling.

Furthermore, natural abundance approaches can be used to determine the production pathways of N_2O. To this end, different isotopocules of N_2O are analyzed according to Verhoeven et al. (2019) using two different scenarios. The first scenario described a reduction of N_2O by denitrification or nitrifier denitrification prior to mixing with the N_2O from other source processes. In scenario 2, mixing takes place before a reduction to N_2. By this method, the contribution of nitrification, denitrification and fungal denitrification can be determined.

Factors like water content, soil temperature, pH and nutrient availability control the dominating production pathway in the soil (Loick et al., 2017). Furthermore, intensively managed soils show higher emissions than soils with less cultivation (Imer et al., 2013). Natural emissions from soils, oceans and the atmosphere are estimated to make up about

11 Tg N_2O per year whereas anthropogenic emissions are estimated at 6.7 Tg N_2O year $^{-1}$ (Ussiri and Lal, 2013).

Soils are usually considered as net sources of atmospheric N_2O, but they can also act as sinks. This depends on the ability to reduce N_2O to dinitrogen (N_2) in denitrification and on its diffusion and dissolution in the soil (Chapuis-Lardy et al., 2007). In particular, wet peatlands are able to reduce N_2O to N_2 for subsequent storage.

1.3 Peatlands – definition, types and drainage

Globally, peatlands cover about 3% of the land area (Blodau, 2002). 87.5% of them are located in the northern hemisphere (Fig. 3). The rest, about 12 %, are located in the southern hemisphere, especially in tropical regions (Bonn et al., 2016). In Europe, peatlands occupy about 5 to 6% of the land area, but more than 60% of them are degraded (Drösler et al., 2008). For an organic soil to be classified as a peatland, the minimum depth of the peat layer has to be between 30 and 40 cm (Rydin and Jeglum, 2013). Peat accumulates quite slowly with an accumulation rate of about 1 millimeter per year (Keddy, 2010).

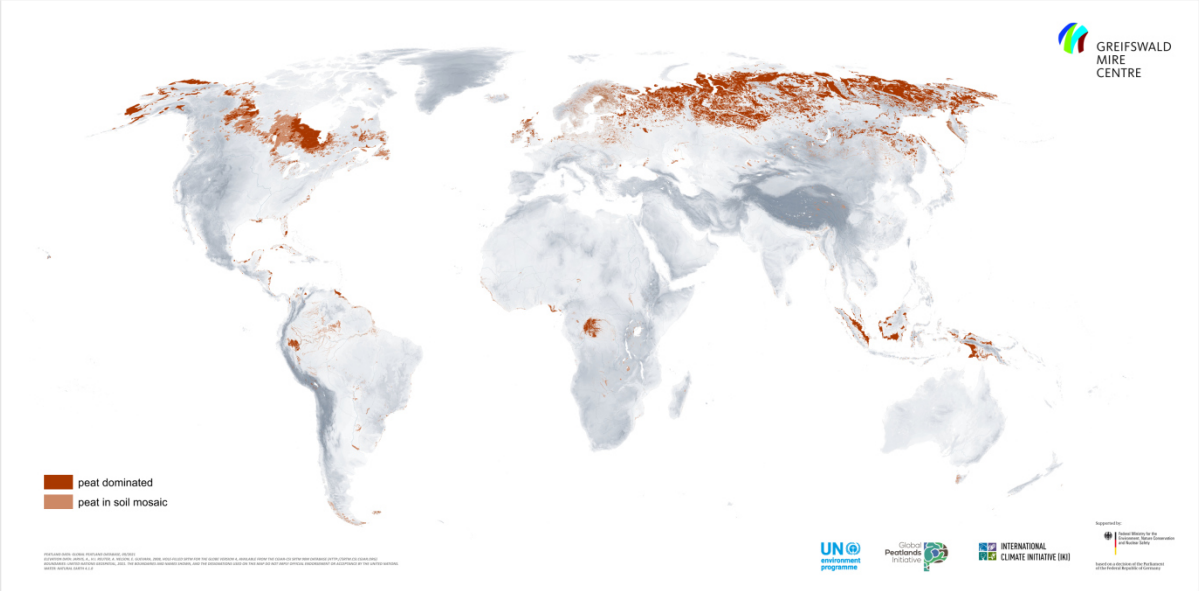

Fig. 3: Global peatland area based on the data of the Global Peatland Database (Greifswald Mire Center, 2022)

Peatlands can be classified into bogs and fens. Fens are fed by groundwater or surface water making them nutrient-rich (Wassen et al., 1990). Bogs, on the other hand, are fed by precipitation leading to nutrient depletion (Gorham, 1957). Factors influencing water conditions in fens are climate, relief, geology, soil and vegetation (Timmermann et al., 2016).

Peatlands are composed of an acrotelm and a catotelm (Clymo, 1984). The acrotelm is a shallow aerobic surface layer and includes the peat soil above the water table and the vegetation layer. Here, fresh organic substances are formed by growth and death of plant material and can be mineralized. The catotelm is located underneath the water surface and is hence anaerobic with less biological activity. These conditions lead to reduced mineralization. In general, undisturbed peatlands have a small acrotelm leading to low greenhouse gas emissions. Since drained peatlands show a lower water table, the acrotelm is consequently considerably larger, leading to more mineralization and greenhouse gas emissions.

A strong peat mineralization leads to the release of CO_2 and N_2O (Gelbrecht et al., 2008). On that account, many fens are supposed to be rewetted for restoring growing peatlands. The aims of the restoration of peatlands include the recreation of biodiversity, production function of peat and plant biomass, independent regulation of nutrients, buffering of flood events and cooling the landscape through evapotranspiration as well as natural processes (Timmermann et al., 2016). This could be achieved either through increased water supply or through reduced water loss.

Peatlands are very important ecosystems in terms of global climate change. They contribute significantly to the global climate system as they store and release greenhouse gases. A few hundred years ago, drainage of peatland started for agriculture, peat extraction and forestry (Jurasinski et al., 2020). Since this time, peatlands developed from sinks to sources of greenhouse gas emissions (Behrendt et al., 2005) as lowering the water table favors conditions for peat mineralization (Vybornova et al., 2019). Climate change and hence, global warming, lead to a further lowering of water table levels, resulting in a larger acrotelm and therefore, in larger greenhouse gas emissions of peatlands. More than 30% of the total global anthropogenic emissions are estimated to originate from land use (Pachauri, 2008).

Due to drainage, only 1% of fens in Germany still show peat accumulation (Joosten and Couwenberg, 2001). The first consequence of drainage is subsidence. As the buoyancy

caused by water in the pores is lacking, the soil subsides, resulting in a loss of peat thickness up to 30% (Blankenburg et al., 2001). As a further consequence, peatlands become more finely porous. Due to the load pressure, subsidence acts primarily vertically (from top to bottom). Drainage also destroys the typical characteristics of peat, as the high water storage capacity is lost and therefore, peat becomes hydrophobic and can store less water, leading to greater water level fluctuations (Timmermann et al., 2016). In turn, this leads to shrinkage, which compacts the peat and therefore shrinks the entire peatland. Shrinkage occurs in all directions, i. e. horizontally and vertically. The reduced water content causes further dehydration significantly increasing the fire hazard in peatlands (Essl et al., 2017)

The drainage of peatlands generally leads to an acceleration of the nutrient cycles resulting in emissions of N_2O into the atmosphere and of NO_3^- into surface water (Timmermann et al., 2016). In Germany, 80% of the total emissions from peatlands originate from fens (Höper, 2007). Fens were often drained for agricultural use, as they are nutrient-rich and were therefore more valuable for such land-use than bogs (Timmermann et al., 2016). Although drained peatlands cover only 0.5% of the world's land area, they cause about 5% of global anthropogenic CO_2 emissions (about 2 Gt CO_2 per year) (Joosten et al., 2016). In Germany, 95% of peatlands are degraded and are responsible for 7% of the total greenhouse gas emissions (53 million tons CO_2 equivalent per year) (Umweltbundesamt). In Mecklenburg-Vorpommern, fens cover 13% of the land surface (Sachs et al, 2015). Here, drained peatlands are even the largest single source of greenhouse gases with 6.2 million t CO_2-equivalents per year (Ziebarth, 2009).

In peatlands, high N_2O emissions often occur at fluctuating water table levels (Flessa et al., 1997; Merbach et al., 2002). Overall, mean annual water table levels of ~40 cm below the surface favor high emissions of N_2O (Couwenberg et al., 2011). Nevertheless, production pathways of N_2O are complex and single drivers of N_2O emissions are difficult to determine.

In addition to their climate effectiveness, natural peatlands are of great importance for biodiversity. Furthermore, peatlands provide diverse ecosystem services like carbon sequestration, biodiversity, water resources, production function, water retention capacity, relaxation and archaeological resources (Bonn et al., 2016). These functions are also lost as a result of drainage.

Rewetting is considered to be the essential component of peatland revitalization (Timmermann et al., 2016). The basic principle of peatland rewetting is simple: rewetted peatlands are characterized by increased water supply and reduced water loss. As a result, rewetting of peatlands reduces the emissions of greenhouse gases, especially CO_2 and N_2O (Augustin and Joosten, 2007). Next to this, rewetting is also an important tool to prevent peatlands from subsidence and nutrient loss (Bonn et al., 2016). The rewetting of peatlands is therefore urgently needed from an environmental and climate policy perspective in order to achieve the 1.5 °C target.

However, in areas where peatland has been drained for agriculture and forestry, rewetting can cause considerable problems and losses for land owners and land users. Therefore, options have to be found to enable profitable land use of rewetted peatlands, especially fens.

An example for the sustainable use of peatlands is the so-called paludiculture. Paludiculture is the agricultural and forestry use of wet bogs and fens (Wichtmann et al., 2016). New innovative and sustainable uses are, for example, the energetic utilization of biomass, the use of reed biomass for new building materials or the cultivation of peat mosses as a peat substitute in horticulture. The wet cultivation of peat soils provides climate protection by reducing CO_2 emissions and by evaporation cooling. It provides alternatives for fossil raw materials without competing with land for food production and enhances biodiversity (Wichtmann et al., 2016).

1.4 Effects of rewetting of fens and paludicultural use on N_2O emissions

In drained peatlands, peat is mineralized and transformed in N_2O. The dynamics of the formation of N_2O and N_2 have not yet been well researched, especially for fens. To date, the effect of rewetting on N_2O sources and emissions is not yet well understood. It has been suggested that rewetting leads to an overall reduction in N_2O emissions (Jordan et al., 2016; Wilson et al., 2016). Studies on processes of N_2O formation in peatlands are almost completely lacking. It is often assumed that denitrification processes lead to N_2O formation, but these are also carried out by different microbial groups (e.g. autotrophic/ heterotrophic bacteria) reacting differently to environmental factors.

Therefore, I analyzed N_2O production as part of the project WETSCAPES, which aimed to provide the scientific basis for a sustainable, i. e. ecologically, economically and socially sound, management of fens, especially degraded and subsequently rewetted areas. The joint project of the Universities of Rostock and Greifswald and the Greifswald Mire Center studied six different sites in Mecklenburg-Vorpommern: a coastal fen near Karrendorf, a percolation fen in the Trebeltal and an alder forest near Wöpkendorf (Fig. 3). Each of the three different types has a drained and a rewetted part.

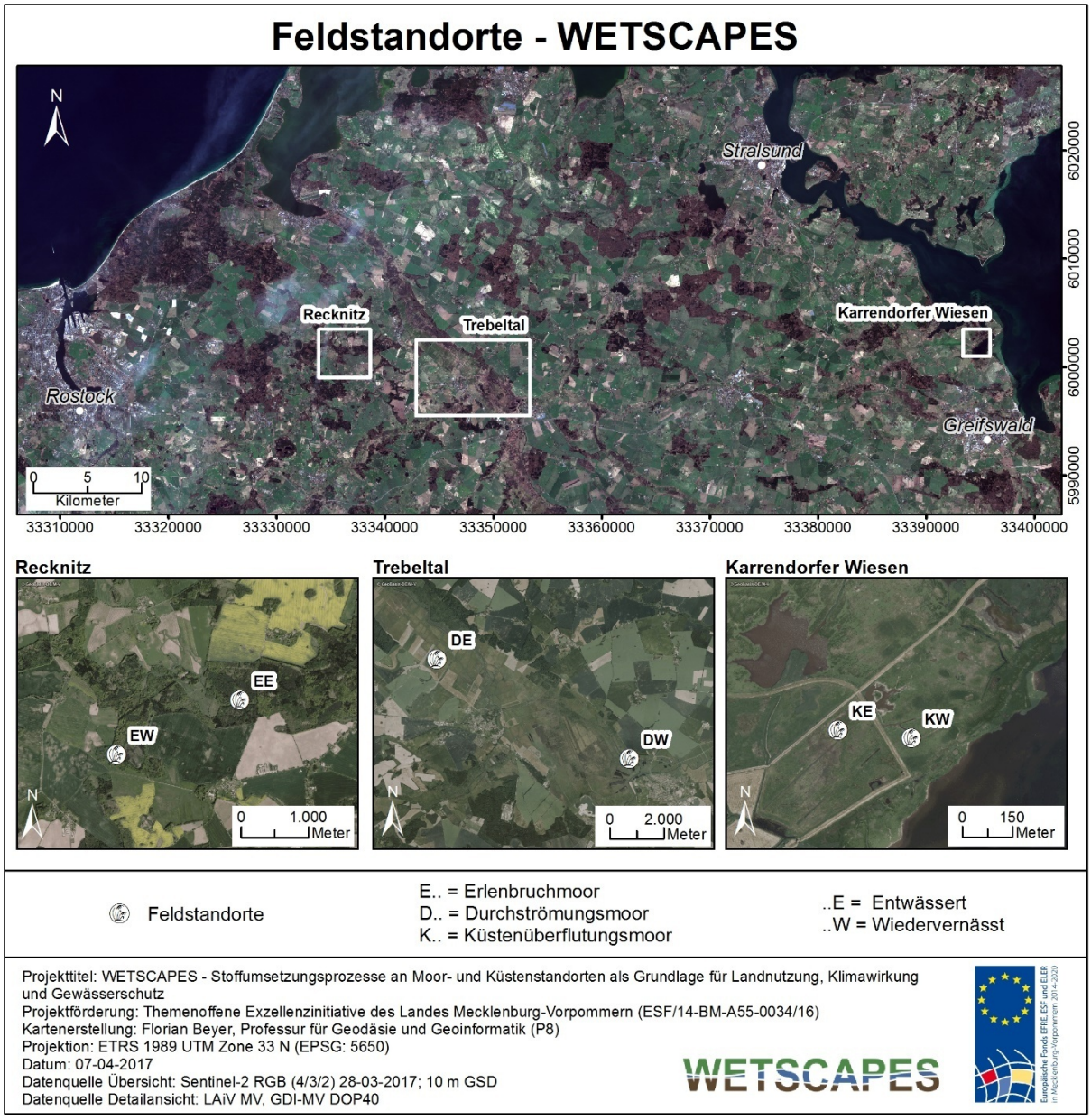

Fig. 3: Location of the different peatland types/ study sites in Mecklenburg-Vorpommern

Coastal fens are influenced by coastal waters. This type of fen originates from marine flooding, but also backwater effects from receiving waters far into the hinterland. In

Karrendorf, this leads to flooding with Baltic Sea water and consequently to saltwater entering the peat body. Coastal fens are located only a few decimeters above sea level and can therefore easily be flooded. Since the 19th century, the study sites of the coastal fen were used as pastures and consequently diked. In 1993, all old dikes were removed and the current dike was built, separating the drained and rewetted study sites. The rewetted coastal fen (CW) is situated in front of the dike, where it is regularly flooded, whereas the drained part (CD) is directly behind. Both study sites have shallow peat and are used as cattle pastures (Jurasinski et al., 2020). The distance between the two study sites is approximately 150 m. As a result of water eutrophication, water retention structures, embankments and river regulations, today there are no longer large areas of coastal peatlands in Central Europe.

Percolation fens are mainly located in valley systems with permanent groundwater supply and have a peat thickness of up to 10 meters. Under natural conditions, they are mesotrophic, acidic to chalky and have an inclined surface (Joosten and Clarke, 2002). They are characterized by low peat decomposition as they are flooded almost permanently by a regular water supply. The percolation fens of the WETSCAPES project are located in river valleys: the rewetted study site (PW) is situated in the Trebel valley, whereas the drained percolation fen (PD) is located in the Recknitz valley. PW was rewetted in the 1990s and is almost in a near-natural condition today (Koch, 2017). The peat thickness at both study sites is between 5 and 6 m. The distance between these two study sites is 8 km.

In the rewetted alder forest (AW), the peat thickness reaches 2 m. The site was initially used as a pasture but at the end of the 19th century, forest pasture was abandoned. In 1900, AW was drained and used as a production forest until its rewetting in 2003. However, the drained alder forest (AD) has a much smaller peat thickness and is strongly degraded as the study site has been in use since 1786 (Jurasinski et al., 2020). The distance between the two sites is 8 km.

1.5 Objectives

The general objective of this thesis is to improve our understanding of the different production pathways of N_2O in fens, especially on rewetted in contrast to drained sites. The

focus was laid on the difference between fen types in the northeast of Germany in order to identify if rewetting of fen sites also results in a reduction of N_2O emissions or if other factors influence N_2O production. Several investigations and experiments were performed to determine these factors.

In order to obtain an overview if rewetting of degraded fens influences the emissions of N_2O from the sites, manual chamber measurements of N_2O emissions were performed every two weeks for three years at all sites (Chapter 2). This survey was intended to identify the difference between the individual peatland types of the study sites and the effect of rewetting on emissions of N_2O. At the same time, seasonal variations as well as hot spots and hot moments (for example after specific weather events like heavy rain) should be identified. In addition, a natural abundance isotopic approach should provide an indication on the potential production pathways of N_2O.

In an incubation study, the dual isotope method was used for a differentiation between N_2O sources (Chapter 3).The aim of this was to improve the understanding of the influence of fen rewetting on sources of N_2O production under controlled laboratory conditions. We expected that peat from rewetted sites would show lower N_2O emissions and that the average water table level height would be the main factor influencing N_2O emissions and sources. Besides, we hypothesized that denitrification would play a more important role on rewetted than on drained sites (Chapter 3).

Soils in the field usually show a highly heterogeneous structure with various microsites. The removal from the field and preparation of the soil for incubation experiments destroys this structure. Therefore, another objective was to enable the use of the dual isotope method directly in the field. Here, a homogeneous distribution of tracers throughout the most active soil volume is important. For this end, field experiments were performed in Lincoln, New Zealand. Different application methods like sprayers or syringes were tested for a homogeneous distribution of the tracers in the soil (Chapter 4).

In the final chapter 5, I summarize my main findings and outcomes and discuss their implications for the rewetting of fens and the needs for future research on the emissions and production pathways of N_2O.

2. Influence of rewetting on N_2O emissions in three different fen types

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ORIGINAL ARTICLE

Influence of rewetting on N_2O emissions in three different fen types

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Abstract In recent years, many peatlands in Europe have been rewetted for nature conservation and global warming mitigation. However, the effects on emissions of the greenhouse gas nitrous oxide (N_2O) have been found to be highly variable and driving factors are poorly understood. Therefore, we measured N_2O fluxes every two weeks over three years on pairs of sites (one drained, one rewetted) of three important peatland types in North-Eastern Germany, namely, percolation fen, alder forest and coastal fen. Additionally, every three months, sources of N_2O were determined using a stable isotope mapping approach. Overall, fluxes were under the very dry conditions of the study years usually small with large temporal and spatial variations. Ammonium concentrations consistently and significantly correlated positively with N_2O fluxes for all sites. Cumulative fluxes were often not significantly different from zero and apart from the rewetted alder forest, which was always a source of N_2O, sites showed varying cumulative emission behavior (insignificant, source, potentially sink in one case) among years. Precipitation was positively correlated with cumulative fluxes on all drained sites and the rewetted alder forest. Isotope mapping indicated that N_2O was always produced by more than one process simultaneously, with the estimated contribution of denitrification varying between 20 and 80%. N_2O reduction played a potentially large role, with 5 to 50% of total emissions, showing large variations among sites and over time. Overall, neither the effect of rewetting, water level nor seasonality was clearly reflected in the fluxes or sources. Emissions were concentrated in hotspots and hot moments. A better understanding of the driving factors of N_2O production and reduction in (rewetted) fens is essential and stable isotope methods including measurements of ^{15}N and ^{18}O as well as site preferences can help foster the necessary comprehension of the underlying mechanisms.

Keywords Nitrous oxide · ^{15}N · Denitrification · Stable isotope mapping · Fen · N_2O reduction

Gerald Jurasinski and Nicole Wrage-Mönnig have contributed equally to this study.

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Introduction

Peatlands are very important ecosystems in terms of global climate change. Drainage of peatlands started a few hundred years ago for activities like agriculture, peat extraction and forestry (Joosten and Couwenberg 2001). Lowering the water table creates favorable conditions for peat mineralization and thus turns peatlands from net carbon sinks to sources (Vybornova et al. 2019). Overall, drained peatlands are a major source of emissions of greenhouse gases (GHG), mainly CO_2 and N_2O (Joosten 2012; Pachauri and Mayer 2015). In Mecklenburg-Vorpommern, drained peatlands emit approximately 30% of the total CO_2 equivalent emissions of this German state (Ziebarth 2009).

Peatlands can be classified as bogs and fens. Fens (as in our study) are fed by groundwater and surface water, making them potentially nutrient- and base-rich (Wassen et al. 1990). Bogs, on the other hand, receive their water from precipitation, leading to nutrient depletion and acidification (Gorham 1991; Keddy 2014). A comparison of bogs and fens under different land use forms with regard to greenhouse gas emissions showed that the emissions of fens (in CO_2 equivalents) were always higher than those of bogs (Höper 2015). Moreover, fens cover an area three times larger (10,800 km^2) than bogs (3360 km^2) in Germany (Große-Brauckmann 1997). However, there are much less studies on N_2O emissions from fens than from bogs.

Nitrous oxide can be produced via various pathways like nitrification, denitrification or nitrifier denitrification (Butterbach-Bahl et al. 2013). Which of these processes dominate in a given soil is controlled by factors such as pH, soil temperature, nutrient availability and water content (Loick et al. 2017). It is often suggested that denitrification is the dominant source of N_2O in organic soils (Beaulieu et al. 2011), especially in peatlands, with a water-filled pore space (WFPS) of 80–95% being ideal for the production of N_2O (Säurich et al. 2019). At higher WFPS, more N_2O can be reduced to dinitrogen (N_2) (Davidsson et al. 2002). An uptake of N_2O into the soil and further reduction to N_2 is also possible. N_2O uptake is expected to typically take place in ecosystems with a high moisture content and limited nitrogen availability (Chapuis-Lardy et al. 2007), such as peatlands.

In the last 30 years, several 100.000 ha of peatlands in Europe have been rewetted in order to reduce greenhouse gas emissions and to re-establish their habitat function (Andersen et al. 2013). This mostly led to a loss of agricultural land (Jurasinski et al. 2020). In Mecklenburg-Vorpommern, peatlands account for 13% of the land area of which 90% are still drained for use in agriculture or forestry (Ziebarth 2009). A complete conversion of this land into restored and protected natural peatlands is not likely achievable. Therefore, the idea of agricultural use of wet peatlands, i.e. paludiculture, is receiving more attention, where the objective is the combination of agricultural use and the maintenance and new formation of peat (Joosten et al. 2016). Examples of the management of peatlands are the growing of reeds as thatching materials or cattail cultivation for feeding cattle (Wichmann 2018).

So far, the effect of rewetting on N_2O dynamics is still unclear. Although only relatively few studies have investigated the effect of rewetting of agriculturally used fens on the emissions of N_2O (Velty et al. 2007; Wilson et al. 2016), it has been suggested that rewetting could cause an overall reduction in N_2O emissions (Jordan et al. 2016). Greenhouse gas emissions after rewetting depend on time since rewetting, climate, peat, nutrient availability, vegetation and hydrology (Wilson et al. 2016), but also on management, which influences several of the before-mentioned factors. The effect of paludicultural management on N_2O emissions is largely unknown since studies are almost absent (but see Günther et al. (2015) for an example).

Here, we investigate the annual and seasonal variation of N_2O fluxes and its sources using stable isotope mapping in drained fens of three peatland types in North-Eastern Germany with agricultural or forestry use and in rewetted counterparts in order to better understand the connection between N_2O fluxes and rewetting. We hypothesized that a) there is a treatment effect of rewetting on N_2O emission from fens; b) seasonal variability influences N_2O emissions; c) the concentration of ammonium (NH_4^+) and nitrate (NO_3^-) as substrates for the production of N_2O has significant effects on N_2O fluxes; and d) denitrification plays a key role in controlling N_2O emissions of rewetted peatlands, with on the one hand denitrifier activity stimulating N_2O emissions and on the other

hand, N_2O reduction by denitrifiers' N_2O reductase reducing N_2O emissions.

Methods

Sites

In the WETSCAPES project (Jurasinski et al. 2020), pairs of drained (D) and rewetted (W) sites of coastal fen (CD and CW; the latter rewetted in 1993 after the building of a new dike, flooded regularly today), percolation fen (PD and PW, rewetted in the 1990s) and alder forest (AD and AW, rewetted in 2003) were set up in early 2017. For a detailed description of the study sites, see Jurasinski et al. (2020). Meteorological stations were installed at four of the six sites in order to monitor weather data, e.g. air temperature, wind speed or precipitation. Due to their spatial proximity, the two sites of the coastal fen and those of the alder forest each shared one meteorological station. As the percolation fen sites are separated by a distance of approximately 8 km, each was equipped with its own meteorological station.

Wooden boardwalks were built at all sites in order to prevent peat compression during field measurements. Two months before starting N_2O exchange measurements, five collars with a diameter of 0.63 m and a distance of 3 m to each other were installed permanently in the soil (0.1 m deep) (Jurasinski et al. 2020) resulting in six sites with five spatial replicates each. The vegetation inside the collars was clipped regularly to simulate management activities like grazing or mowing.

N_2O flux measurements and explanatory variables

Between August 2017 and August 2020, N_2O fluxes were measured every two weeks with static closed chambers in order to detect small fluxes characteristic for N_2O. Usually, measurements were performed between 8 a.m. and 4 p.m. Due to a national Covid-19 lockdown, there is a gap in the measurements between March and July 2020. After this period, we stopped measuring in the alder forest sites.

The custom made chambers follow the design described by Günther et al. (2014) with flexible polyurethane walls and a height of approximately 0.63 m, however, depending on the conditions and the vegetation of the study site, the height of the chambers can be adapted variably. On our study sites, the height of the chambers mostly varied between 0.55 and 0.65 m, but when PW and AW were flooded, chamber height was adapted to up to 0.9 m. The chambers were equipped with a fan for mixing the air in the headspace and a thermometer for recording the temperature inside.

During measurements, the chambers were closed for 40 min, and gas was sampled from the headspace every 10 min (0–10–20–30–40 min). Gas samples were taken using a 60 ml syringe with a two-way stopcock. The syringe was first flushed repeatedly with air from the headspace before taking the sample. Each sample (about 30 ml) was immediately transferred into evacuated 12 ml Exetainer® vials producing overpressure. The samples were analyzed for N_2O concentrations using a gas chromatograph (Shimadzu Auto System) calibrated with standards of 203, 304, 502, and 1037 ppb N_2O in synthetic air. Concentrations below 203 ppb could be measured linearly until 50 ppb, but were likely overestimated (data not shown). Since no measured N_2O concentrations were below 50 ppb and very few below 200 ppb, the potentially introduced error is small.

In addition to the greenhouse gas measurements, water table levels and temperature as well as NH_4^+ and NO_3^- contents were measured as potential control variables. The temperature was measured with a calibrated temperature probe every 10 min when a gas sample was taken. The water table levels were recorded every 15 min on Campbell Scientific CR300 or CR1000 (AW/AD) data loggers by different sensors. At CD, PW and PD, we used Seba Dipper PT-water level loggers, at CW, we used a Seba Dipper-APT water level logger. For NH_4^+ and NO_3^- determination, soil samples were taken every three months and extracts were prepared. Afterwards, the NH_4^+ and NO_3^- concentrations were determined colorimetrically using a Photometry CFA method (Skalar SAN, Skalar Analytical B.V., The Netherlands). The analysis was performed according to EN ISO 13395 and EN ISO 11732.

Isotopic measurements and mapping approach

Since October 2018, additional gas samples were taken every three months and analyzed for isotopocules of N_2O – isotopically substituted molecules

$^{14}N^{15}N^{16}O$, $^{15}N^{14}N^{16}O$, and $^{14}N^{14}N^{18}O$ of the main $^{14}N^{14}N^{16}O$—for determining the production pathways of N_2O. For this purpose, 110 ml Exetainer® vials were used, while the sampling procedure remained as described above, only with larger syringes (200 ml). The gas samples were analyzed with an isotope ratio mass spectrometer (IRMS, IsoPrime 100, Elementar, Langenselbold), with TraceGaspreconcentrator (Elementar, Langenselbold). For calibration, we used two working standards (0.9 and 1.8 ppm N_2O in synthetic air, with 0.15‰ for $\delta^{15}N$ and 40.66‰ for $\delta^{18}O$ and a site preference (SP) of 1.42‰ for the 0.9 ppm standard, and 0.02‰, 40.32‰ and 1.47‰ for 1.8 ppm, respectively) calibrated against the standards of the laboratory of the Department of Environmental System Science, ETH Zürich (Verhoeven et al. 2019) that were run in triplicates at the beginning and end of the batch of each site. An N_2O reference gas peak (100% N_2O, Air Liquide, Germany) was used for calibration of the sample peak ratios with every sample. Afterwards, the ratios were corrected for drift and span via the working standards. Stability (≤ 0.01‰) and linearity (≤ 0.02‰) of the IRMS were measured by injection of 10 gas pulses of similar or varying amount, respectively, using pure N_2O. Determination of external precision for N_2O was done by four samples per run of our standard gas mixture containing 1.8 ppm N_2O with an average standard deviation of 0.22‰ for ^{15}N, 0.62‰ for ^{18}O and 0.86 for SP. The SP was calculated based on the relation of $^{15}N_\alpha$ and $^{15}N_\beta$ (which was calculated of $^{15}N_\alpha$ and bulk ^{15}N-N_2O).

We analyzed the isotopic data following Lewicka-Szczebak et al. (2017) and Verhoeven et al. (2019). The isotope ratio data was interpreted for sources of N_2O using two different scenarios (Lewicka-Szczebak et al. 2017; Verhoeven et al. 2019). The first scenario described a reduction of N_2O by denitrification or nitrifier denitrification prior to mixing with the N_2O from other source processes. In scenario 2, mixing takes place before a further reduction of N_2O. For scenario 2, the model did not provide reasonable solutions, since, for example, the contribution from denitrification processes was often in a negative range. This was also observed by Verhoeven et al. (2019). Therefore, we only considered the results of scenario 1. In May 2019, negative site preferences were measured for all sites, resulting in both scenarios showing implausible solutions. For this reason, we discarded these measurements from analysis, but included them in a graphical interpretation using an isotope map.

Flux estimation

N_2O fluxes were estimated based on the rate of change of gas concentrations in the headspace of the chamber using the package flux 0.3–0.1 (Jurasinski et al. 2014) to fit linear regressions to the data in R version 3.6.1 (R Core Team 2020). The function flux tries to find the best fitting linear regression to the change of concentration over time and finds outliers by running regressions for all possible variations of n—x data points (n = total number of gas concentration measurements for one chamber placement, x = number of possible outliers). This is controlled by setting *min.allowed*, the minimum number of concentration measurements to be retained. We often see clear outliers from an otherwise obvious linear increase. These are well captured by the algorithm. In addition, the algorithm may well retain more points (and in fact, often does). Due to a change in the number of concentration samples taken for each flux measurement over the course of the study (first 3, then 4, then 5 concentration measurements; the latter covering the majority of the fluxes) driven by discussions among authors, we adapted *min.allowed* to the situation: For 3 and 4 concentration measurements, *min.allowed* was set to 3, while for 5 concentrations it was set to 4.

flux uses the normalized root mean square error (NRMSE) as the quality criterion for the outlier detection and elimination procedure. The model (and, therefore, configuration of concentration data points) with the lowest NRMSE is chosen unless the complete model (contains all concentration data points) has an NRMSE ≤ 0.1. The slope of the resulting linear regression is then used to estimate the flux via Eq. 1; with F the N_2O flux (ng m^{-2} h^{-1}), M the molar mass of N_2O (g mol^{-1}), p the air pressure (101,300 Pa), V the chamber volume (m^3), R the gas constant (m^3 Pa K^{-1} mol^{-1}), T the average temperature in the chamber during closure (K), A the surface area of the measurement collar (m^2) and dc/dt the change of concentration over time, i.e. the aforementioned slope. We use the atmospheric sign convention, meaning that positive fluxes indicate a release from the ecosystem to the atmosphere and negative fluxes indicate uptake by

the ecosystem. No fluxes were discarded since fluxes with high NMRSE are typically those that are very small anyway.

$$F = \frac{MpV}{RTA} * \frac{dc}{dt} \quad (1)$$

We calculated the minimum detectable flux for our setup (defined by a combination of the size and area of the chambers, sampling time and precision of measuring device) according to the robust linear regression approach (Hüppi et al. 2018). As the size of the chambers was adjustable, we took the largest volume used in order to have a conservative estimate of the flux detection limit.

Cumulative gas fluxes were calculated as the integral under the connected flux estimates over one year. We used fluxes (both positive and negative) below the minimum detectable flux as measured for the cumulative flux calculation. As there was a gap in the data due to national Covid-19 lockdown, cumulative fluxes for the third measurement year were calculated from March 2019 to March 2020 and not like in the other years from August–July of the following year. Therefore, the cumulative data of the second and the third year overlap partially. Since every measurement year is arbitrarily defined anyway (see Beetz et al. (2013) for an example of addressing the variability of annual GHG flux estimates), we think that this approach is helpful here to enable us to compare three annual values.

Statistics

For each measuring day, means and standard deviations of N_2O fluxes were calculated per site as well as for cumulative fluxes. Data were tested for normality using the Shapiro–Wilk-Test. ANOVA was used to check for differences ($\alpha = 0.10$) among fen types or between water management varieties. An ANOVA with repeated measurements was used to find significant differences among measuring days. If the requirements for ANOVA were not fulfilled, the Kruskal–Wallis-test was used to determine effects of rewetting or of the different fen types. The Tukey-, Holm-Sidák- and Dunn-tests were used as post-hoc tests to test for significant differences among values applying the most appropriate test suggested by SigmaPlot 13.0. Next to this, a two-tailed t-test was used to check for significance of difference of cumulative fluxes from 0. Correlation of (cumulative) fluxes was calculated with environmental variables, e.g. temperature, water table level and NH_4^+ and NO_3^- concentrations. Statistical analyses were performed with SigmaPlot 13.0.

Results

N_2O fluxes

Overall, N_2O fluxes were small (Fig. 1 a-c), usually below 500 µg N_2O-N m^{-2} h^{-1}, with one peak emission event in AW reaching 2030 µg N_2O-N m^{-2} h^{-1} (inset plot Fig. 1c). Many fluxes (both positive and negative) were below the minimum detectable flux. N_2O exchange followed a slight seasonal trend, with fluxes being marginally larger in summer than during winter, but the differences between the seasons were not significant (data not shown).

At both coastal fens CD and CW, the N_2O fluxes of the two sites were small and not significantly different between sites ($p = 0.990$, Fig. 1a). In both CW and CD, there were significant differences among measuring days ($p \leq 0.001$ in both cases). At the percolation fen sites PW and PD, we measured small fluxes without large variations until August 2018 (Fig. 1b). Thereafter, fluxes were considerably larger. Only PD showed significant differences among measuring days ($p \leq 0.001$). In the alder forest sites AD and AW, N_2O fluxes were more variable than in the other sites, ranging from −229.3 to 2030.3 µg N_2O-N m^{-2} h^{-1} in AW and 2.1 to 250.3 µg N_2O-N m^{-2} h^{-1} in AD (Fig. 1c). In both AW and AD, fluxes varied significantly among measuring days ($p \leq 0.001$).

Overall, only AW differed significantly in N_2O emissions from all other sites ($p \leq 0.001$), showing larger N_2O emissions. Furthermore, CW showed significant differences to PD and AD ($p = 0.040$ and $p = 0.026$, respectively). The other sites were not significantly different from each other (p between 0.128 and 0.999).

The cumulative fluxes differed largely among the individual sites during the different measurement years (Fig. 2). While AW was a source of N_2O throughout the study period, cumulative fluxes for all other sites were either insignificant or slightly positive, depending on the year. The drained site AD even

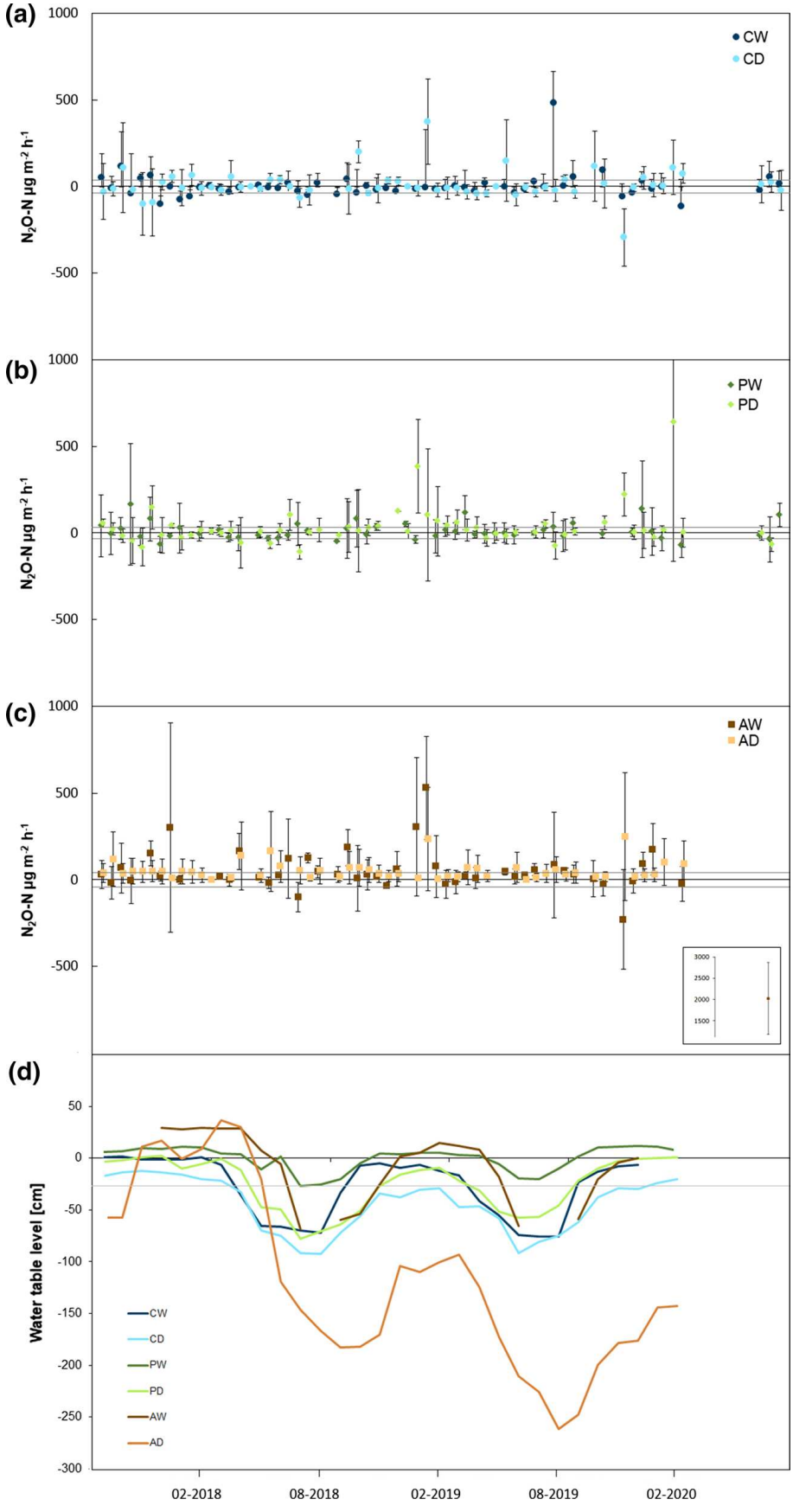

Fig. 1 N_2O-N emissions (µg m^{-2} h^{-1}) of the rewetted (CW) and drained (CD) coastal fen (**a**), the rewetted (PW) and drained (PD) percolation fen (**b**) and the rewetted (AW) and drained (AD) alder forest (**c**) over three years and the water table level for all sites (**d**). In figure a-c, grey lines mark the minimum detectable flux, in figure d, the water table level of −0.25 m. In figure c, the inset plot shows the outlier value measured for AW in the week of August 5 2019. Data points are means (n = 5) ± standard errors

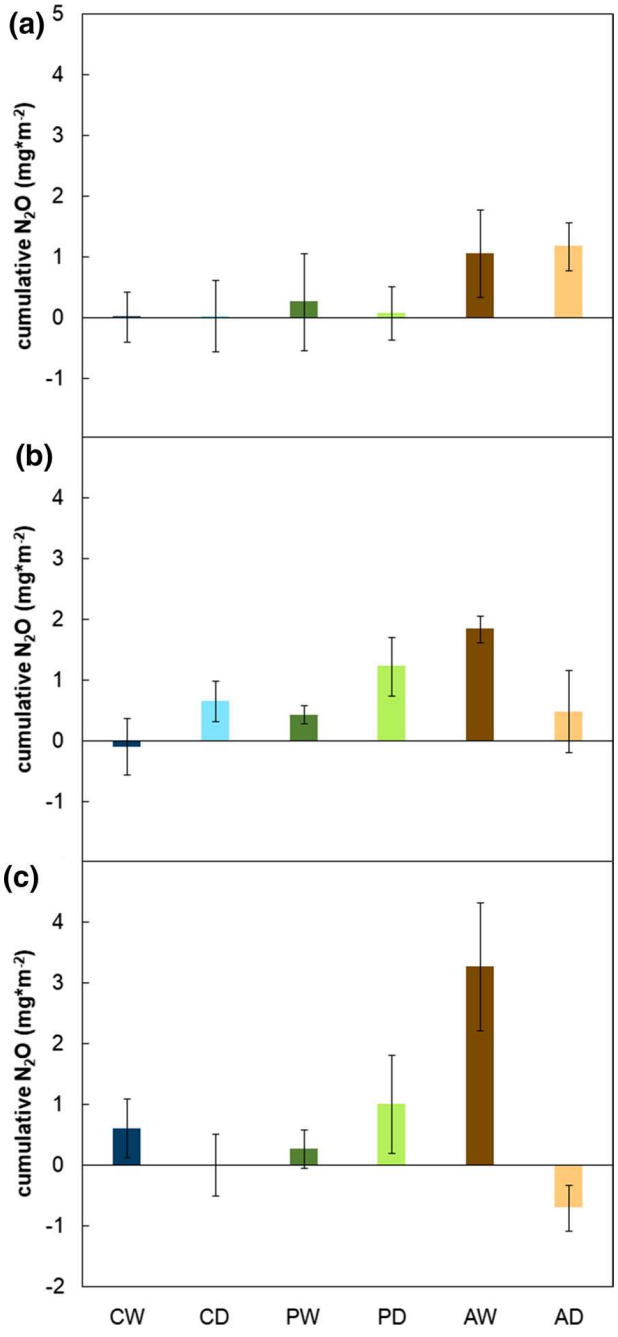

Fig. 2 Cumulative fluxes of N_2O-N emissions (g ha^{-1}) of the six sites (rewetted (CW) and drained (CD) coastal fen, rewetted (PW) and drained (PD) percolation fen, rewetted (AW) and drained (AD) alder forest) for **a** the first measurement year (August 2017–July 2018), **b** the second measurement year (August 2018–July 2019), and **c** the third measurement year (March 2019–March 2020). Data points are means (n = 5) ± standard errors; asterisks indicate whether results are significantly different from zero (*0.1 ≥ p > 0.01, **0.01 ≥ p > 0.001, ***p ≤ 0.001)

showed overall negative fluxes that were significantly different from zero in the third measurement year. All sites except PW showed significant differences among measurement years (between $p = 0.002$ and $p = 0.046$).

Environmental conditions

Although the rewetted sites usually had higher water table levels than the drained ones, seasonal fluctuations were large (Fig. 1d). Even on the rewetted sites, water table levels sank to below 0.25 m (maximally down to 0.75 m) below the surface in summer 2018 and 2019, reflecting drought conditions in those years. These were most pronounced in the drained site AD, where the water table sank to below 2.5 m in 2019. From July 2019 onwards, the drained site PD interestingly had the second highest water table level, higher than or similar to that of the rewetted sites with exception of PW, which showed the most stable wet conditions throughout.

Overall, the NH_4^+ concentrations were of the same range as the NO_3^- concentrations, with the latter showing some concentration peaks especially in the second half of the experiment (Fig. 3a, b). The NH_4^+ concentrations fluctuated strongly, without clear patterns. Initially, NH_4^+ concentrations at AW were comparatively large (up to 54 µg N g^{-1} DM), but dropped sharply as of summer 2018 (Fig. 3a). At this time, NH_4^+ concentrations were small at all sites. Afterwards, especially rewetted sites showed larger concentrations of NH_4^+. The drained sites AD and PD, on the other hand, showed the smallest concentrations of NH_4^+ over the entire measurement period. This was reflected in the ^{15}N enrichments of NH_4^+, which were usually comparatively large in AD and PD (Fig. 3c). However, also enrichments fluctuated over time, without consistent patterns.

In contrast, NO_3^- concentrations were small at all sites until summer 2018 (Fig. 3b). Thereafter, an increase in NO_3^- concentrations occurred at PD and AW, while the other sites still showed smaller concentrations. The largest NO_3^- concentrations were measured at AW in October 2019 (273 µg N g^{-1} DM). Generally, NO_3^- was more enriched in ^{15}N than NH_4^+ (on average over all sites 22.6 ± 11.1‰ and 7.6 ± 9.6‰, respectively; Fig. 3d, c). Also here, there were large temporal fluctuations without consistent patterns.

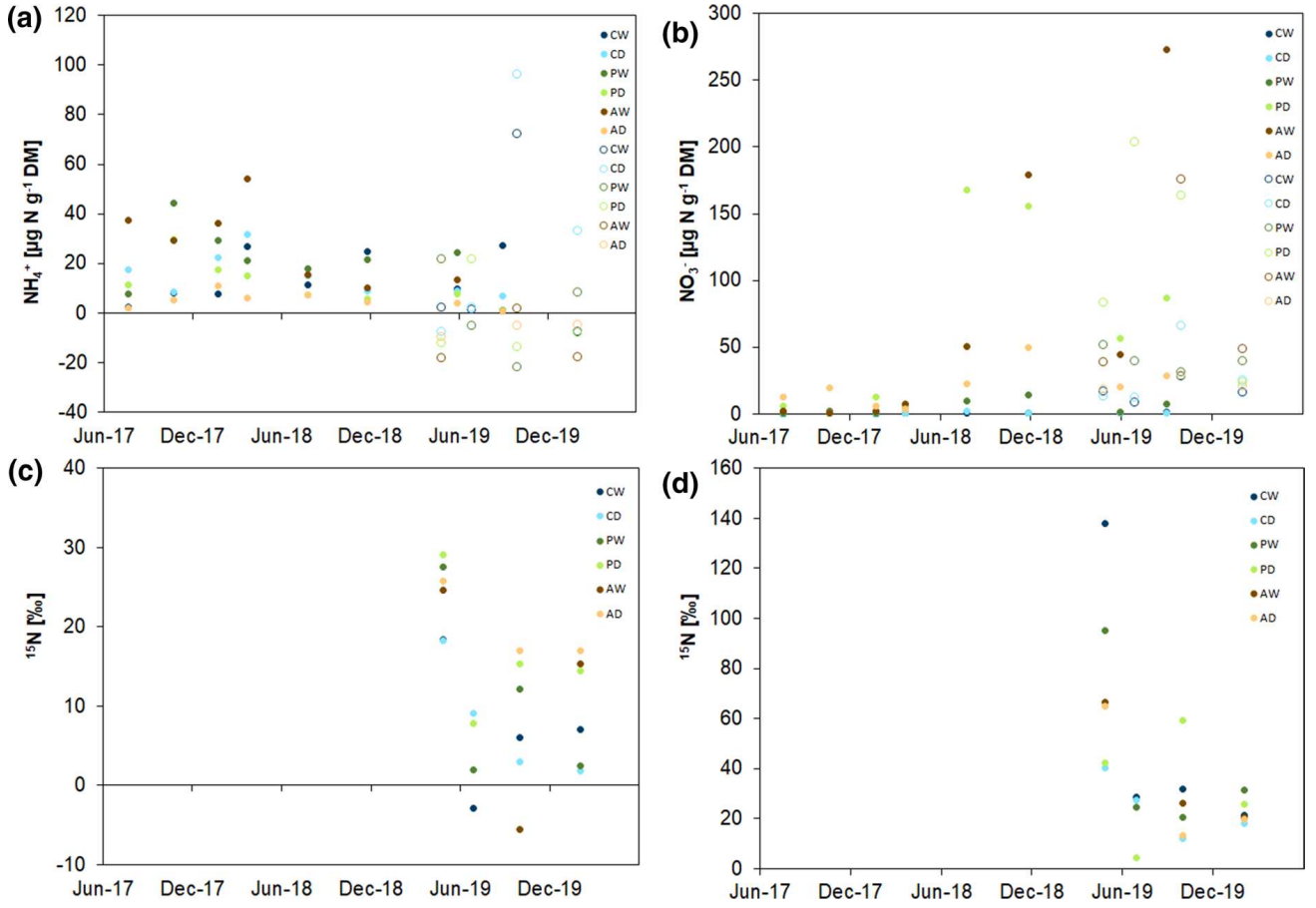

Fig. 3 NH_4^+ (**a**) and NO_3^- concentrations (**b**) (µg N g^{-1} dry mass) of the rewetted (CW) and drained (CD) coastal fen, the rewetted (PW) and drained (PD) percolation fen and the rewetted (AW) and drained (AD) alder forest over 2.5 years. Unfilled circles represented concentrations calculated based on dry mass averages, as no dry mass measurement was performed at this point of time. Data from ^{15}N from NH_4^+ (**c**) and ^{15}N from NO_3^- (**d**) was only available from May 2019 to February 2020

Correlations of N_2O fluxes with environmental conditions

Correlations with temperature and water table were mostly not significant (Table 1). Only in CW and PD, the temperature was significantly correlated with N_2O fluxes, once positively and once negatively ($p = 0.081$ and $p = 0.002$, respectively). Water table showed no significant correlations with N_2O emissions. At all sites, there were significant positive correlations with NH_4^+ concentrations ($p = 0.016$–$p = 0.038$). In both coastal sites as well as in PW and AD, NO_3^- concentrations had (marginally)significant positive correlations with N_2O emissions ($p = 0.033$—$p = 0.095$).

Generally, correlations between water table levels and NH_4^+concentrations were positive, while those with NO_3^- concentrations were negative (Table 1). For both alder forest sites, these were significant for NH_4^+ ($p = 0.035$ for AW and $p = 0.055$ for AD). On all drained sites, NO_3^- concentrations were (marginally) significantly negatively correlated to water table levels. Especially on CD, a strongly significant negative correlation was observed ($p \leq 0.001$), whereas those on PD and AD were marginally significant ($p = 0.052$ and 0.061, respectively). We did not find significant correlations between NO_3^- concentrations and water table levels on any of the rewetted sites.

The correlations between precipitation and cumulative fluxes were positive for all sites and significant for all drained sites and AW (Table 1, $p \leq 0.001$–$p = 0.015$).

N_2O sources and N_2O reduction

The isotope map of site preference versus ^{18}O signatures of all measurements of all locations (Fig. 4)

Table 1 Statistical analysis of the correlations between environmental conditions and N_2O fluxes, mineral nitrogen and water level, and cumulative N_2O fluxes and precipitation of the different sites (rewetted (CW) and drained (CD) coastal fen, rewetted (PW) and drained (PD) percolation fen, rewetted (AW) and drained (AD) alder forest): shown are correlation coefficients (with * and in bold when they were significant)

	CW	CD	PW	PD	AW	AD
Correlation with N_2O fluxes						
Temperature	**0.273***	−0.098	0.036	**−0.449****	−0.022	−0.029
Water table	−0.072	0.041	0.058	0.211	0.267	0.070
NH_4^+	**0.568***	**0.608***	**0.570***	**0.538***	**0.553***	**0.584***
NO_3^-	**0.539***	**0.507***	**0.463***	0.423	0.349	**0.553***
Correlation with water levels						
NH_4^+	0.239	0.300	0.305	0.556	**0.843***	**0.745***
NO_3^-	−0.279	**−0.968****	−0.328	**−0.749***	−0.622	**−0.734***
Correlation with cumulative N_2O fluxes						
Precipitation	0.133	**0.740***	0.470	**0.735***	**0.866***	**0.989***

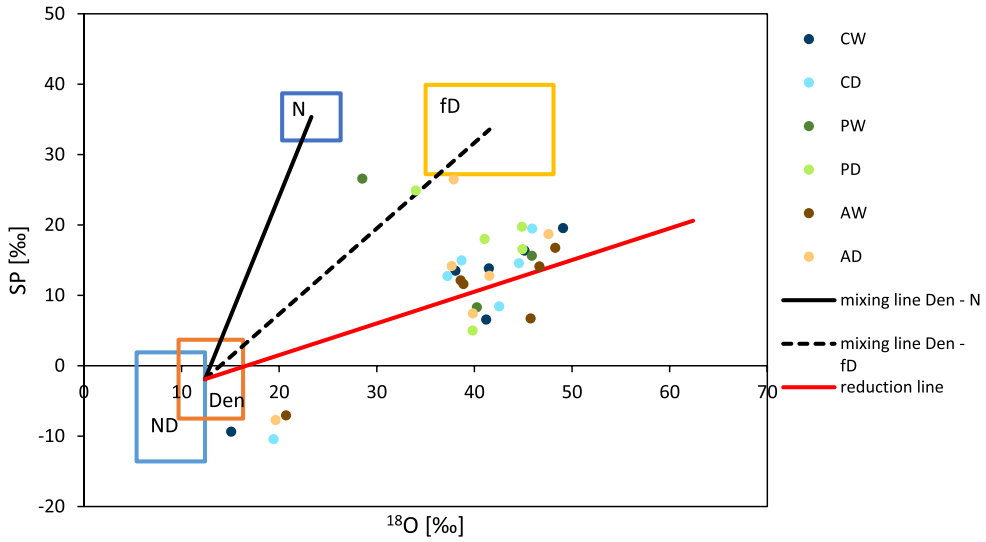

Fig. 4 Isotope map of site preference (SP) versus $\delta^{18}O$ signatures of measured N_2O. Boxes indicate endmember values according to Yu et al. (2020), but corrected for nitrification and nitrifier denitrification using a $\delta^{18}O$ signature of water samples in fen sites in Mecklenburg-Vorpommern of approximately 7‰ according to Böttcher (oral communication). Please note that there is quite some seasonal and depth variation in $\delta^{18}O$ signature of water samples (Koebsch et al. 2019)

shows most values outside of endmember value boxes, but around the N_2O reduction line from N_2O produced by nitrifier denitrification or denitrification. Some values (all from May 2019) with negative site preferences are grouped just outside the endmember box of nitrifier denitrification, on a (not drawn) reduction line from the most negative site preference endmember values of nitrifier denitrification. Three further values from November 2019 (AD) and March 2020 (PD and PW) were more enriched in site preferences and grouped along a mixing line of (nitrifier) denitrification and fungal denitrification. This pattern could also derive from a mixing of bacterial denitrification pathways and nitrification, with additional N_2O reduction.

N_2O fluxes on the days of isotopic measurements reflected the overall variations well (Fig. 5a), capturing both small fluxes around the minimum detectable flux as well as the large fluxes of especially AW. Further analysis of the isotopic signatures on these days indicated that at the beginning of the measurements, there were smaller differences in the estimated

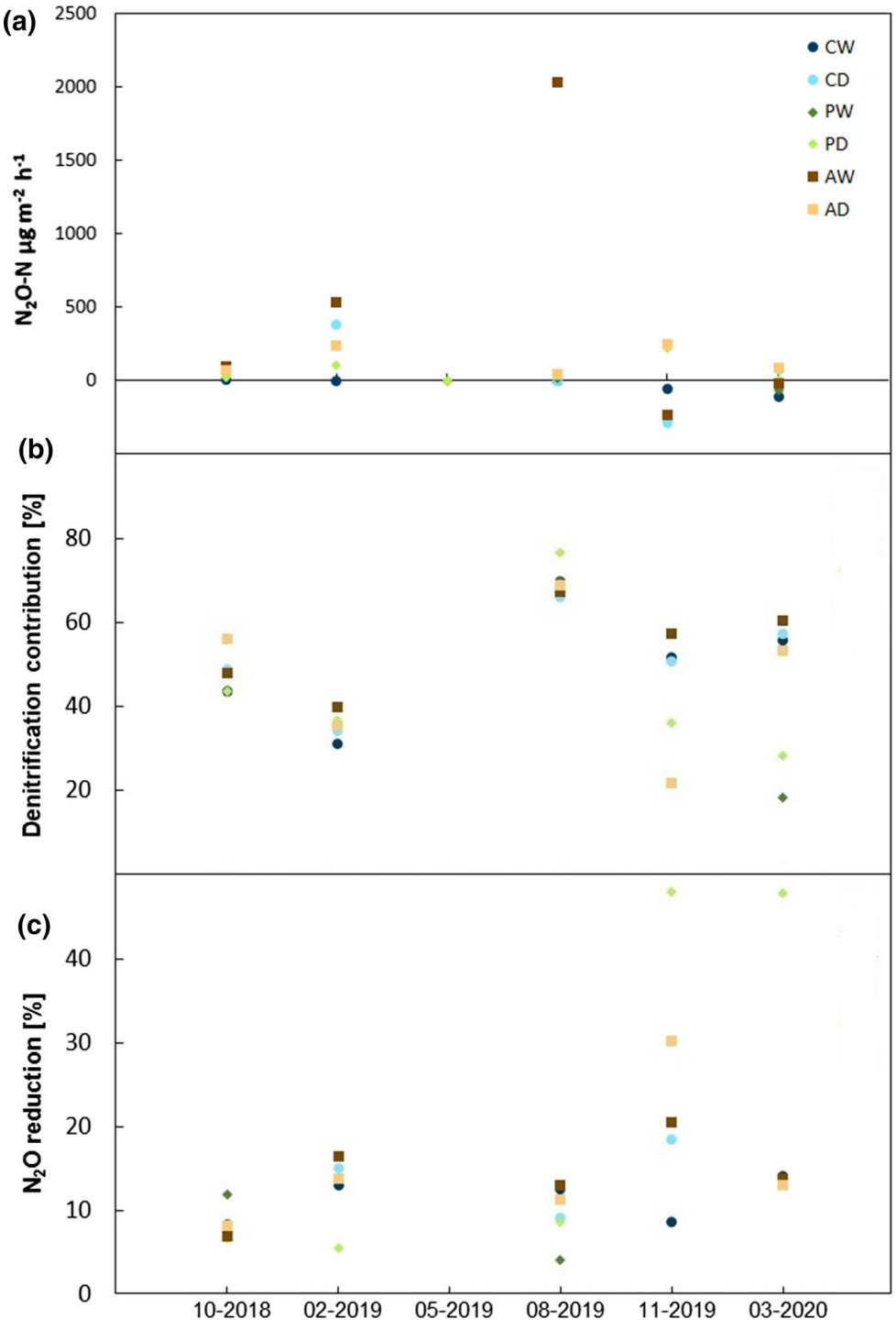

Fig. 5 **a** N_2O fluxes (µg m^{-2} h^{-1}) of the six different sites from October 2018 until March 2020 on the days of isotopic measurements. **b** Denitrification contribution (%) of the six different sites from October 2018 until March 2020. **c** N_2O reduction (%) of the six different study sites from October 2018 until March 2020. The calculation of N_2O fluxes as well as the contribution of denitrification and N_2O reduction was based on average values

contribution of denitrification (COD) to N_2O production among sites than at the end (Fig. 5b). In October 2018, all sites showed a similar estimated COD (between 43.7% and 49.0%), with slightly larger values in AD (55.9%). In August 2019, estimated COD was large at all sites. The largest estimated COD was observed at PW with 76.6%, whereas the remaining sites varied between 65.9%—69.9%. On the last two measurement occasions, the estimated COD was between 20.0%—60.0% (Fig. 5b). Correlations with water table level were negative, but displayed no significant correlations for any site (data not shown).

Estimated N_2O reduction varied over time and among sites (Fig. 5c). After August 2019, the results

were widely spread, with CW showing the smallest estimated N_2O reduction with 8.7%. In PD, we recorded a noteworthy estimated N_2O reduction on the last two measurement occasions of 48.0%, more than double that of most other sites. In March 2020, the estimated N_2O reduction of the other sites showed almost identical estimated N_2O reductions of 13.1–14.2% (Fig. 5c). Overall, correlations between estimated N_2O reduction and water table level were mostly positive, except for AD ($r = -0.138$). However, only in CD, estimated N_2O reduction and water table level were significantly positively correlated ($p = 0.043$). Furthermore, a trend to a significant positive correlation was found in PD ($p = 0.098$).

Discussion

N_2O emissions did not differ significantly between drained and wet sites

Compared to other studies, our cumulative fluxes were extremely small and often below the minimum detectable flux. Other studies (Flessa et al. 2002; Yao et al. 2009) mostly reported fluxes between 1 and 2 kg N_2O-N ha^{-1} yr^{-1} from agricultural systems, whereas our largest cumulative fluxes were about one order of magnitude smaller. Nykanen et al. (1995) also measured very small N_2O fluxes from natural peatlands, in a similar range to our fluxes, whereas those from drained peatlands were much larger, in line with the results from Vybornova et al. (2019). Huth et al. (2012) confirmed generally small N_2O emissions from a wet fen, corresponding to our results.

In contrast to our first hypothesis, N_2O emissions were not necessarily larger on drained compared to rewetted sites. Drought conditions in 2018 and 2019 that were also visible in the precipitation and temperature records (Jurasinski et al. 2020) severely influenced water table levels (Fig. 1d). From July 2019 onwards, the drained site PD even had a higher water table level than most rewetted sites. Thus, the categorization into ‘rewetted’ and ‘drained’ sites may be too simple to explain fluxes, especially under the extreme conditions experienced here.

According to Mosier et al. (1998), drained peatlands are relevant sources of N_2O. We, however, estimated the largest cumulative fluxes for the rewetted site of the alder forest (AW). The comparatively large cumulative fluxes in AW might have been caused by the exceptionally dry conditions that led to effects similar to draining and, thus, potentially to mineralization of peat especially in rewetted sites, where new peat may have built up in previous years (at least for PW this was shown by Mrotzek et al. (2020)). However, they were driven by very few larger positive fluxes. As measurements could only be carried out every two weeks, the uncertainty connected with the calculated cumulative fluxes is large.

In summary, the categorization into rewetted or drained sites seems too simplistic here to explain fluxes, mainly because our measurements covered two consecutive dry years that were part of an extremely dry period across Europe since 2015, which likely „is unprecedented in the past 2,110 years “ (Büntgen et al. 2021). Consequently, other factors such as substrate availability of N or oxygen content in the soil are also expected to influence N_2O fluxes.

Water table variations did not explain N_2O fluxes

Water table level and soil moisture are well known to strongly affect N_2O fluxes. In general, based on analyzing all data irrespective of site, N_2O fluxes were not significantly correlated with water table levels in this study ($p = 0.728$), showing that other factors must have been driving fluxes.

In general, all water table levels fluctuated considerably (Fig. 1d), which is known to trigger N_2O emissions (Flessa et al. 1997; Merbach et al. 2002; Couwenberg et al. 2008). In another study (Augustin and Chojnicki 2008), areas with a fluctuating water table showed small, but highly variable N_2O fluxes. Such unstable conditions may in oxic conditions cause rapid mineralization of peat newly formed in anoxic conditions. This suggests that N_2O fluxes result from a delicate interplay of production and reduction processes being influenced by a range of factors, with N_2O reduction being very important under these conditions.

Emissions were concentrated in hotspots and hot moments

Although the observed seasonal variation was in line with our second hypothesis, we expected it to be much stronger, based on other studies (Imer et al. 2013; Vybornova et al. 2019). In general, on

measurement days and the days before, there was only little precipitation, obviously influencing N_2O fluxes. The long-term average (period from 1981 to 2010) precipitation for Mecklenburg-Vorpommern was 619 mm (DWD 2018). For our study period, weather stations recorded the largest annual precipitation in PW (533.6 mm in 2018, 537.0 mm in 2019), whereas the coastal peatlands and PD only received around 450 mm of precipitation in 2018 and 2019, respectively (Jurasinski et al. 2020). For the alder forest, we recorded the lowest annual precipitation sums with 382.9 mm and 388.8 mm in 2018 and 2019, respectively (ibid.), clearly below the annual average. Both coastal fen sites with intermediate precipitation showed a climate-neutral behavior, with no or very limited N_2O production (Fig. 2). On all drained sites and the drought-influenced rewetted alder fen AW, significantly positive correlations between precipitation and cumulative fluxes were observed (Table 1), again hinting at fluctuating wetness triggering emissions. N_2O hot moments can appear shortly after an increase in soil moisture and in soil NO_3^- (Ruser et al. 2006), as e.g. observed in AW in August 2019 when it had rained after a long dry spell.

In general, however, the N_2O fluxes in our study varied considerably over the entire measurement period, so that a clear seasonal trend was not detectable. Correlations between N_2O fluxes and temperatures or water table levels might have been masked by the high spatial variability (Lohila et al. 2010) or other factors, like nutrient availability.

In line with our third hypothesis, the correlations of NH_4^+ and NO_3^- concentrations with N_2O fluxes were more frequent and slightly stronger than with water table level and temperature (Table 1). It is important to keep in mind that NH_4^+ and NO_3^- concentrations fluctuated strongly and are the result of production and consumption processes, including processes leading to N_2O production, and that mineral N is produced via local mineralization. Precipitation is not a source of mineral N in these sites. Thus, smaller concentrations of mineral N in the soil could either indicate small production or high consumption in relation to production. The higher enrichment of NO_3^- than NH_4^+ (Fig. 3) indicated consumption of NO_3^-—probably via denitrification pathways—to be more important than its production via mineralization and nitrification.

No measurement location displayed consistently larger N_2O emissions or mineral N concentrations over the whole measurement period. Instead, all locations showed strong fluctuations over time. This indicates a large spatial variability among the individual measurement locations (Imer et al. 2013; Landry and Rochefort 2012), despite their spatial proximity. Thus, there were small hotspots of N_2O emissions, which were, however, variably distributed over the sites, indicating hot moments. These hotspots could occur for example where more NH_4^+ or NO_3^- was available in the soil and continue until the substrate was consumed (Flessa and Beese 2000; Ruser et al. 2006), or where aeration changed in microsites. Such dynamic situations require that the interpretation of discontinuous measurements is done cautiously.

Pronounced N_2O reduction observed under various conditions

Our ^{15}N and ^{18}O analysis showed that N_2O reduction played an important role in our observations (Fig. 5c). In line with previous publications (Clough et al. 2005; Ostrom et al. 2007), it was detected on all isotope sampling days, under various conditions. Chapuis-Lardy et al. (2007) compared reports of N_2O uptake from various studies and registered distinctly larger values compared to our study. N_2O reduction to N_2 happens under conditions that favor complete denitrification and depends on several factors like soil moisture, temperature, pH and N availability (Clough et al. 2005). Higher water table levels typically lead to slower diffusion of oxygen into the peat and, thus, may create anaerobic conditions resulting in more complete denitrification with a reduction of N_2O to N_2 (Davidsson et al. 2002; Ambus and Zechmeister-Boltenstern 2007). Interestingly, this effect likely occurred on the drained sites of the coastal and percolation fens, where we found marginally significant positive correlations between water table level and N_2O reduction ($p = 0.043$ and $p = 0.098$, respectively), supporting our first hypothesis. This is also concurrent with the results of the last two days of ^{15}N and ^{18}Oisotope measurements, both recorded in winter with higher water table levels, favoring reduction of N_2O. Furthermore, less nitrogen was available in the soil at this time, again leading to smaller fluxes due to less production and stimulated N_2O reduction (Vybornova et al. 2019).

The lowest measured water table levels associated with N_2O reduction in this study ranged from −0.21 m (PW) to −0.91 m (CD) at most sites. AD showed N_2O reduction at even lower water table levels (still above 10% at a water table level of −2.58 m). This is especially interesting, as we assessed N_2O reduction based on gas fluxes at the surface. Thus, N_2O reduction in the upper soil might have taken place despite the low water table level, or N_2O reduction in deep layers might have been so strong that it was still measureable at the surface. As deep soil layers are usually water saturated, it is more likely that N_2O reduction took place in microsites in the upper soil. Whichever way it happened, our results show that N_2O reduction needs to be taken into account in these peatlands even under unsaturated conditions.

In addition, at all sites, negative fluxes were recorded, in line with atmospheric N_2O being absorbed and reduced to N_2 (Regina et al. 1996). In those cases where these were larger than the minimum detectable flux, this suggests that also N_2O uptake may happen at a range of conditions. So far, N_2O uptake is not yet well understood and further studies need to systematically investigate this process.

Estimating sources of N_2O in conditions with pronounced N_2O reduction

Measurements of ^{15}N and ^{18}O helped us better understand the sources of N_2O. However, the N_2O fluxes were usually around zero, also on the days used for isotope sampling (Fig. 5a). As a result, distributing these small N_2O fluxes among the different processes is prone to errors. So far, the effects of pronounced N_2O reduction on the interpretation of N_2O signatures have not been studied. Isotopic signatures of N_2O are often corrected before interpretation with a method developed by Keeling for ^{13}C of CO_2 (Keeling 1958). This so-called 'Keeling plot' method consists of a plot of measured isotopic composition against the reverse of the measured mole fraction of N_2O, with the intercept of the linear regression line (at quasi infinite N_2O production from the sources) interpreted as the isotopic composition of soil-derived N_2O (Pataki et al. 2003). However, this method requires an increase in N_2O mole fraction over measurement time (Wolf et al. 2015). With N_2O reduction, this prerequisite is not fulfilled.

In theory, as the influence of N_2O reduction on isotopic signatures is larger where a large proportion of the N_2O is reduced (i.e. its remaining mole fraction is small), this will cause an enrichment of remaining N_2O at small concentrations of N_2O, but not much difference to the original signature at large N_2O concentrations. Thus, the intercept of the above-mentioned 'Keeling plot' would theoretically still be the same as without N_2O reduction (although the slope would be different). In practice, however, the largest measured N_2O concentration on sites (or in incubations) with overall N_2O uptake would be equal to the ambient concentration, which is not infinitesimally large and thus far from the intercept with the y-axis in the Keeling plot. In this case, N_2O reduction would lead to a line with a positive slope and thus a smaller value for the intercept, i.e. the corrected isotopic composition of soil-derived N_2O. While this might lead to the correct value for the process producing N_2O, the influence of N_2O reduction would again be lost. Thus, in cases with N_2O reduction, a correction of isotopic signatures cannot be recommended. The interpretation of data with isotope maps is still feasible, as the influence of N_2O reduction on the signatures is explicitly considered by these methods.

Denitrification not always the main source of N_2O emissions

Isotope sampling for ^{15}N and ^{18}O took place every three months in this study. For this reason, the derived source estimation should not be overinterpreted, since every season was reconstructed based on the data derived during a single measurement day.

Denitrification is usually considered the main source of N_2O from peat soils (Pihlatie et al. 2004). However, in this study, the estimated COD varied between 21.7% and 76.6% over the measurement period, contradicting the fourth hypothesis of this process being the largest source (although still being important as a sink). Interestingly, the largest estimated COD occurred on all study sites in August 2019 (Fig. 5b), which was characterized by first rain after dry summer conditions with low water table levels, i.e. conditions not considered typical for denitrification. The method used for identifying sources of N_2O production by ^{15}N and ^{18}O does not allow for differentiating between denitrification and nitrifier denitrification (Verhoeven et al. 2019).Thus, this

emission peak might have also been caused by nitrifier denitrification (see also Fig. 4).

Nitrification and denitrification processes seem to have taken place simultaneously in all conditions observed, irrespective of water levels. Denitrification occurring under unsaturated conditions could have happened in remaining anaerobic microsites (Renault and Stengel 1994) or via aerobic denitrification (Robertson et al. 1995) or nitrifier denitrification (Wrage-Mönnig et al. 2018).

The measurement from May 2019 differed especially in values for site preferences from all other data (on average −8.66‰, see Supplementary Information). There have been reports of such negative site preferences likely caused by nitrifier denitrification (values of −13.6 to +1.9‰ according to Yu et al. (2020). This is in line with the ^{18}O signatures (Fig. 4), but not quite with the ^{15}N signatures, which were too enriched compared to the endmember values of nitrifier denitrification (data shown in the Supplementary Information). Since endmember values for ^{18}O (see Fig. 4) and ^{15}N have been corrected with average values measured over all sites and dates, values for single days might have deviated from this, potentially explaining discrepancies. Besides, site preferences from nitrifier denitrification were derived from two pure culture studies (Frame and Casciotti 2010; Sutka et al. 2006). Clearly, more data is required here to make progress regarding this pathway. Besides, plants have also been observed to produce N_2O as a by-product during the reduction of NO_3^- with a negative site preference: the C_4-plant *Miscanthussinensis* yielded N_2O with a site preference of −6.25‰ and rather enriched ^{18}O-signatures (Lenhart et al. 2019). The latter does not correspond to our results, as the ^{18}O-signatures ranged between 15 and 20‰ at that time. However, N_2O production by plants is a rather new topic and other (C_{3-}) plants might also yield different results.

Conclusion

In conclusion, our results suggest that a categorization into rewetted and drained sites does not necessarily offer a straightforward explanation of the variations in N_2O fluxes. Dry conditions in the upper peat layers of rewetted sites due to drought conditions may stimulate N_2O production more than further lowering the water table in already drained sites. Interestingly, fluxes of N_2O were around zero on all sites over most parts of the measurement period. N_2O reduction, derived by isotopic measurements of ^{15}N and ^{18}O, was positively correlated with water table levels, showing the importance of rewetting. However, the factors influencing N_2O reduction are not yet completely understood. Thus, more studies should be performed on fen sites, ideally combining continuous measurements with isotopic analyses to get more information on the drivers of N_2O fluxes under these conditions and causes of hotspots or hot moments. Both insignificant and negative N_2O fluxes need to be systematically investigated to make full advantage of the potential of N_2O reduction for mitigation.

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Availability of data and materials Data is available in the data portal of the Wetscapes project.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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3. Denitrification is not necessarily the main source of N_2O in rewetted fens

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ORIGINAL PAPER

Denitrification is not Necessarily the Main Source of N_2O from Rewetted Fens

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Abstract
Drained agricultural peatlands are being increasingly rewetted for global warming mitigation. This creates novel ecosystems, with unclear effects on nitrogen cycling. Therefore, we aim to understand the impact of rewetting on nitrous oxide (N_2O) production and its sources. Soil samples from pairs of sites differing in water regime (drained [D] and rewetted [W]) and peatland type (coastal fen [C], percolation fen [P] and alder forest [A]) in North-Eastern Germany were analyzed for microbial production pathways of N_2O using the dual-isotope method with four tracers ($H_2{}^{18}O$, $N^{18}O_3{}^-$, $^{15}NO_3{}^-$, $^{15}NH_4{}^+$) in a laboratory incubation experiment. Unexpectedly, the largest N_2O fluxes were found for rewetted sites. In four sites, denitrification dominated N_2O production (80—90%). Only CW and AD displayed almost equal contributions of N_2O from $NO_3{}^-$ and $NH_4{}^+$, showing also largest maximum contributions of nitrifier denitrification (44–48%). Nitrification contributed less than 8% in all soils. Less than 20% of N_2O was from nitrification-coupled denitrification. Soil samples with high initial water content, requiring drying prior to preincubation, displayed largest emissions, irrespective of peatland type or field water regime. Interestingly, if field conditions were dry and water was added for the preincubation, the contribution of nitrifiers to N_2O production was increased, in line with larger concentrations of $NO_3{}^-$. The results confirm the enhancing effect of drainage on N_2O fluxes. However, they also indicate a legacy effect of previous conditions on sources of N_2O. Overall, short-term changes in water content had strong effects on fluxes, but not sources of N_2O.

Keywords Nitrous oxide · ^{15}N · ^{18}O · Nitrification · Denitrification · Dual-isotope method

1 Introduction

Drainage of peatlands started a few hundred years ago for activities like agriculture, peat extraction and forestry (Joosten and Couwenberg 2001). Drainage generally leads to aerobic decomposition and thus, to greenhouse gas emissions (Canadell et al. 2007; Page et al. 2002; Wösten et al. 1997).

In Europe, peatlands account for about 5 to 6% of the land area, and more than 60% of them are drained (Drösler et al. 2008). In Germany, even 95% of peatlands are drained, causing 5% of Germany's total anthropogenic greenhouse gas emissions (46 million tons of carbon dioxide equivalents per year) (Hahn-Schöfl 2015). Of these peatland emissions, 80% originate from fens (Höper 2007): their larger nutrient contents compared to bogs made them preferred drainage targets (Timmermann et al. 2016).

Peat mineralization leads to the release of carbon dioxide and nitrous oxide (N_2O) (Gelbrecht et al. 2008). N_2O is a long-lived greenhouse gas with an average concentration of about 331 ppb in the atmosphere (Tian et al. 2020). In the stratosphere, its decomposition products are involved in ozone destruction (Crutzen 1991; Ravishankara et al. 2009).

There is a range of processes and pathways producing N_2O in soils (Butterbach-Bahl et al. 2013). They can take place simultaneously in different soil microsites, making them difficult to distinguish (Heil et al. 2015; Stein 2019; Wrage-Mönnig et al. 2018) and important to understand in order to develop N_2O mitigation strategies. In wet fens, denitrification (Fig. 1) is usually considered to be the main source of N_2O (Augustin et al. 2001; Lohila et al. 2010). However, especially under drained conditions, also nitrification (Fig. 1) can contribute to N_2O production (Martikainen et al. 1993; Regina

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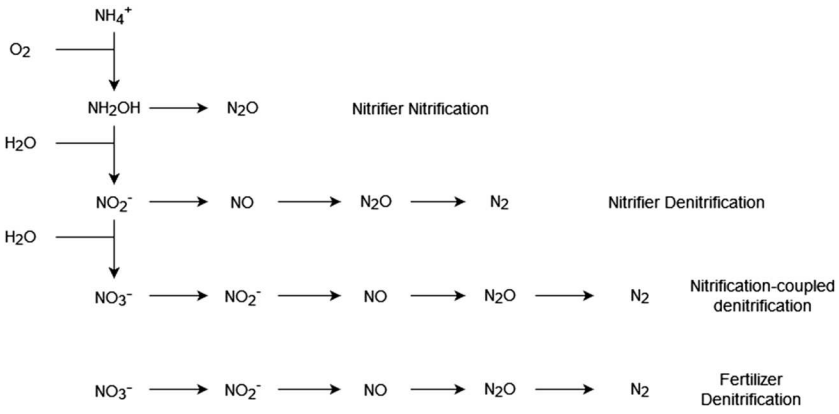

Fig. 1 Major pathways of N_2O production: nitrifier nitrification, nitrifier denitrification, fertilizer denitrification and nitrification-coupled denitrification. The difference between fertilizer denitrification and nitrification-coupled denitrification is the different source of the nitrate used from either external sources or nitrification

et al. 1996). Another important pathway is nitrifier denitrification, where NO_2^- is reduced to N_2O and potentially N_2 as in denitrification, but by autotrophic ammonia oxidizers (Kool et al. 2007; Wrage et al. 2004). Furthermore, there are various other pathways producing N_2O, like heterotrophic nitrification, co-denitrification or fungal denitrification. Various methods exist to distinguish among these sources, but none covers all processes and pathways.

So far, the effect of rewetting on overall N_2O emissions and on soil sources of N_2O is not well understood. Research suggests that rewetting causes an overall reduction in N_2O emissions (Jordan et al. 2016; Wilson et al. 2016). Thus, direct comparisons of drained and rewetted peatlands demonstrated that drained sites showed larger N_2O emissions (Davidsson et al. 2002; Vybornova et al. 2019). However, Gelbrecht et al. (2008) observed that while rewetting of drained fens to a water table level of 0.3 – 0.8 m above ground strongly decreased N_2O emissions, a fluctuating groundwater level (-0.3 m ± 0.3 m above ground level) led to their increase (Berendt et al. 2022). Studies systematically investigating sources of N_2O from (rewetted) fens under controlled conditions are missing.

Therefore, the aim of this study was to improve our understanding of the influence of fen rewetting on N_2O production and its sources under controlled laboratory conditions. We incubated soil of pairs of drained and rewetted sites of three different fen types, using the dual-isotope method according to Kool et al. (2011). With this method, it is possible to distinguish among nitrification, nitrifier denitrification and denitrification as sources of N_2O. We chose this method as we suspected that nitrifier denitrification might be important under the conditions encountered. We hypothesized that a) peat from rewetted sites would show smaller N_2O fluxes than from drained ones, b) that the average water table height in the field would be the main influencing factor for N_2O emissions as it determines both peat mineralization (and thus substrate availability) and microbial community composition, and c) that denitrification would be a larger source of N_2O on rewetted sites than on drained ones.

2 Material and Methods

2.1 Material

Soil (0 – 20 cm) was collected from the six study sites (pairs of drained (D) and rewetted (W) sites on a coastal fen (C), percolation fen (P) and alder forest (A)) of the WETSCAPES project (Jurasinski et al. 2020) and stored cool (8—10 °C) until the start of the experiment. One week after soil sampling – which was used for preliminary tests to determine the water content and water-holding capacity (WHC) – the preincubation started. For more information about the study sites and the soil properties, see Supplementary Material and Jurasinksi et al. (2020).

2.2 Methods

Incubation Experiment The WHC was determined for each soil according to Vengadaramana and Thairiyanathan (2012) using a funnel with filter paper (Whatman No. 1) instead of a perforated tin box.

After a two-day preincubation with 50 g soil (dry mass) in 750 ml Weck jars ($n = 5$) at room temperature (between 20 and 22 °C) and with a water content of 85% WHC, the main incubation was started by adding isotopic tracers dissolved in distilled water to reach 95% WHC and mixing the dissolved tracers into the soil with a glass rod. All treatments received equal amounts of mineral N in form of 7.14 mg of ammonium nitrate (NH_4NO_3). These conditions were chosen as a compromise between creating comparable conditions for all sites and not changing site conditions too much, while being able to add isotopic tracers. Incubations were carried out according to the dual-isotope method (Kool et al. 2011). In brief, the method used treatments (TR) with the following isotopic tracers: $H_2{}^{18}O$ (TR1), $N^{18}O_3^-$ (TR2), $^{15}NO_3^-$ (TR3) and $^{15}NH_4^+$ (TR4), with the ammonium and nitrate tracers enriched at 10 at% and H_2O enriched at 1 at%. In contrast to the initial method, the soil samples were not homogenized

or dried in order not to destroy the peat properties, unless the peat was too wet initially: soil that had a larger water content was dried to approximately 85% WHC at room temperature before the start of the pre-incubation. This was the case in three soils: AW, PW and PD. Especially the site AW was completely flooded at sampling. The additional water was included in the calculation of water content, resulting in AW having a calculated water content of 120% WHC. The jars were closed directly after tracer addition with air-tight lids containing a septum.

Gas Measurements At 3 h, 6 h and 24 h after tracer application, gas samples were taken with a 20 ml syringe and transferred into evacuated exetainer vials for analyses of N_2O concentration and its isotopic enrichments. The gas samples were analyzed with a TraceGaspreconcentrator (Elementar, Langenselbold, Germany) coupled to an isotope ratio mass spectrometer (IRMS, IsoPrime 100, Elementar, Langenselbold, Germany). For calibration, we used two working standards (0.9 and 1.8 ppm N_2O in synthetic air, $\delta^{15}N$ 0.15 and 0.02‰, $\delta^{18}O$ 40.66 and 40.32‰, respectively) calibrated against the standards of the laboratory of the Department of Environmental System Science, ETH Zürich (Verhoeven et al. 2019). At the time these experiments were carried out and samples were measured, no official reference materials existed for N_2O (Mohn et al. 2022) and also no N_2O with known enrichment in ^{15}N in the atom% range expected with tracer addition was available. We regularly measured isotopically enriched as well as natural abundance ^{15}N in solids (see below), finding the IRMS linear over this range. Therefore, we assumed linearity also for N_2O. The working standards were run at the start and end of each run and in duplicate every 20 samples. For calibration of the sample peak ratios, an N_2O reference gas (100% N_2O, Air Liquide, Germany) was run with every sample. Afterwards, the ratios were corrected for drift and span via the working standards. Stability (≤ 0.01‰) and linearity (≤ 0.02‰) of the IRMS were measured by injection of 10 N_2O reference gas pulses of similar or varying amount, respectively. Determination of external precision for ^{15}N in N_2O was done using at least four samples of our 1.8 ppm N_2O working standard per run and was on average 0.22‰.

Soil Extractions After 24 h, soil KCl extractions (150 ml 1 M KCl per 40 g soil, 1 h shaking, filtration over Whatman No. 1 filter paper) were carried out and extracts prepared for ^{15}N isotopic analyses of $NH_4^+$and NO_3^- using microdiffusion (Brooks et al. 1989).The samples were then measured on an elemental analyzer (vario PYRO cube, Elementar, Germany) coupled to the above IRMS. The external precision for ^{15}N in solid samples, determined as the standard deviation of 7 to 20 natural abundance samples of sulfanilamide during one run with samples intermixed was on average over the lifetime of the used source 0.16‰. As internal standards, we used sulfanilamide and wheat flour. These were calibrated against IAEA-600 and IAEA-NO-3 for ^{15}N, as well as IAEA-311 for samples enriched in ^{15}N. Isotopic values are reported in at% excess for the tracer study.

Calculations and Statistics N_2O fluxes were calculated based on linear regressions of the gas concentrations over time. Calculation of sources was done according to Kool et al. (2011). According to this method, N_2O produced from NH_4^+ is divided into nitrification-coupled denitrification (NCD), nitrifier nitrification (NN) and nitrifier denitrification (ND) using ^{15}N and ^{18}O as tracers. The frequently used ^{15}N tracer method was not able to differentiate between pathways related to nitrification (nitrifier nitrification, nitrification-coupled denitrification and nitrifier denitrification). Here, we also used ^{18}O as a tracer to quantify the O exchange in the different pathways. The method yields maximum and minimum values per pathway. In the results, we present the maximally possible amounts of these production pathways for reasons of clarity. A one-way ANOVA was used to check for differences in variables among sites ($\alpha \leq 0.05$). Data were tested for normality using the Shapiro–Wilk-Test and for equal variances with the Brown-Forsythe-Test. If the requirements for ANOVA were not fulfilled, the Kruskal–Wallis-Test was performed. The Tukey- or Holm-Sidák-Test were used as post-hoc tests. Statistical analyses were performed with SigmaPlot 13.0.

3 Results

3.1 NH_4^+ and NO_3^- Concentrations

In all soils, there was less NH_4^+ at the end of incubations than NO_3^- (Fig. 2). With the exception of the coastal wetland, drained sites contained significantly more NO_3^- and less NH_4^+ than the rewetted one ($p \leq 0.001$). CD also had the largest NH_4^+ concentration of all sites, 25.8 mg NH_4^+-N kg^{-1} ($p \leq 0.001$), and CW the significantly largest NO_3^- concentration (Fig. 2), 67.5 mg NO_3^--N kg^{-1} ($p \leq 0.001$).

As expected, in TR3 and TR4, considerable ^{15}N enrichments in mineral nitrogen were measured at the end of the incubations (data in the supplement). In TR3, enrichments in ^{15}N-NO_3^- ranged from 1.4 to 3.0 at%. There were no enrichments of NH_4^+ in this treatment. TR4 showed smaller enrichments of 0.6 – 1.4 at% for ^{15}N-NH_4^+. Furthermore, enrichments in ^{15}N-NO_3^- of between 0.5 and 1.1 at% were also detected in this treatment (data in the supplement).

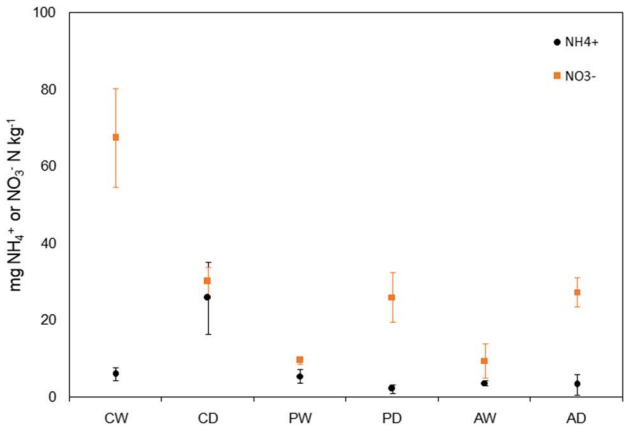

Fig. 2 NH_4^+ and NO_3^- concentrations (mg NH_4^+-/NO_3^--N kg^{-1}) in soil samples of the rewetted (CW) and drained (CD) coastal fen, rewetted (PW) and drained (PD) percolation fen and rewetted (AW) and drained (AD) alder forest at the end of the incubation experiment. Shown are means and standard deviations

3.2 Gas Fluxes

Incubations of soil from all sites showed N_2O production (Fig. 3). Generally, fluxes were larger in rewetted than in the respective drained sites ($p \leq 0.001$). The largest flux with 516.1 ± 390.8 µg N_2O-N kg^{-1} h^{-1} occurred in AW, followed by PW with 323.2 ± 205.6 µg N_2O-N kg^{-1} h^{-1} ($p = 0.099$). N_2O production from incubations of CW, CD and AD was small (17.8 ± 21.6 µg N_2O-N h^{-1} kg^{-1}; 0.8 ± 6.2 µg N_2O-N h^{-1} kg^{-1}; 6.5 ± 18.6 µg N_2O-N h^{-1} kg^{-1}, respectively), and significantly different from that of AW ($p \leq 0.001$). PD, however, showed a slightly larger N_2O flux than the other drained sites with 38.9 ± 53.2 µg N_2O-N h^{-1} kg^{-1}, significantly different from all sites except of CW ($p \leq 0.001 - p = 0.028$, $p = 0.534$ for CW).

3.3 N_2O Source Determination

All sites produced at least half of the N_2O from labelled NO_3^-, i.e. from denitrification (Fig. 4). Interestingly, rewetting produced no clear patterns concerning the contribution of the different sources to N_2O production. AD and CW produced the smallest amount of N_2O from NO_3^- (with $52.3 \pm 17.1\%$ and $56.1 \pm 14.0\%$, respectively) compared to the other sites ($p = 0.001 - 0.008$). The contribution of denitrification to N_2O production was almost identical for both sites of the percolation fen ($84.4 \pm 11.4\%$ in PW; $85.8 \pm 4.9\%$ in PD, respectively) and AW ($81.0 \pm 7.4\%$). These values were not significantly different ($p = 0.841$ between PW and PD; $p = 0.590$ between PW and AW, and $p = 0.269$ between PD and AW). In CD, N_2O was formed almost entirely from NO_3^- under the conditions tested, representing with $90.2 \pm 5.2\%$ the largest contribution and showing significant differences to CW and AD ($p = 0.001$), but not to the other sites ($p = 0.054 - 0.690$).

The largest maximum contributions of ND to the production of total N_2O were estimated for CW ($43.9 \pm 14.0\%$) and AD ($47.7 \pm 17.1\%$) ($p = 0.710$, Fig. 5). At the remaining four sites, the maximum amounts of ND were between 10 – 20% ($p = 0.054 - 0.841$), with significant differences to CW and AD ($p \leq 0.001$ to $p = 0.009$).

At CW, the maximal contribution of NCD was equal to that of ND and significantly larger than that of all other study sites ($p \leq 0.001$ to $p = 0.008$). The smallest maximal contributions of NCD were calculated for CD and AD ($4.4 \pm 7.6\%$, $7.6 \pm 10.8\%$, respectively), showing a significant difference between CD and PD ($p = 0.042$), with PW, PD and AW having intermediate values for the maximal contribution of NCD (Fig. 5).

NN did not contribute to N_2O production from CW, PW and PD under the conditions tested (Fig. 5). For the other sites, the maximally possible contributions from NN were

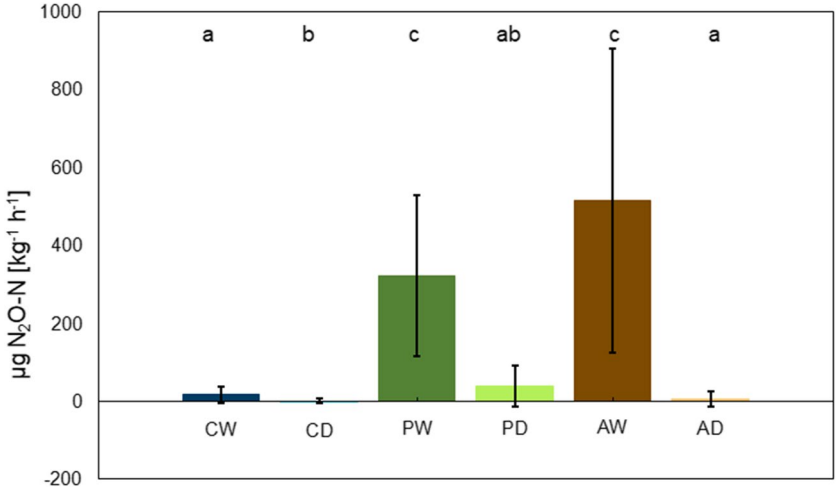

Fig. 3 N_2O-N fluxes (µg kg^{-1} h^{-1}) of incubated soil samples of the rewetted (CW) and drained (CD) coastal fen, the rewetted (PW) and drained (PD) percolation fen and the rewetted (AW) and drained (AD) alder forest. Shown are means and standard deviations

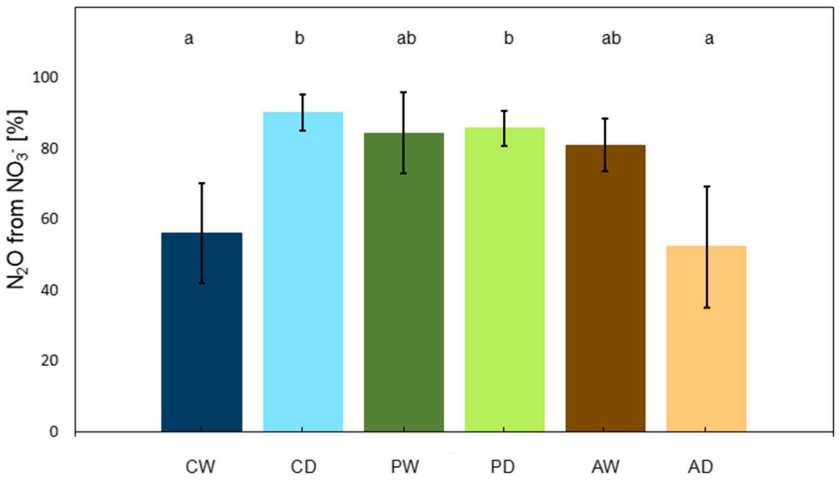

Fig. 4 N_2O produced from ^{15}N-$NO3^-$ (%) in soil incubations of the rewetted (CW) and drained (CD) coastal fen, the rewetted (PW) and drained (PD) percolation fen and the rewetted (AW) and drained (AD) alder forest. Shown are means and standard deviations

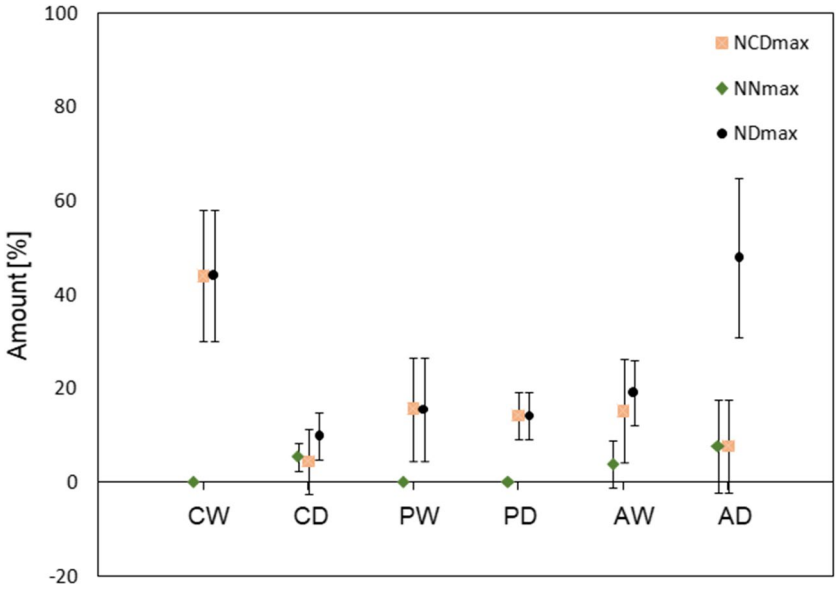

Fig. 5 Maximum N_2O production (%) from nitrification-coupled denitrification (NCDmax), nitrifier nitrification (NNmax) and nitrifier denitrification (NDmax) of the rewetted (CW) and drained (CD) coastal fen, the rewetted (PW) and drained (PD) percolation fen and the rewetted (AW) and drained (AD) alder forest. Shown are means and standard deviations. For further information on calculations, see text

also small ($5.4 \pm 3.6\%$, $3.8 \pm 5.6\%$, $7.6 \pm 10.8\%$, respectively), showing no significant differences among sites ($p = 0.503 - 0.841$).

4 Discussion

In contrast to the first hypothesis, N_2O fluxes were larger from the rewetted sites than from the respective drained ones (Fig. 3). This was remarkable, as considerably larger fluxes are normally expected from drained peatlands than from wet ones (Augustin et al. 1998). In this experiment, however, all soils were incubated at the same water content, making adaptations in water content necessary at the beginning of the incubation. When comparing the change in water content between field conditions at sampling and the start of the incubation, it is striking that the sites that had to be dried before the incubation all showed substantive N_2O emissions (Fig. 7a). This reinforces that drainage increases N_2O fluxes, even if some water was added again to start the incubation. The N_2O fluxes of the other sites, where hardly any drying was required or even a considerable amount of water had to be added, were almost negligible. This indicates that further wetting of the soils did not lead to larger N_2O fluxes, but drying of the soils just before the addition of water, i.e. quick reduction in water content, did. This is in line with studies showing increasing N_2O emissions with fluctuating water regimes (Gelbrecht et al. 2008; Jørgensen and Elberling 2012) and suggests that drying causes the onset of emissions, even lasting into concurrent wetter conditions. This is important for the management of rewetted sites, where fluctuating water regimes are more usual than in pristine fen peatlands (Kreyling et al. 2021).

When regarding the water table level of the sites previous to sampling (Fig. 6), it was evident that although rewetted sites usually had a higher water table level than drained ones, seasonal fluctuations were large, in line with other findings on fens (Kreyling et al. 2021). The largest fluctuations in the water table level were found on AD, where it dropped to more than -2.5 m in summer 2019 (Fig. 6), reflecting the drought conditions in that year. However, other sites also showed large variations in water table level among the seasons, fluctuating up to 1 m. Even on all rewetted sites, the water table level was more than 0.25 m below the surface in summer. Based on the findings of this current incubation study, such drying could cause increased fluxes of N_2O. In field measurements at those sites, larger N_2O emissions were measured particularly at PW and AW in summer 2018 (Berendt et al. 2022). At that time, the water table level for AW was more than 60 cm below surface, resulting in large N_2O emission during that season.

Despite the low water level in the field and rewetting of the soil before incubation, emissions from AD and CW (as well as CD, were no change in water content had to be carried out) were very small. This is remarkable, as many studies showed large emissions from drained sites (Augustin et al. 1998; Merbach et al. 2001). Nevertheless, there are also some studies that reported small fluxes from drained alder sites (Eickenscheidt et al. 2014). Based on the results seen here, short-term decreases in water content seem to be more important for N_2O emission events (Dinsmore et al. 2009; Jørgensen and Elberling 2012) than long-term site conditions, even if substantial overall changes in water content occur over time.

In order to incubate all soils under the same conditions, we used a moisture intermediate between all soils, meaning some soils had to be air-dried and others wetted for preincubation. As our results show, the soils that had to be dried the most showed the largest N_2O emissions. It is likely that the soils would have produced considerably smaller N_2O emissions without prior drying. This is a methodological effect, but it also shows the large impact of short-term drying of fens on N_2O production.

Here, we only concentrated on some production pathways of N_2O (denitrification, nitrifier nitrification, nitrifier denitrification and nitrification-coupled denitrification) based on the dual isotope method chosen. Nevertheless, there are many other pathways that can produce N_2O. So far, these are not captured by the present methods and efforts should be taken to find a method that differentiates all known major sources of N_2O, potentially in a combination of isotope approaches.

Denitrification was an important source of N_2O, but not in all cases more important in rewetted than in drained sites (Fig. 4). In contrast to our second hypothesis, the largest contribution of denitrification with more than 90% was found for the drained site CD. When comparing the change in water content before the start of the incubation with the amount of N_2O from denitrification (Fig. 7b), it was noticeable that the sites that had to be dried before incubation (AW, PW and PD) showed a larger amount of denitrification. Since these sites were also very moist before incubation, they were probably tending towards denitrification (Lohila et al. 2010). In contrast, soil samples of the sites CW and AD were drier than 85% WHC before the start of the preincubation. These two sites produced smaller amounts of N_2O from denitrification. Thus, the soils seem conservative in the main source of N_2O, despite short-term changes in conditions before the incubation. This is in line with results from an acidic fen experimentally dried or flooded, which did not show large reactions to experimental conditions in terms of N_2O production or denitrifier community structure (Palmer et al. 2016). Another recent study showed that the predominant N_2O production pathway of a (mineral) soil

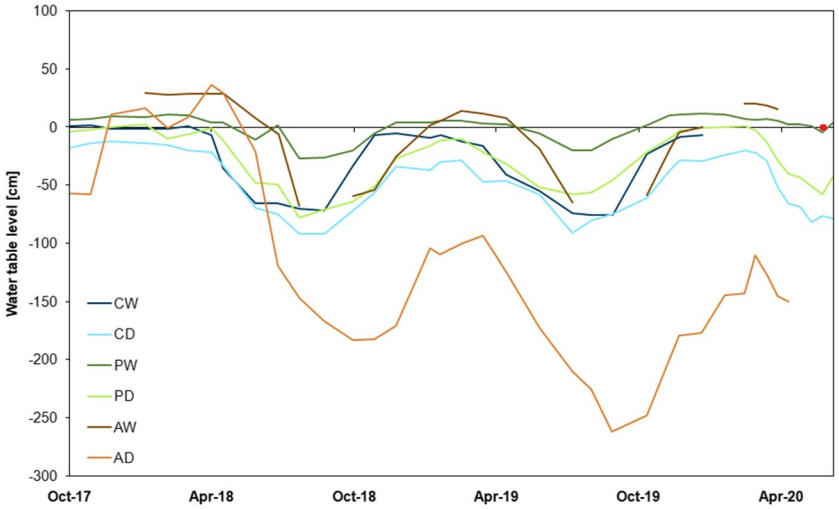

Fig. 6 Water table level of all six sites (rewetted (CW) and drained (CD) coastal fen, rewetted (PW) and drained (PD) percolation fen, rewetted (AW) and drained (AD) alder forest) from October 2017 to June 2020. The red dot marks the time of soil sampling for the incubation experiment. The interruptions in the shown water table levels were due to failures of the measuring instruments

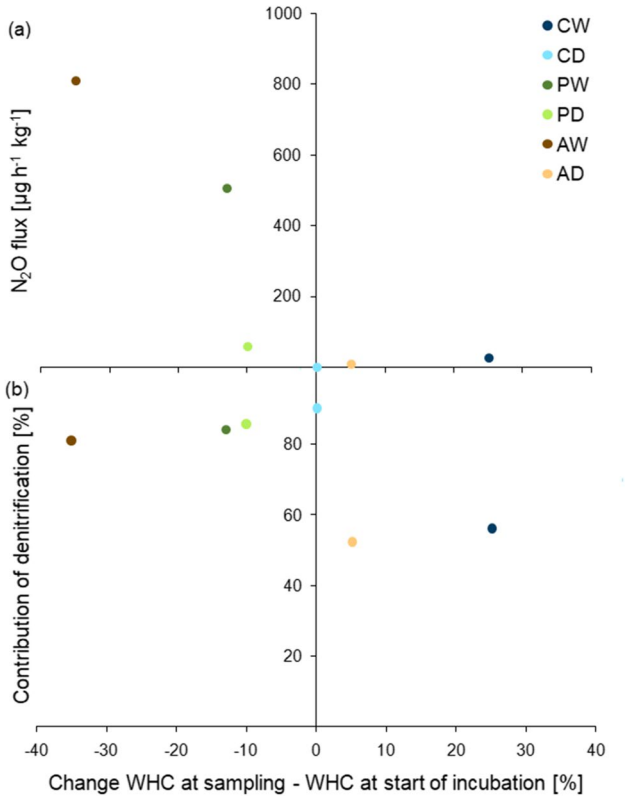

Fig. 7 Change of water contents between sampling time and start of the incubation experiment: shown are the changes in % water-holding capacity (WHC) against the N_2O flux (µg h^{-1} $kg.^{-1}$) (**a**) and changes in % WHC against the contribution of denitrification (%) (**b**)

determined the effect of biochar on N_2O production (Ji et al. 2020). Thus, a (molecular) fingerprint of the dominant N_2O production pathway(s) of a soil might help to better understand its behavior in changing conditions.

The sites CW and AD showed small NH_4^+ concentrations in relation to NO_3^-, with almost half of the N_2O produced from NH_4^+. The small NH_4^+ concentrations indicate that nitrification was fast here in relation to mineralization, and also to denitrification, as large NO_3^- concentrations as well as ^{15}N enrichments of NO_3^- in incubations with added $^{15}NH_4^+$ suggest. Since N_2O emissions were relatively small in CW and AD, either little N_2O was produced or the N_2O produced was largely reduced to N_2. For CW, N_2O produced from NH_4^+ could originate from either ND or NCD. In contrast, up to 50% of the N_2O produced in AD from NH_4^+ originated from ND. These results were very surprising since we expected that most of the N_2O in peatlands would be produced via denitrification at a WHC of 95%. Interestingly, there are other studies reporting a remarkable contribution of nitrification at 80% water-filled pore space (Pihlatie et al. 2004), but most of the studies showed that denitrification was the dominant process of N transformation in the soil under water-saturated conditions (Wolf 2000). Again, the predominant conditions and thus predominant microbial pathways might play a role here. Probably, the dry conditions in the field prior to incubations led to this large contribution of nitrification processes, even after over 24 h at wetter conditions. Even at water contents of 95% WHC, peat soils can still have dry pores, as pores can be very large, draining quickly, making peat a dual-porosity medium (Rezanezhad et al. 2016). Large contributions of nitrifiers to N_2O production in rewetted fens were also shown by Masta et al. (2022). In all soils studied here, either ND or NCD could explain N_2O production from NH_4^+, with negligible potential contributions of NN as these showed no or extremely small contributions (smaller than 10%) to the production of N_2O (Fig. 5). Thus, pure nitrification does not seem to play a large role for N_2O production in these soils.

5 Conclusion

Our results suggest that contrary to our hypothesis, a categorization into drained and rewetted fen sites cannot be used as an indicator for the microbial production pathways of N_2O: as largest contributions of denitrification to N_2O production were observed on a drained site. Short-term reductions in water content immediately prior to incubation resulted in largest N_2O emissions, not rewetting of soil that had been comparatively dry in the field for a longer time. Thus, such quick drainage appears to stimulate N_2O production more than lower long-term water table levels. Interestingly, all sites showed contributions to N_2O production from both nitrification and denitrification processes, with water addition to field-dry peat soils leading to large contributions of nitrification pathways to N_2O emissions.

Interestingly, although short-term changes in water content overruled longer-term conditions in the field in terms of N_2O fluxes, its sources were determined by longer term conditions and predominant microbial communities.

This is interesting for the management of rewetted peatlands: It could enable a fingerprint of microbial communities to help predict N_2O dynamics and develop an informed management of rewetted peatlands. For this, the stability of such communities over time needs to be investigated. Furthermore, the results underline that short-term changes in water content of rewetted peatlands need to be reduced to minimize N_2O emissions.

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Data Availability Data is available in the data portal of the Wetscapes project.

Code Availability Not applicable.

Declarations

Conflicts of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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4. Application methods of tracers for N_2O source determination lead to inhomogeneous distribution in field plots

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FULL ARTICLE

Application methods of tracers for N_2O source determination lead to inhomogeneous distribution in field plots

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Abstract

Source determination of N_2O has often been performed using stable isotope incubation experiments. In situ experiments with isotopic tracers are an important next step. However, the challenge is to distribute the tracers in the field as homogeneously as possible. To examine this, a bromide solution was applied as a stand-in tracer using either a watering can, a sprayer, or syringes to a relatively dry (25% gravimetric moisture content) or wet (30%) silt loam. After 1 h, samples were taken from three soil depths (0-10 cm), and analyzed for their water content and bromide concentration. The application with syringes was unsuccessful due to blocked cannulas. Therefore, further laboratory experiments were conducted with side-port cannulas. Despite a larger calculated gravimetric soil moisture difference with watering can application, more Br^- tracer was recovered in the sprayer treatment, probably due to faster transport of Br^- through macropore flow in the wetter conditions caused by the watering can treatment. The losses of Br^- (33% for the watering can, 28% for the sprayer treatment) are equivalent to potential losses of isotopic tracer solutions. For application of 60 at% $^{15}NH_4^+$, this resulted in theoretical enrichments of 44-53 at% in the upper 2.5 cm and 7-48 at% in 5-10 cm. As there was hardly any NO_3^- in the soil, extrapolations for $^{15}NO_3^-$ calculated enrichments were 57-59 at% in the upper 2.5 cm and 26-57 at% in 5-10 cm. Overall, no method, including the side-port cannulas, was able to achieve a homogeneous distribution of the tracer. Future search for optimal tracer application should therefore investigate methods that utilize capillary forces and avoid overhead pressure. We recommend working on rather dry soil when applying tracers, as tracer recovery was larger here. Furthermore, larger amounts of tracer lead to more uniform distributions. Further studies should also investigate the importance of plant surfaces.

KEYWORDS
^{15}N, bromide, nitrous oxide, spraying, stable isotope, syringe, watering can

1 | INTRODUCTION

Nitrous oxide (N_2O) is a long-lived greenhouse gas with an average concentration of 324 ppb in the atmosphere.[1] Since pre-industrial times, this concentration has risen by about 19%.[2] In the stratosphere, N_2O is decomposed to active components involved in stratospheric ozone destruction.[3–5] The main terrestrial sources of N_2O production are natural soils and agricultural land.[6] Nitrification and denitrification are the most important microbial pathways for the production of N_2O in soil.[7] However, there are many other microbial and chemical sources that are challenging to distinguish as they may take place simultaneously.[8–10]

To aid in the differentiation among these pathways, isotopic tracer methods have been developed.[8] Commonly used isotopic tracer methods include the triple labeling approach[11] and the dual-isotope method.[12] These methods are based on the addition of ^{15}N (in form of NH_4NO_3 in the triple labeling) or of ^{15}N and ^{18}O tracers (in the dual isotope method). Both assume a homogeneous distribution of tracers in the soil. So far, these methods have usually been carried out in laboratory incubation experiments, where isotopic tracer solutions can be mixed into the soil. Future improvements in our understanding of N transformations in undisturbed soils requires that such experiments can also be performed in the field.

Previously, studies have applied isotopic tracers to intact soils using a variety of methods. Ideally, ^{15}N tracer should be evenly distributed vertically and horizontally in the soil.[13] To achieve this, Wang et al[14] recommended a multi-injector consisting of 10 syringes with side-port cannulas, while Sgouridis et al[15] injected ^{15}N tracer with syringes fitted with normal cannulas (about 10 to 15 cm long). When labeling larger experimental plots in the field with ^{15}N tracer, it is also important that the labeling can be performed rapidly. Accordingly, some studies have applied ^{15}N tracer solutions using watering cans.[16] However, it is not clear what depth of soil becomes labeled using such a technique, or how homogeneously the label is distributed.

Ionic charge on the ^{15}N tracers may also cause bias in the ensuing distribution through the soil. For example, when using ammonium (NH_4^+) and nitrate (NO_3^-) as tracers, NH_4^+ may remain in the upper layers of the soil while NO_3^- is displaced with water and possibly leached down the profile. Such a biased distribution of the NH_4^+ and NO_3^- tracers could ultimately affect the calculation of N_2O sources.

To date, the effects of different tracer application methods on the distribution of ^{15}N tracers in field plots has not been studied. Therefore, we compared different methods for applying ^{15}N tracers to soil using a bromide (Br^-) solution as a stand-in tracer and measuring gravimetric soil moisture content and soil Br^- concentrations as proxies for initial isotopic tracer distributions. The Br^- ion is considered a conservative tracer because it occurs at relatively small concentrations in natural soils and no chemical alterations take place when Br^- comes into contact with soil or water.[17,18] Due to the negatively charged Br^- ion, this experiment simulated more the distribution of NO_3^-. If a homogeneous distribution is not achieved for this, it will certainly not be achieved for NH_4^+.

We hypothesized that there would be differences among the application treatments using watering can, sprayer, or syringes concerning the time needed for application as well as the homogeneity of application. We hypothesized a trade-off between time needed and homogeneity, with injection and watering can applications at the extremes. Furthermore, a higher run-off is possible with the watering can, but we hypothesized that the solution might reach deeper soil layers more quickly than with the sprayer due to the larger hydraulic head. Although we expected larger run-off, more dilution of the label and more macropore flow in wet than in dry soil, leading to less label recovery and more heterogeneity, we hypothesized that in general, the Br^- recovery would be linked to an increase in moisture content after application in both dry and wet soil. The observed Br^- tracer distribution was used to assess the theoretical distribution and enrichment of isotopic tracers.

2 | MATERIALS AND METHODS

This experiment was performed at the experimental station of Lincoln University (43° 38′ 54.35″ S, 172 ° 28′ 06.01″ E), New Zealand, in November 2018, on a Wakanui silt loam (Mottled Immature PallicSoil [New Zealand classification[19]]; AericEpiaquept [USDA]). The grassland site, comprising of *Lolium perenne* and *Trifolium repens*, had not been grazed for 10 years, with a history of mowing over this time. The NH_4^+ content in the soil was 7.82 mg kg^{-1} whereas the NO_3^- content was only 1.18 mg kg^{-1}. The following treatments (n = 4) were compared: application of Br^- solution (2.5 L, equivalent to 10 mm of precipitation) by either (a) watering can, (b) hand-held sprayer (hereafter called "sprayer"), or (c) injection with syringes. In addition, there were four controls without added tracer. The experimental design comprised a randomized complete block with 20 plots of 0.25 mš each (0.5 m × 0.5 m). Before applying the solution, the vegetation on all plots was cut to 10 cm by a lawn mower to minimize the interception of the solution by the plants.[16] The experiment was performed on dry soil with an average gravimetric soil moisture content before application of 25.0% ± 1.4% (Experiment 1). In order to analyze the effect of the initial soil moisture on tracer homogeneity, the experiment was repeated with the watering can and control treatments after a rain event that delivered 65.6 mm precipitation over several days and resulted in the soil having an average gravimetric soil moisture content of 30.4% ± 3.4% (Experiment 2). In addition to that, a third experiment was also performed with a "brilliant blue" tracer dye applied by watering can on to the wet soil[20] to visualize the penetration into the soil.

The application of Br^- solution (0.5 g Br^- L^{-1} as KBr) was calculated to achieve a soil Br^- concentration of ~100 μg Br^- g^{-1} dry soil (assuming a uniform distribution to 10 cm depth) and thus ensure detectability. For Experiment 1 and 2, Br^- tracer solution was first applied as uniformly as possible to the whole plot using either the watering can or sprayer methods. Then the application of the Br^- tracer via syringe was performed in Experiment 1 in a linear pattern of seven rows with six injections per row, with the total solution volume for the plot being

divided into 42 syringes of 60 mL each. The length of the cannula reached a depth of 3.8 cm and had an inner diameter of 0.8 mm (BD PrecisionGlide™ needle). This was shorter than used in a previous study:[15] since we took samples 1 h after Br^- tracer application, we wanted to apply the Br^- tracer as homogeneously as possible to the upper soil layer to reach comparability with the other application methods.

One hour after Br^- tracer application, 10 soil samples were randomly taken from Experiment 1 and 2 with a soil corer (inner diameter 2.5 cm by 10.0 cm deep) from the inner part of the plot. These soil cores were divided into three depths: 0–2.5 cm, 2.5–5.0 cm, and 5.0–10.0 cm, hereafter referred to as depths 1, 2, and 3, respectively.

For Experiment 3, "brilliant blue" tracer dye was mixed with water (6 g/L) and as in Experiments 1 and 2, a watering can was used to apply 2.5 L of solute onto a plot. One hour after dye application, the soil was excavated to a depth of 30 cm in 1 cm thin increments and the soil was visually observed and photographed.

To determine the soil gravimetric water content, 5 g of field moist soil was dried at 105°C for 24 h. For the determination of the soil Br^- concentration, 3 g of field moist soil was extracted with 20 mL distilled water: soil and water were shaken in a Falcon tube (60 min), then centrifuged (3300 rotations/min, 20 min) prior to filtering through a glass fiber syringe filter (pore size of 0.45 μm). The filtered extracts were analyzed for Br^- on an Ion-Chromatograph (Dionex ICS-2100, ThermoFisher Scientific). The detection limit for Br^- was 20 μg/L in the water extract (0.133 mg kg^{-1}).

The observed Br^- tracer distribution was used to determine the theoretical distribution and enrichment of isotopic tracers (^{15}N as either NH_4^+ or NO_3^-, and ^{18}O in H_2O). It was assumed that the isotopic tracer solutions would migrate through the soil in a similar manner to the Br^- tracer solution (this is probably an overestimation for NH_4^+). By calculating the amount of tracer solution that reached a given soil depth, based on variations in Br^- recovery, we calculated the isotopic distribution in the soil. We assumed the tracer solution volume was equivalent to the Br^- solution applied (2.5 L); with 0.912 g N tracer L^{-1} and an enrichment of 60 at% ^{15}N, equivalent to 40 kg N ha^{-1}.[16] By correcting for antecedent NH_4^+ and NO_3^- dilution already in the soil, with natural abundance values of 0.385 and 0.380 at%, respectively, the theoretical isotopic ^{15}N enrichments (at%) were calculated. The method was also used in an analogous way for calculating the potential ^{18}O enrichment after application of $H_2{}^{18}O$ with an original enrichment of 10 at%.

2.1 | Laboratory experiment

Since the syringes were directly clogged during the test and thus, an application of the tracer was not possible with this method in this field, application with cannulas was further tested in the laboratory in Germany. One intact soil block (loamy sand) of 0.3 m × 0.3 m (depth 0.25 m) was extracted from ungrazed grassland at the experimental station of the University of Rostock. In the laboratory, this block was further cut into four blocks of 0.15 m × 0.15 m. Four cannulas were constructed from 10.3 cm long stainless-steel tubes (outer diameter 3 mm, inner diameter 2 mm) closed and formed to a tip at one end. In each tube, eight holes were drilled with a longitudinal distance of 1.25 cm and a 90° turn between holes to optimize distribution (diameter of 2 mm for the top four holes and 1.5 mm for the four holes close to the tip).

All four cannulas were attached in a 7.5 cm square to the bottom of a sealed aluminum chamber (14.0 × 10.3 × 7.2 cm length × width × height), which served as a combined reservoir. To avoid blockage of the cannulas during insertion, compressed air was applied with an air gun attached to the top of the chamber. This might change structure and gas diffusivity of the soil. The chamber was aligned horizontally using a spirit level to ensure the same overhead for each cannula.

After insertion, 200 mL of stirred TiO_2-suspension (8.8 L/mš) was added to the chamber to trace infiltrated water by coloration.[21] One soil block with four infiltration points each was evaluated after 30, 60, 90, and 120 min, respectively, to check for a temporal effect of the infiltration. To this end, each soil block was cut along a line directly connecting two cannulas as well as half way in between these two cuts.

As the infiltration into the soil was not homogeneous, the flow characteristics through the infiltration system and the newly designed cannulas was further tested using individual 45 mL pots for each cannula. Seven tests were performed using three different materials inside the pots to provide increasing flow resistance without clogging of needles (empty pots < cotton wool < sand). The cotton wool (~2 g) was pushed into the pots by hand, the sand (dry, Ø 2 mm) was slightly compressed by tapping the pots on a table. Each material test was carried out with water and with TiO_2-suspension, except sand that was only tested with water. After applying 100 mL of solution, time for infiltration was measured and the amount of solution reaching each pot was measured gravimetrically. Each test was repeated three times for each material. Values of the outflow per pot were normalized by calculating their percentage shares of the total outflow volume of each repetition.

2.2 | Statistics

For the measured variables, means and SDs were calculated for each soil depth. Furthermore, coefficients of variation were calculated for the horizontal as well as for the vertical resolution of selected variables within the soil. ANOVA was used to check for treatment effects and for differences in variables with soil depth ($\alpha \leq .05$). Data were tested for normality using the Shapiro-Wilk test. If the requirements for ANOVA were not fulfilled, the Kruskal-Wallis test was performed to determine treatment effects. The Tukey, Holm-Sidák, and Dunn's test were used as post hoc tests. Statistical analyses were performed with SigmaPlot 13.0.

Concerning the flow characteristics, test means and SDs of the normalized outflow were calculated.

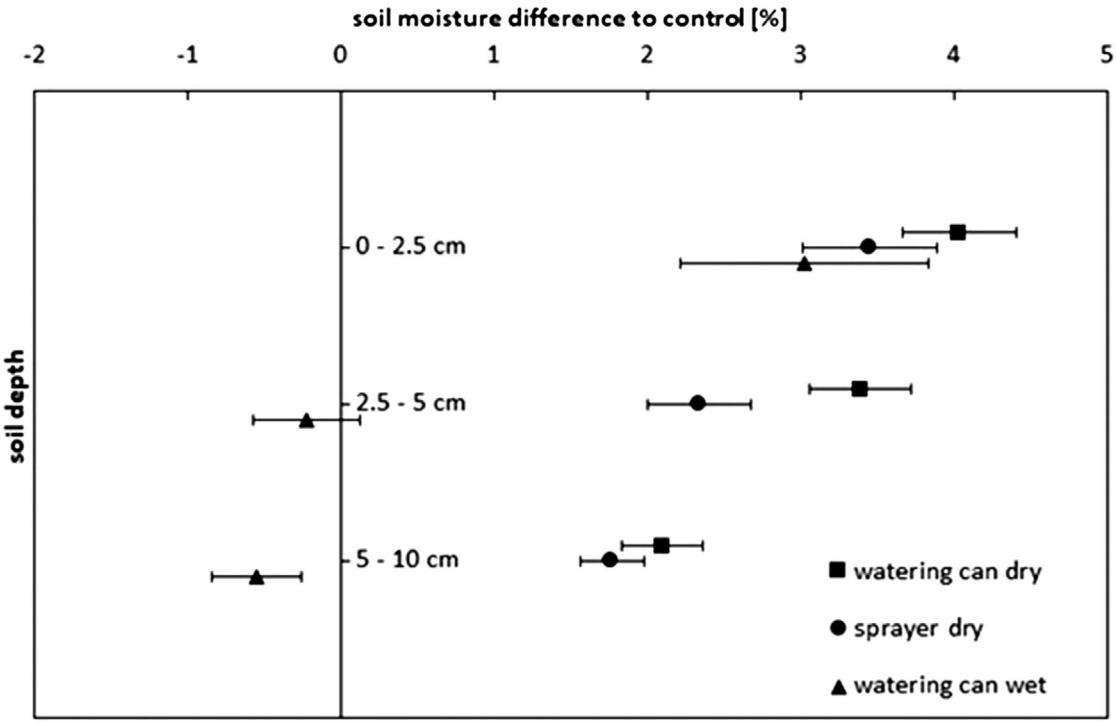

FIGURE 1 Comparison of the difference in gravimetric soil moisture content relative to the control treatment (%) over depth following Br^- solution application by watering can (square, Experiment 1), or sprayer (circle, Experiment 1) to dry soil, and by watering can to wet soil (triangle, Experiment 2). Data points are means (n = 4) ± standard errors

3 | RESULTS

3.1 | Field experiment

The watering can treatment had the shortest application time. Here, the total solution volume of 2.5 L was applied within 1 min (10 mm/min). Since the solution could be applied most quickly, this treatment also had the greatest potential for run-off. For the application by sprayer, 3-4 min per plot were required for solution application.

The treatment with the syringes was not successful under the given soil conditions, as the cannulas were blocked directly upon insertion into the soil. Even pre-drilling the holes for the syringes did not lead to any further progress. In order to apply the volume of two syringes to the soil, about 10 min were needed. As this was not feasible under field conditions for larger areas, the treatment was abandoned. An experiment with syringes was instead performed in the laboratory to determine how homogeneously the tracer solution is distributed by syringe application (see below). The following field results only refer to the watering can and sprayer treatments.

3.1.1 | Soil water content dynamics

In Experiment 1, delivering the Br^- tracer solution using either the watering can or sprayer treatments increased the soil moisture relative to the control (Figure 1). After applying the Br^- tracer solution to dry soil, there was a slight, but significantly, larger soil moisture content when using the watering can compared with the sprayer ($P = .047$), when averaged over all depths. When comparing the watering can and sprayer treatments at individual depths, there were no significant treatment effects on soil moisture at depths 1 and 3 ($P = .152$; $P = .344$, respectively). However, in depth 2, application by watering can led to significantly wetter soil than with the sprayer ($P = .030$). Variation in soil moisture decreased with depth. The largest gravimetric water content, 30-31%, was measured in depth 1: this held 3.5-4.0% more water than the control (Figure 1). This difference decreased at depth 2 (2.3-3.4%) and further at depth 3 (1.8-2.1%). If the complete tracer solution had been recovered, the increase in soil moisture in contrast to the control would be 5.0-5.5%, 3.6-4.3%, and 2.6-2.8% in depths 1 to 3, respectively.

The antecedent soil moisture had a significant effect ($P \leq .001$) on changes in gravimetric soil water content caused by tracer application with watering can (Figure 1). This was especially remarkable in soil depths 2 and 3: following solution application by watering can to the wet soil, there was no increase in soil water content compared to the control; increases only occurred when solution was applied to dry soil.

In Experiment 1, the increase in soil moisture content following solution application to dry soil in the watering can treatment accounted for a total of 2 ± 1.3 L (80% ± 52%; here and in the following: means ± standard deviation) of the applied solution, corresponding to a loss of ~0.5 L solution. On the plots where the sprayer was used, 1.6 ± 1.3 L (64 ± 52%) was accounted for, equivalent to a doubling of the loss

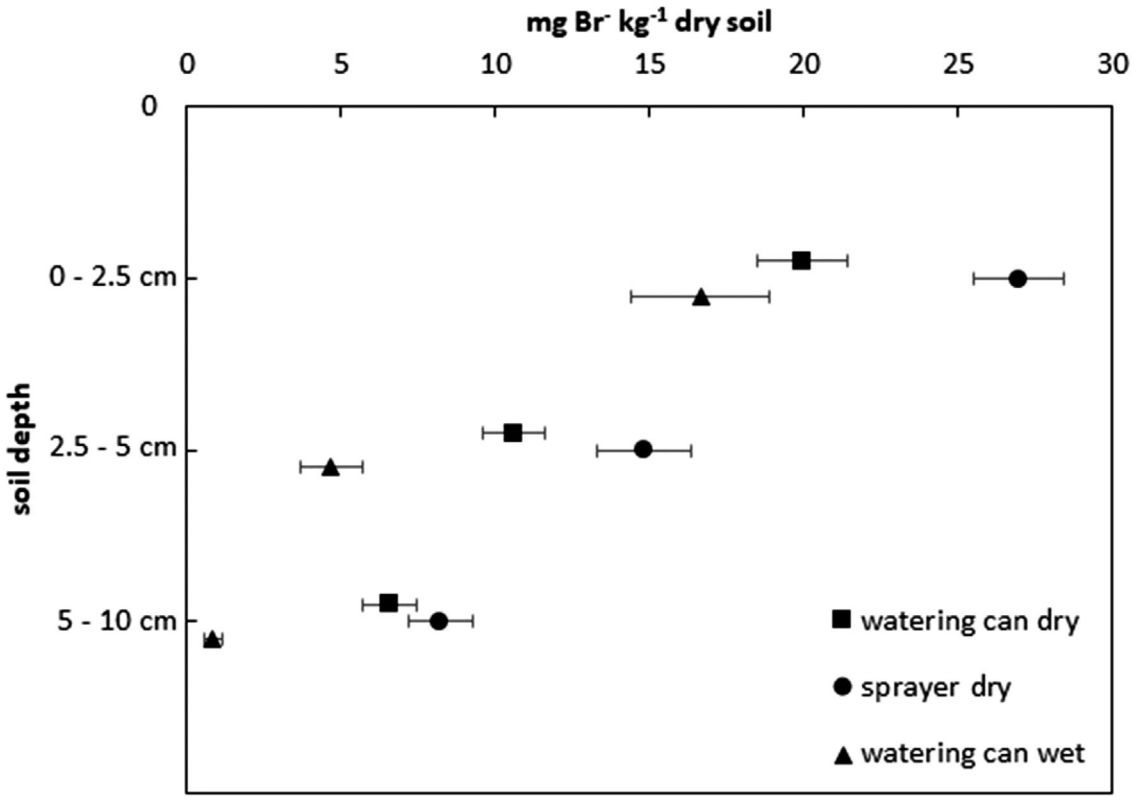

FIGURE 2 Comparison of Br^- concentration relative to control treatments (mg kg^{-1}) over depth after tracer application by watering can (square) or sprayer (circle) to dry soil (Experiment 1) and by watering can (triangle) to wet soil (Experiment 2). Shown are means (n = 4) ± standard errors

compared to the watering can application. In Experiment 2, based on the increase in soil moisture, applying the solution to the wet soil resulted in only 0.324 ± 1.9 L (13 ± 76%) of the solution being recovered, with the largest recovery in the top soil layer (0.309 L).

3.1.2 | Bromide concentration

In the control treatment, soil Br^- concentrations averaged 0.941 ± 0.081 mg Br^- kg^{-1}. Elevated soil Br^- concentrations were found in all depths and treatments where Br^- was applied (Figure 2). When applied to dry soil, there was a significant difference ($P = .016$) between watering can and sprayer applications with, on average over all depths, 12 and 17 mg Br^- kg^{-1}, respectively. When comparing the effect of the application treatments at individual depths, soil Br^- concentrations were only different at depths 1 and 2 ($P = .001$; $P = .017$, respectively). The largest differences between applications were observed in depth 1: the sprayer application led to a Br^- concentration of 27 mg Br^- kg^{-1}, whereas the watering can treatment had a concentration of 20 mg Br^- kg^{-1} (Figure 2). At soil depth 2, the concentrations had declined by ca. 50% and ranged from 11 mg kg^{-1} (watering can) to 15 mg Br^- kg^{-1} (sprayer). In soil depths 1 and 2, both treatments had relatively large SDs, with those in the sprayer treatment larger than from the watering can (up to ± 6 mg Br^- kg^{-1}). In soil depth 3, there was no significant difference between the watering can and sprayer treatments, which averaged 7 mg Br^- kg^{-1} ($P = .226$).

Over all depths, application of Br^- solution by watering can to wet soil (Experiment 2) led to significantly smaller Br^- concentrations than application to dry soil ($P \leq .001$, Figure 2). The difference was not significant at depth 1 ($P = .058$). Here, the SD of Br^- concentrations in the wet soil was larger than at deeper depths. Also, the mean concentration decreased with depth, with no significant difference between the control and watering can application at depth 3 after Br^- application to wet soil ($P = .066$, 1.0 mg Br^- kg^{-1}).

Overall, more Br^- was recovered in the plots where the solution was applied with the sprayer than with the watering can to dry soil. In Experiment 1, for the watering can treatment, 830 ± 330 mg of the 1.25 g Br^- applied (67 ± 26%) was recovered, while 1085 ± 391 mg of Br^- (87 ± 31%) was recovered in the plots with sprayer application. In Experiment 2, 490 ± 709 mg of Br^- (39 ± 57%) was recovered in the wet soil. Compared to the results of soil moisture, it was obvious that the watering can treatment in dry soil had the highest recovery of the applied water, but a lower Br^- recovery than the sprayer treatment.

The coefficients of variation for the Br^- concentrations with depth (vertical resolution) were consistently smaller than those for soil moisture (Table 1). The treatments applied to dry soil resulted in very similar coefficients of variation. The application by watering can to wet soil generated the largest coefficients of variation (Table 1). In the

TABLE 1 Coefficients of variation for the different treatments pooled over all depths (n = 12) (watering can on dry and wet soil, sprayer on dry soil) in vertical resolution

Treatment	Soil Moisture	Br^- Concentration
Watering can on dry soil	0.479	0.403
Sprayer on dry soil	0.402	0.355
Watering can on wet soil	2.546	0.599

horizontal direction, the coefficients of variation were larger for soil moisture than for soil Br^- concentrations, with an exception being the deepest soil layer following applications to dry soil (data not shown). On wet soil, the horizontal coefficients of variation were overall very large. All horizontal variation coefficients increased in the Br^- method with depth, while they tended to decrease for soil moisture (data not shown).

To determine homogeneity of distribution of tracer solution, we took 10 samples per soil depth in this experiment. Figure 3 shows the development of the coefficients of variation with the number of samples taken. The coefficients of variation of the watering can treatment on wet soil and the sprayer treatment on dry soil varied more with a smaller number of samples than those of the watering can treatment on dry soil. The largest coefficients of variation were found in the wet soil, whereas the smallest were observed in the watering can treatment on dry soil. From six to seven soil samples onward (per 0.25 mš), the coefficients of variation remained stable.

3.1.3 | Experiment with blue dye

In Experiment 3, the blue dye provided clear visual evidence of run-off, with dye found outside of the plot. The depth profiles clearly demonstrated that the tracer dye was not uniformly distributed (Figure 4), even though most of the tracer could be found in the upper 10 cm. In some places, the tracer could be observed at a depth >20 cm, especially in combination with earthworm burrows and roots.

3.1.4 | Theoretical isotopic tracer enrichments

The calculated enrichments of $^{15}NH_4^+$ generally showed a slight decrease with increasing soil depth (Figure 5A). In the upper 2.5 cm, the $^{15}NH_4^+$ tracer enrichment was diluted to a median value of 51 at% in the sprayer treatment, with values ranging from 44 to 53 at%. For all soil depths, the values above the median showed rather little variation, whereas those below varied over a range of 20 at%. In the upper 2.5 cm, variation was mostly found in the lowest quartile, whereas in 5-10 cm, the variation was mainly in the quartile below the median.

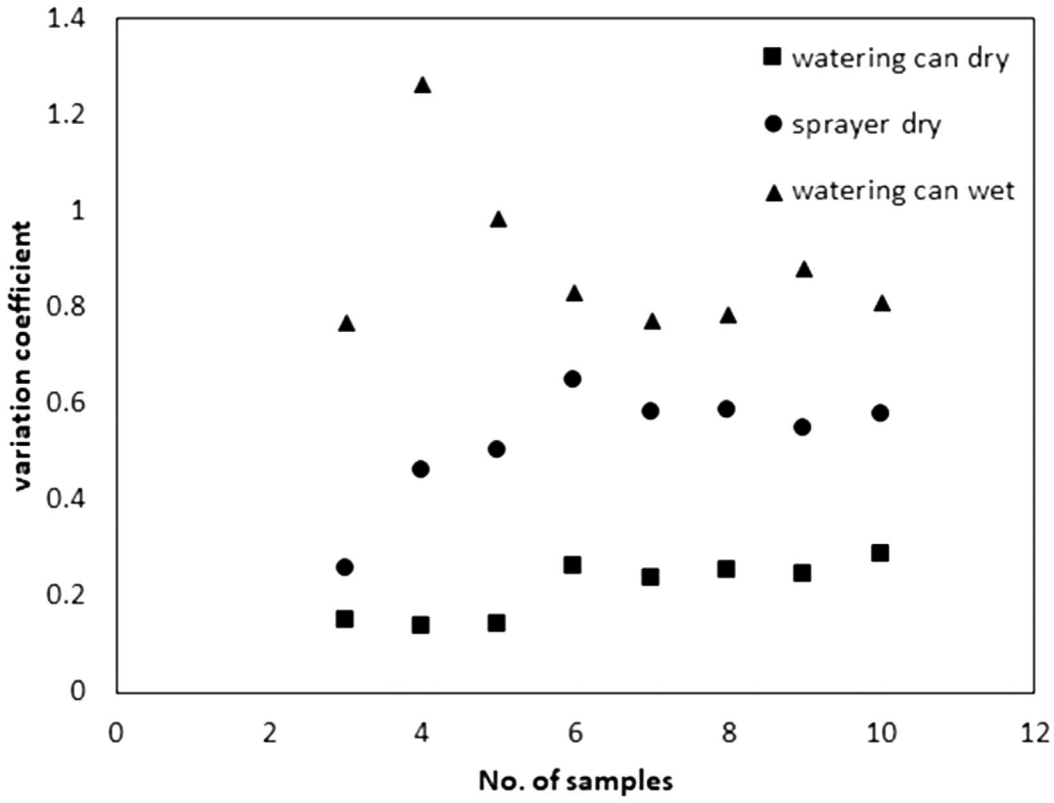

FIGURE 3 Variation coefficients depending on the number of soil samples. Shown are three different treatments (watering can and sprayer on dry soil, watering can on wet soil)

FIGURE 4 Photo of a cross section of a typical soil depth profile (depth = 30 cm) showing penetration of "brilliant blue" dye in wet soil

In the deepest soil depth, both treatments showed a median potential enrichment of 34 at%. Overall, the potential enrichments with the sprayer treatments were slightly but not significantly larger than with the watering can ($P = .097$).

The calculated potential enrichments for $^{15}NO_3^-$ showed a very similar pattern (Figure 5B), but the data were less dispersed (median range 56-59 at%). The theoretical dilution of the $^{15}NO_3^-$ tracer was not as strong as with $^{15}NH_4^+$, as the antecedent NO_3^- concentration was very small. The median enrichment was at 58 at% in the upper 2.5 cm and even the lowest soil depth reached potential enrichments of more than 50 at%, with large variations. Again, the distribution of data was negatively skewed at all three depths and no significant differences were found ($P = .699$). The calculated theoretical enrichments of NO_3^- in the soil did not differ between tracer application methods.

In the third theoretical scenario, with $H_2{}^{18}O$, the calculated potential enrichment also decreased over soil depth (Figure 6). Due to the antecedent soil moisture content, the dilution was larger than for $^{15}NO_3^-$ and $^{15}NH_4^+$, being, on average, a 1/9th dilution. The largest calculated ^{18}O enrichment was just 2.2 at%, and the smallest only 0.2 at%. As for the ^{15}N enrichments, the potential calculated ^{18}O enrichments with the sprayer treatment were slightly larger than with the watering can, however, the different application methods did not show a significant difference ($P = .232$).

3.2 | Laboratory experiment with multi-hole cannulas

Only seconds after starting infiltration, substantial amounts of TiO_2-suspension were flowing out of macropores from the sides and bottom of the soil blocks. The soil profiles matched this observation: Only very small or no visible coloration by TiO_2 in the area close to the cannulas was recognizable (Figure 7). Concurrently, well-colored hotspots were identified along earthworm holes and decaying roots.

The test of the flow characteristics showed large variations in outflow volume of individual cannulas (Figure 8), especially when testing with water in empty pots (0.3-46% share of total volume per cannula). Variations in outflow volume per cannula resembled the variations among all cannulas. There was no consistent effect of cannula, water versus TiO_2 or material in the pots on the amount of outflow per cannula. The duration of infiltration was similar for empty pots and those filled with cotton wool (47-67 s). With sand in the pots, the first repetition with water took 76 s and fully infiltrated the volume of the 45 mL pots. In the second and third repetition, water infiltrated in the top of the pots and about one-third of the water flowed to the surface and spilled over the pots' edges.

4 | DISCUSSION

In this study, we compared the practicality of and resulting homogeneity after applying a tracer solution to a pasture soil, in situ, using either a sprayer, watering can or syringe. Under the soil conditions at our test site, the cannulas of the used syringes were blocked immediately, even in pre-drilled holes. Of course, pre-drilling should be avoided as the solution would otherwise follow this macropore. Nevertheless, there are other soil conditions where syringe application is feasible. As also the tests with side-port cannulas in another soil and the very standardized laboratory setups showed very large variation among cannulas and preferential flow though macropores into deeper soil layers, we also expect a heterogeneous distribution of water and any solved tracers. The only benefit over the other application methods might be less runoff. Basically, to avoid preferential flow, it seems that the hydraulic head has to be minimized. Additionally, it has to be verified if flushing the soil with compressed air might change structure and gas diffusion of the soil.

As the outflow volume of the multi-hole cannulas strongly fluctuated among cannulas but also per cannula among repetitions, this was

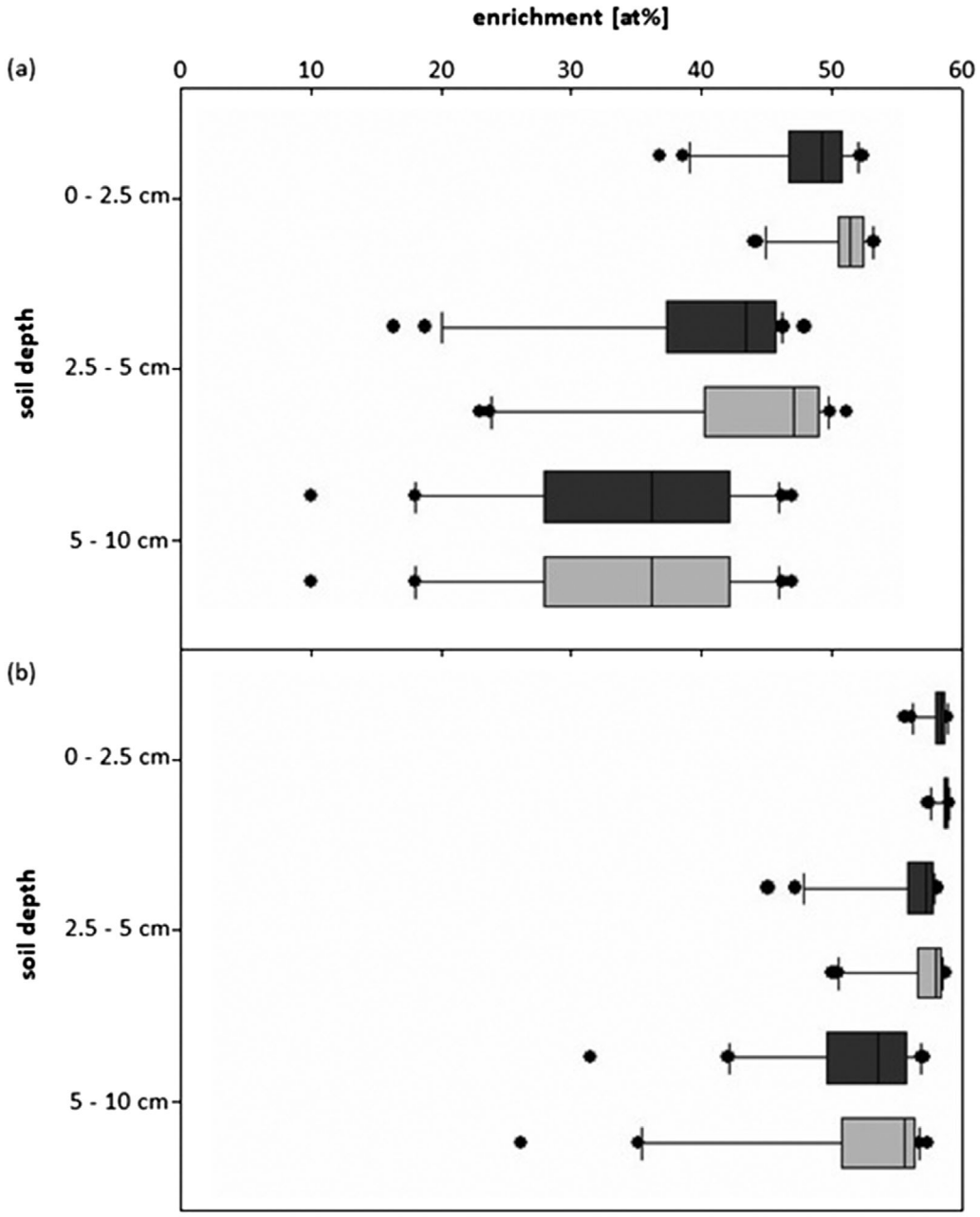

FIGURE 5 Resulting theoretical enrichments (at%) of $^{15}NH_4^+$ (a) and $^{15}NO_3^-$ (b) for the watering can (dark grey) and the sprayer (light grey) treatments on dry soil for three soil depths, calculated based on the distribution of Br^- tracer solution assuming an application of 60 at%

probably not caused by design or the chamber not being perfectly horizontal, but by reasons concerning the flow characteristics of the cannulas themselves. An assumption is that small bubbles inside the cannulas cause a strong resistance due to surface tension in a small diameter tube. Although equipping each cannula with its own reservoir might somewhat reduce heterogeneities, blocking of cannulas or sections thereof by air bubbles would still be possible. Therefore, the concept of multi-hole soil cannulas does not appear very promising. Using single or dual-hole cannulas with their own reservoir and used in several steps in multiple depths may avoid problems from blocked sections, but the biggest problem caused by preferential flow through macropores will likely persist. Slowing the flow through the cannulas might mitigate the flow through macropores and lead to a better distribution. We therefore suggest an application method that is avoiding overhead pressure and is utilizing the capillary forces of the soil to achieve a homogeneous distribution of dissolved substances (tracers).

In line with the first hypothesis, there were differences among the application treatments using watering can, sprayer, or syringes concerning the time needed for application as well as the homogeneity of application. The watering can allowed the fastest application, requiring 1 min per plot, whereas the sprayer application required 3-4 min. The difference in these application times would be significant if the

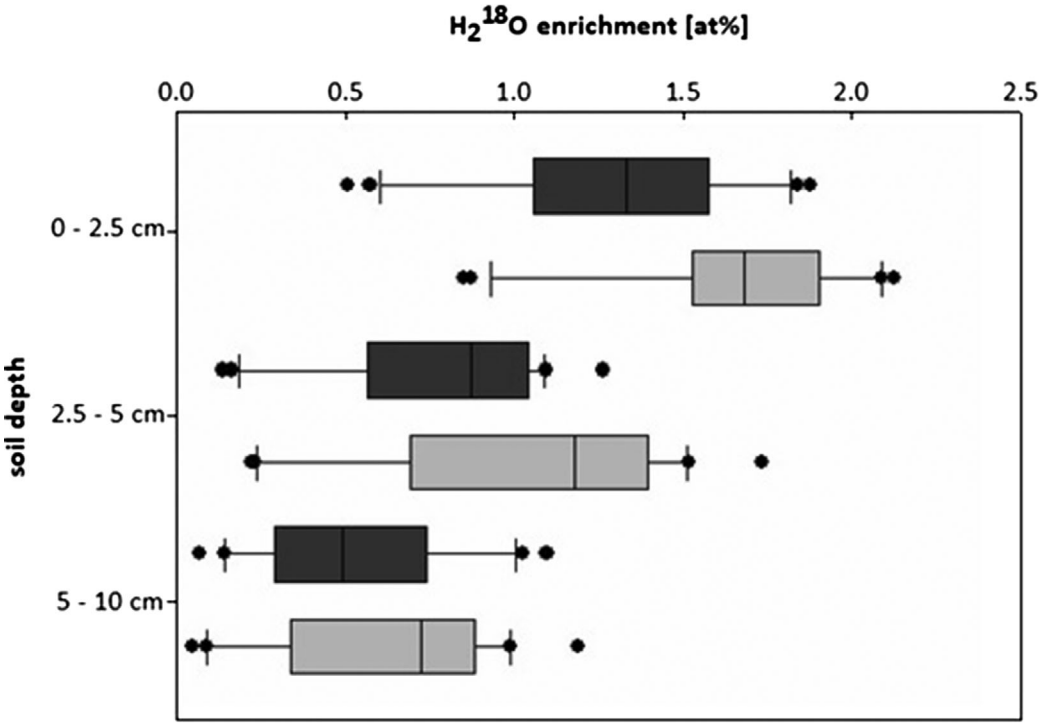

FIGURE 6 Resulting theoretical enrichments of $H_2{}^{18}O$ (at%) for the watering can (dark grey) and the sprayer (light grey) treatments on dry soil for three soil depths, calculated based on the distribution of Br^- tracer solution assuming an application of 10 at%

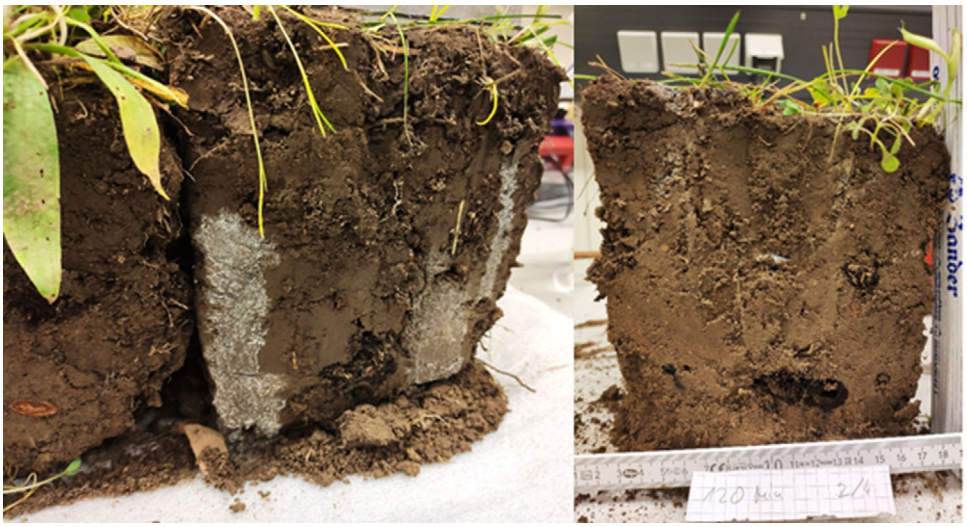

FIGURE 7 Left: Intact soil block (15 cm × 15 cm) after infiltration of TiO_2-suspension with three white colored areas marking points of extensive outflow of TiO_2-suspension from the sides of the block. Right: Cut though the infiltration points 2 and 4 showing only minor white coloration in the areas of the cannulas and one intensely colored hot spot (center of the soil block) that was identified as earthworm hole

first soil samples were required to be taken immediately after application of solutions and if resources for sampling were limited. Large standard errors for mean Br^- concentrations (Figures 1 and 2) and horizontal variation coefficients (data not shown), as well as decreasing tracer concentrations with increasing depths (Figure 1) demonstrate that the Br^- tracer was not homogeneously distributed. The vertical gradients will change over time due to diffusion (we took samples one hour after application) and leaching following precipitation events. As the used soil was under permanent grassland, the formation of macropores is favored,[22] increasing the potential for preferential flow.[23] This influences both the distribution of the tracer upon application and the changes in distribution after precipitation events.

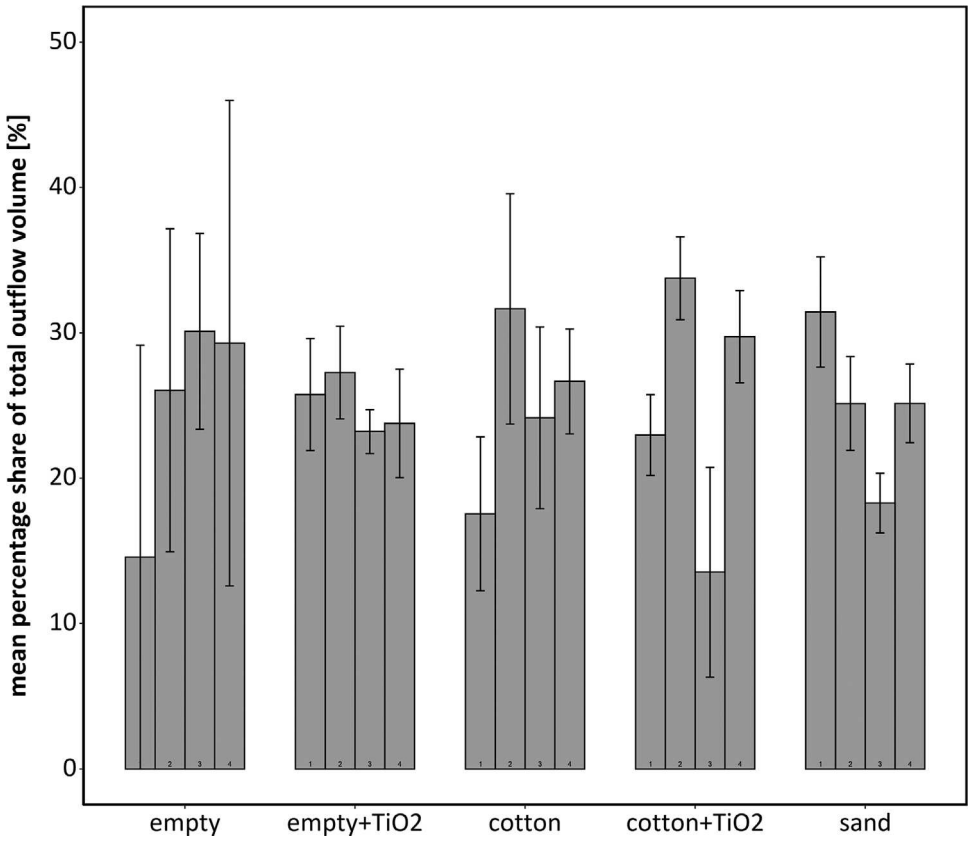

FIGURE 8 Mean percentage share of individual cannulas (1-4) of the total outflow volume of the respective repetition (n = 3) ± standard errors

Although we took great care when applying tracer solution, some horizontal inhomogeneity might have been caused by the manual application. To avoid this, tracers might be applied mechanically, for example with a sprinkler system with a constant flow. This would enable a more uniform application of the solution and could contribute to minimizing errors. However, this would also not prevent preferential flow of solution, for example, along roots or earthworm burrows (Figure 4).

Contrary to our hypothesis, there was no significant correlation between the measured Br^- and the observed change in moisture caused by the treatment ($P = .879$). There were, however, clearly significant correlations between gravimetric moisture content and measured Br^- concentration ($P \leq .001$). The poorer correlation between relative change in soil moisture between treated and control plots and soil Br^- concentrations could have been due to heterogeneities in soil moisture distribution, causing initial differences between measured control and treated plots.

The results show that in general, the distribution of the Br^- solution was more homogeneous when applied by sprayer than by watering can. The coefficients of variation were smaller than those of the watering can treatment, both overall and in depths 1 and 2, and recovery of tracer was larger. Blue dye application also clearly showed that run-off to outside the plot could occur when using the watering can. We assume that run-off was larger with the watering can treatment than the sprayer and therefore, more Br^- was deposited in the soil inside the plot with the sprayer treatment. In the watering can treatment, ~67% of the Br^- was recovered versus 82% in the sprayer treatment. Losses might have occurred due to either run-off or penetration to deeper layers. The faster application in the watering can treatment will have resulted in a larger hydraulic head, potentially making it possible for the solution to reach deeper soil layers more quickly. Elrick and Parkin[24] suggested that a larger hydraulic head would lead to a larger flow rate into the soil. This could cause greater macropore flow and consequently more leaching of solution, increasing the tracer solution loss. In the dye experiment, the tracer was found below 20 cm in combination with earthworm borrows and roots. We did not find significant differences in Br^- concentration or soil moisture changes between sprayer and watering can application in the deepest soil layer of the dry soil studied here (Figures 1 and 2). Therefore, the potentially larger hydraulic head caused by the watering can application apparently did not lead to deeper soil penetration, but potentially more run-off. Another factor potentially affecting infiltration of tracer solution is the presence of plants. The vegetation at our test site consisted mainly of grasses, which can intercept tracer solution, especially at lower precipitation intensity[25] as in the sprayer treatment. For further studies, vegetation should be considered, as leaf surfaces, for example, intercept more solution and thus reduce infiltration into a soil, whereas surfaces with less vegetation would also show less interception and therefore, a larger soil infiltration.

TABLE 2 Sensitivity analysis of inhomogeneity of isotopic tracer application: Shown are standard errors (S. E.) depending on (a) variations in the enrichment of isotopic tracer and (b) variations of the amount of tracer applied

(a) Depending on enrichment (0.912 g/L applied)					
	60 at%	70 at%	80 at%	90 at%	100 at%
S. E.	0.559	0.652	0.745	0.838	0.931
(b) Depending on the tracer amount (60 at% applied)					
	0.912 g/L	1.2 g/L	1.5 g/L	1.7 g/L	2 g/L
S. E.	0.559	0.467	0.397	0.361	0.318

As hypothesized, the wetter the soil, the less Br^- was recovered. The upper soil depth showed a distinct change in soil moisture and consequently an increase in Br^- concentrations. Since hardly any tracer solution infiltrated the deeper soil depths 2 and 3 when applied to the wetter soil, the Br^- concentration here was also clearly smaller. Timlin et al[26] suggested that in dry soil, the solution moves with large pressure gradients into the smaller pores. These smaller pores drain more slowly than larger pores and therefore, the tracer remains longer and vertical transport is reduced. Also Heathman et al[27] showed that in soils with dry aggregates, the downward movement of Br^- tracer was delayed.

Given that the Br^- recoveries were larger and coefficients of variation were significantly smaller in the dry soil, it is clear that isotopic tracer should be applied to dry soil if possible, and that a spray application is better suited. However, in order to investigate denitrification, experiments are commonly performed on soils with larger water contents. In this case, the results indicate that extra water should be added with the tracer in order to generate denitrifying conditions, rather than adding tracer to a wetted soil. To assist in interpretation of the results, the volume of soil affected by tracer additions must also be identified using Br^- or dye.

The coefficient of variation of measured variables (soil water, soil Br^- concentration) varied with sample number (Figure 3). It became stable when at least six or seven soil cores were taken from an area of 0.25 mš, reflecting the inhomogeneity of the soil. An understanding of the potential variation due to sample numbers, as attained here, should also be a requirement for a field study.

Concerning theoretical isotopic enrichments, the SDs for $^{15}NO_3^-$ were much smaller than those for $^{15}NH_4^+$ since considerably less NO_3^- than NH_4^+ was initially present in this soil, leading to less dilution. Thus, the homogeneity of tracer distribution depends – in addition to the application technique – also on soil parameters like the water content or the antecedent NH_4^+ and NO_3^- concentrations.[28] Although about 50% of the soil volume would have a ^{15}N enrichment similar to that aimed at, the remaining 50% would have a reduced enrichment, especially where soil stocks dilute the tracer. Variations in enrichments of mineral N will lead to equivalent variations in the enrichment of N_2O and in the following also in the calculated processes responsible for N_2O emissions. Of course, ^{18}O-H_2O can be expected to be more diluted due to water already present in the soil. On average, it was calculated to reach only 10% of the added enrichment. However, the heterogeneity was calculated to be much less than for ^{15}N tracers. Importantly, the variation in calculated potential enrichments cannot be decreased by increasing the enrichment of the used tracer, but by adding more tracer to the soil (Table 2).

In summary, this study shows that there are clear differences in homogeneity of tracer application and time needed among methods. Neither application method, watering can, sprayer nor injection led to a homogeneous distribution of tracer due mainly to macropore flow and dilution as well as needle clogging by clay or air bubbles for injection. Spraying led to slightly better results than the other methods. However, so far, the tested methods do not provide a sound basis for differentiating soil processes leading to N_2O production.

To improve homogeneity, we suggest to reduce the hydraulic head during application, but to use larger volumes of isotopic tracers. Applying tracers to dry soils should be preferred. Possibility of injection needs to be tested in the given soil. Run-off, or overland flow, and leaching to deeper soil layers needs to be assessed using dyes and Br^- tracer. Then, isotopic values might be corrected for heterogeneity of tracer application.

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5. General Discussion

N_2O emissions from drained and rewetted fens are influenced by complex interactions between hydrological conditions, microbial processes and nutrient availability. Understanding the pathways of N_2O production in these ecosystems is crucial for effective peatland management and greenhouse gas mitigation strategies.

5.1 N_2O fluxes from drained and rewetted fens

To record N_2O emission and uptake events, measurements were carried out over three years on pairs of drained and rewetted fens. According to our first hypothesis, we expected larger N_2O emissions from drained sites compared to the rewetted ones. In Berendt et al. (2022), we show that the N_2O fluxes were small with large spatial and temporal variations on all sites. Also Nielsen et al. (2023) found small N_2O fluxes close to zero on their studied fens. In Berendt and Wrage-Mönnig (2023), we expected that peat from rewetted sites would show smaller N_2O fluxes than from drained sites. Contrary to this hypothesis and other studies (e.g. Augustin et al., 1998), N_2O fluxes in our study were larger from rewetted sites. Besides, very dry years prevailed over the measurement period (Jurasinski et al., 2020), making the division into drained and rewetted sites too simple, as PD, for example, had a more shallow water table level than some rewetted sites. Overall, all water table levels fluctuated strongly over the entire measurement period and thus probably influenced N_2O fluxes (Flessa et al., 1997; Merbach et al., 2002; Couwenberg et al., 2008), but there was no correlation found between N_2O fluxes and water table level in our study. Consequently, there have to be other important factors that influence N_2O emissions and uptake events.

Other studies reported large cumulative fluxes from agricultural sites (Flessa et al., 2002; Yao et al., 2009), which we did not observe at our sites. In Berendt et al. (2022) we show that the cumulative fluxes of the sites were extremely small and often below the minimum detectable flux. Thus, our results were more comparable to findings from natural or rewetted peatlands (see Nykanen et al. (1995), Huth et al. (2012)), despite the dry conditions. However, it is important to note that the measurements were performed only every two weeks, leading to a high probability of missed hot moments.

According to our second hypothesis, we expected the seasons to influence N_2O emissions. We observed a trend towards this, but we expected it to be more pronounced. The N_2O fluxes fluctuated strongly over the entire measurement period. Positive significant correlations between precipitation and N_2O fluxes suggested that precipitation had a stronger influence on N_2O fluxes than water table levels. Precipitation could lead to hot moments, which can occur shortly after an increase in soil moisture (Ruser et al., 2006).

The availability of nitrogen compounds also plays a crucial role. It has been hypothesized that the concentration of NH_4^+ and NO_3^- as substrates for N_2O production has a significant effect on N_2O fluxes (Berendt et al., 2022). Here, we found slightly stronger correlations than with water table level or temperature. It is important to note that NH_4^+ and NO_3^- concentrations fluctuate strongly with production and consumption processes in the soil. If little N can be found in the soil, it is a hint for either a small production or a high consumption in relation to production processes. In addition, nutrient availability (here in form of NH_4^+ and NO_3^-) could lead to hot spots of N_2O emissions, as there could be locations where more N was available (due to inhomogeneous distribution in the soil), resulting in larger N_2O emissions (Flessa and Beese, 2000; Ruser et al., 2006). Nevertheless, it is important to keep in mind that there was a high spatial variability within and among sites.

5.2 N_2O pathways

In Berendt et al. (2022), a natural abundance approach of Lewicka-Szczebak et al. (2017) was used for determining N_2O production pathways and potentially N_2O reduction. Several noteworthy observations were made. First, N_2O reduction played an important role as it was detected in all samples. N_2O reduction to N_2 takes place under conditions that favor complete denitrification and depends on various factors like soil moisture, pH, temperature and N availability (Clough et al., 2005). N_2O reduction was also observed during low water table levels, but it was highest in winter, when water table levels were high (Vybornova et al., 2019). As N_2O reduction also occurred at low water table levels, this could be explained either by a reduction in the upper soil (despite low water table level and aerobic conditions) or in the lower soil, whereby the N_2O reduction here would have to be so intense that it was still measurable at the surface, where most N_2O production would be expected. In addition,

we observed negative N_2O fluxes on all sites, indicating uptake of atmospheric N_2O and further reduction to N_2 in the soil (Regina et al., 1996).

In addition, in Berendt et al. (2022), we observed that under all environmental conditions, nitrification and denitrification contributed to N_2O production. This was also supported by results of Berendt and Wrage-Mönnig (2023), where we studied soil samples from the sites in the laboratory and analyzed the different microbial production pathways of N_2O. We found in both experiments that both nitrification and denitrification processes contributed to the production of N_2O at every site. Thus, both processes proceeded simultaneously in the soil (Wrage-Mönnig et al., 2018), despite high water contents. Furthermore, the contribution of these processes in the field seems to have not been affected by soil moisture or seasons. At this point, we have to emphasize again that the measurements were performed once every three months due to logistics and efforts and thus represent a snapshot of the conditions on the sites. Consequently, it is difficult to extrapolate one single measurement day to a whole season.

Furthermore, we expected that denitrification is a larger source on rewetted sites than on drained sites. We observed that the contributions of denitrification varied strongly (between 22 – 77%) over seasons in Berendt et al. (2022). The largest contribution of denitrification was observed in summer (August) despite a low water table level, which does not reflect the typical or optimal conditions under which denitrification occurs. Our results in Berendt and Wrage-Mönnig (2023) showed that denitrification was an important source on our sites, but not at every site. Due to the originally high water content of the soil samples of AW and PW in the field, these sites also displayed a large amount of denitrification processes. Also other studies (e. g. Lohila et al. (2010)) reported that moist soils tend towards denitrification. CW and AD, on the other hand, were distinctly drier in the field, resulting in a smaller amount of denitrification processes (approx. 50%). This leads to the conclusion that the dominance of N_2O producing pathways did not appear to be very sensitive to short-term changes in water content (Palmer et al., 2016), despite changes in overall N_2O production. In addition, these two sites also showed small concentrations of labelled $^{15}NH_4^+$, which indicates rapid conversion due to nitrification processes. This was supported by the presence of large NO_3^- concentrations.

In Berendt and Wrage-Mönnig (2023), the dual isotope method was used for determination of microbial production pathways of N_2O in the soil. In order to enable an incubation of the soils at the same water content, some of the soil samples had to be dried first after sampling, as the different sites showed different water contents. It was noticeable that the soils that had to be dried for the incubation (especially AW and PW) showed larger N_2O emissions. The samples from the sites that did not have to be dried before the start of the incubation showed almost negligible N_2O fluxes. Other studies also showed larger N_2O emissions at fluctuating water levels (Gelbrecht et al., 2008; Jørgensen and Elberling, 2012). It can therefore be assumed that the soils would probably have shown smaller N_2O fluxes without prior drying (or 'simulated draining') as N_2O emissions from drier soils/ soils with a lower water table level in the field measurements were almost negligible from our sites (Berendt et al., 2022).

Overall, we can summarize that permanently drier conditions in the field seemed to play a greater role for sources of N_2O than short-term changes. In general, the largest source of N_2O production on all our sites was denitrification, although it was not the single source. The largest nitrification pathways were nitrifier denitrification or nitrification-coupled denitrification, whereas nitrifier nitrification seemed to be negligible (smaller than 10%) on our sites.

5.3 Tracer application in the field

Berendt et al. (2020) investigated the feasibility of performing isotopic tracer incubation experiments directly in the field. Various application methods (watering can, sprayer and syringes) were used to achieve the most favorable distribution of the tracer (in our study Br^-) in a heterogeneous undisturbed soil. It was found that syringes or their cannula became clogged directly when inserted into the soil, even if we had pre-drilled small holes for them. Nevertheless, this application method may be useful for other soils with less fine particles than the soil we tested. The first hypothesis that a difference is achieved both in time required and in homogeneity of distribution between the individual application methods was supported: there were large temporal differences in the application of the tracers, as the watering can was the quickest possibility to apply the tracers to the field, whereas application by sprayer and especially by syringes took significantly longer. In addition, it was

found that Br^- concentrations decreased with increasing soil depth even in the top soil with all methods and consequently, no homogeneous distribution in the field could be achieved.

Clay et al. (2004) and J. D. Jabro et al. (1991) observed that macropores (created by worm burrows or roots, for example) can cause preferential flow. We also noticed the same effect in our experiment with blue dye solution, where the colored tracer was better distributed along the macropores than in the remaining soil. An additional rain event could also lead to a change in distribution due to leaching. Furthermore, it is likely that the manual application also led to an inhomogeneous distribution in the soil. We would therefore suggest that tracers should be applied automatically, for example by some kind of a sprinkler system, to enable a more uniform application.

In Berendt et al. (2020), no correlation was found between Br^- and the water content of the soil. Br^- was applied more homogeneously overall by the sprayer, whereas the watering can apparently led to more surface run-off, as a total of 82% of the Br^- could be recovered during application with the sprayer, but only 67% with the watering can. It was assumed that the loss can be explained either by surface run-off or as a result of the tracer penetrating into deeper layers than investigated in the study. This was supported by the observation that the tracer was applied more quickly using the watering can and therefore, a higher hydraulic head was present, penetrating the deeper layers more rapidly. This was also confirmed by the experiment with the blue dye, in which the tracer could also be determined underneath the soil depth that was analyzed.

The vegetation on the studied site can cause a further problem. The vegetation in this study consisted mainly of grasses. It is conceivable that vegetation could lead to a further loss of the tracer, as it can remain on it in small droplets, especially if there is a lower precipitation intensity, such as with the sprayer. Especially if there is a high density of vegetation (e. g. many leaves), it is recommended that the vegetation should be mowed before the start of the experiment in order to reduce tracer loss. This should also be considered in further studies.

Berendt et al. (2020) found that the wetter the soil, the less Br^- was recovered. This leads to the conclusion that the tracer should be applied rather to dry than to moist soils and preferably not immediately after a rain event. It was also observed that the soil was highly inhomogeneous as it was characterized by different grain sizes and pores (due to worm

burrows or roots). As a result, the number of samples should be increased in order to achieve the best and most reliable results.

Overall, the sprayer provided the best results of the methods tested in the study of Berendt et al. (2020). Nevertheless, none of the methods could provide an ideal basis for tracer application in the field. In order to improve homogeneity, the hydraulic head should be reduced, a higher concentration of the tracer should be used and the soil should be as dry as possible as boundary conditions for the experiment.

5.4 Conclusion and outlook

Rewetting of peatlands has gained significant attention as a strategy to mitigate greenhouse gas emissions, particularly CO_2 and CH_4. However, the effects of peatland rewetting on N_2O emissions are less well understood. We observed that N_2O was not a particular issue under our conditions, as there seemed to be a strong reduction to N_2 in our studied sites. As stated in Berendt et al. (2022), N_2O emissions on our sites were consistently bouncing around 0 and thus, almost negligible. Consequently, these results were significantly smaller than described in other studies on peatland sites. As Flessa et al. (1997) and Couwenberg et al. (2008) already found, fluctuating water table levels and in particular a sharp drop and following rise of water table levels lead to large N_2O emissions. We also observed this at our sites, where the water table level fluctuated up to 300 cm over the entire measurement period. This was also supported by our results from Berendt and Wrage-Mönnig (2023): the largest N_2O emissions were measured at samples that we had to dry before the start of the incubation experiment.

As a result, it seems essential to establish a good water management on peatland sites in order to reduce or even prevent severe water table level fluctuations as far as possible. However, under the current conditions caused by climate change, this is very difficult to achieve. As temperatures keep rising, the water table levels in peatlands are dropping continuously, resulting in increasingly dry conditions in peatlands (Breeuwer et al., 2009). In addition, extreme weather events such as heavy rainfall or storm tides occur more frequently, complicating a good water management in peatlands. These aspects should be considered when rewetting peatland sites. A well-managed and controlled rewetting

strategy can influence the mitigation of N_2O emissions by reducing rapid water table level fluctuations.

In addition to a controlled water management, other factors may also support mitigation. A proper nitrogen management is also required: managing nitrogen inputs through controlled fertilization and minimizing nitrogen deposition can also help mitigate N_2O emissions from peatlands. Balanced nitrogen inputs can prevent excessive availability of nitrogen compounds that drive N_2O production. The studied sites were all non-fertilized. Even the coastal fen, which is used as pasture, did not receive any additional excrement. In the incubation experiment, all soils received additional nitrogen. Even the addition of nitrogen to our soils did not appear to have had a major impact on N_2O fluxes, as these were almost negligible. Therefore, the addition of fertilizer does not seem to play a major role on our sites. Nevertheless, it is known that fertilization can lead to increased N_2O production. Hence, this should definitely be considered in terms of climate change mitigation on other sites.

Despite the findings from this thesis, there are still many factors that are not yet completely understood and therefore need to be focused on in the future. First, long-term measurements are needed to capture the temporal variability of N_2O emissions following peatland rewetting. Seasonal and interannual variations can provide insights into the factors influencing N_2O production and fluxes over different hydrological and climatic conditions. Second, upscaling findings from plot-level studies to ecosystem scales is essential for accurately estimating the overall impact of rewetted peatlands on N_2O emissions. For this purpose, lots of measurements and studies should be performed at various different sites in order to include the great variability of sites. Third, the methods for determining microbial production pathways should be further developed. So far, the dual isotope method only covers denitrification, nitrifier nitrification, nitrifier denitrification and nitrification-coupled denitrification. However, there are numerous other N_2O production pathways that cannot be detected by the above-mentioned method. Furthermore, more efforts should be invested in the analysis of N_2O reduction in managed peatlands in order to improve the quantification of the contribution of reduction and potentially its use to further reduce N_2O emissions. Finally, modeling approaches would definitely be helpful: Integrating process-based models with empirical data can improve our understanding of N_2O dynamics in peatlands. Modeling

can help to predict the effects of different management scenarios and inform decision-making.

If all these aspects are considered and further research is invested, our understanding of N_2O fluxes and production pathways in peatlands can be further improved. As a result, rewetting strategies and the management of peatlands can be adapted and optimized to the specific needs and requirements of this ecosystem.

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Konferenzen, Workshops und Auslandsaufenthalte

08/2017	AGGF 2017 Posterbeitrag: *„WETSCAPES: Stoffumsetzungsprozesse an Moor- und Küstenstandorten als Grundlage für Landnutzung, Klimawirkung und Gewässerschutz“*
10/2017	Advanced Measurements and Analyses of Greenhouse Gas fluxes from Soils
06/2018	Academic Writing in Natural Sciences
09/2018	Peat under Water Workshop
11/2018 – 12/2018	Forschungsaufenthalt in Lincoln, Neuseeland
06/2019	8th International Symposium on Non-CO_2 Greenhouse Gases Posterbeitrag: *“N_2O emissions in drained and rewetted peatlands”*
09/2019	Wetscapes Conference Posterbeitrag: *“Nitrous Oxide Emissions of rewetted, agriculturally used temperate peatlands”*
10/2019	What can we learn from stable N_2O isotope data? Posterbeitrag: “*Sources of N_2O production in drained and temperate peatlands and paludiculture determined by the dual-isotope method*”

Besondere Kenntnisse

Sprachen:	Deutsch: Muttersprache Englisch: sehr gute Kenntnisse Französisch: Grundkenntnisse
EDV-Kenntnisse:	Gute Anwenderkenntnisse in MS Office-Anwendungen (Word, Excel, Power Point), GIS, R, Sigmaplot
Scheine:	Führerschein Klasse B, M und L, Tauchschein „Scuba Diving“
Exkursionen:	Hitzacker (Oktober 2011, Geomorphodynamik) Südengland (März – April 2014, Große Geländeübung zu Sedimentbecken) Allgäuer Alpen (August – September 2014, Kartierkurs) Mallorca (September – Oktober 2014, Sedimentdiagenese)

Hamburg, 21.05.2024

Jacqueline Berendt