Journal of Soil Science and Plant Nutrition (2023) 23:3705–3713
https://doi.org/10.1007/s42729-023-01291-7

ORIGINAL PAPER

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

Jacqueline Berendt[1,2] · **Nicole Wrage-Mönnig**[1]

Received: 26 November 2022 / Accepted: 8 May 2023 / Published online: 22 May 2023

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

✉ Jacqueline Berendt
jacqueline.berendt@uni-rostock.de

[1] Grassland and Fodder Sciences, Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany

[2] Present Address: Behörde für Umwelt, Klima, Energie und Agrarwirtschaft, Hamburg, Germany

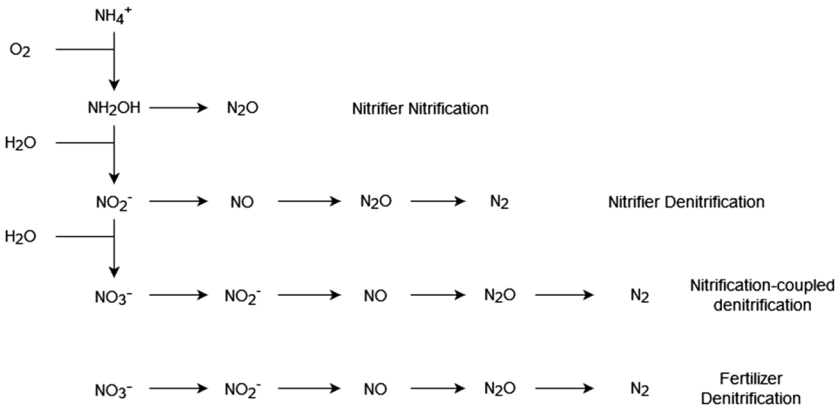

Fig. 1 Major pathways of N_2O production: nitrifier nitrification, nitrifier denitrification, fertilizer denitrification and nitrification-coupled denitrification. The difference between fertilizer denitrification and nitrification-coupled denitrification is the different source of the nitrate used from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

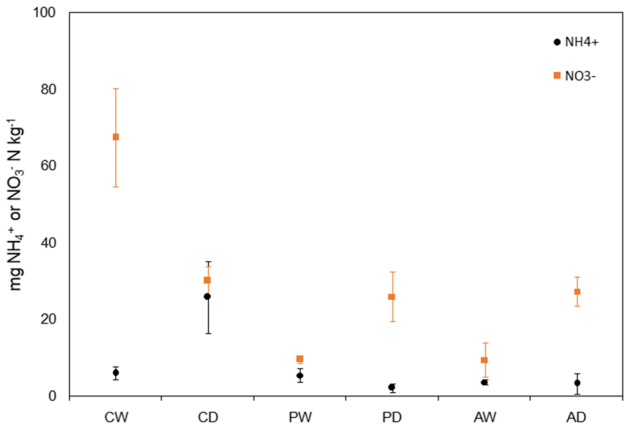

Fig. 2 NH_4^+ and NO_3^- concentrations (mg NH_4^+-/NO_3^--N kg^{-1}) in soil samples of the rewetted (CW) and drained (CD) coastal fen, rewetted (PW) and drained (PD) percolation fen and rewetted (AW) and drained (AD) alder forest at the end of the incubation experiment. Shown are means and standard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

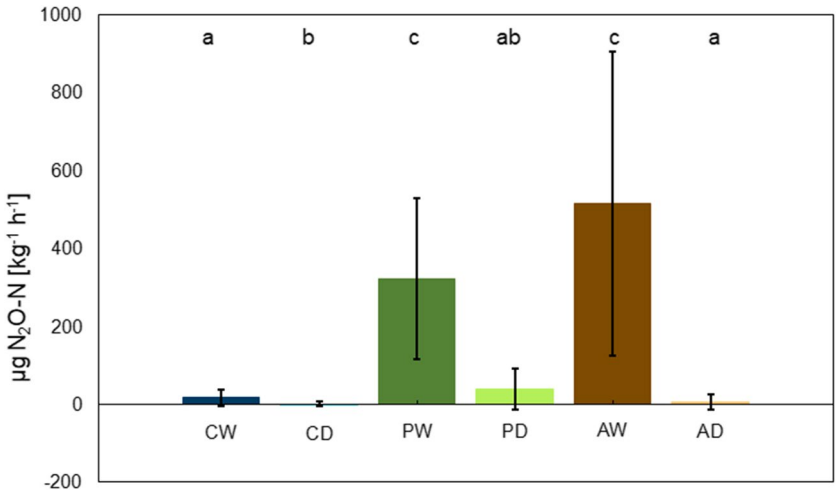

Fig. 3 N_2O-N fluxes (µg kg^{-1} h^{-1}) of incubated soil samples of the rewetted (CW) and drained (CD) coastal fen, the rewetted (PW) and drained (PD) percolation fen and the rewetted (AW) and drained (AD) alder forest. Shown are means and standard deviations

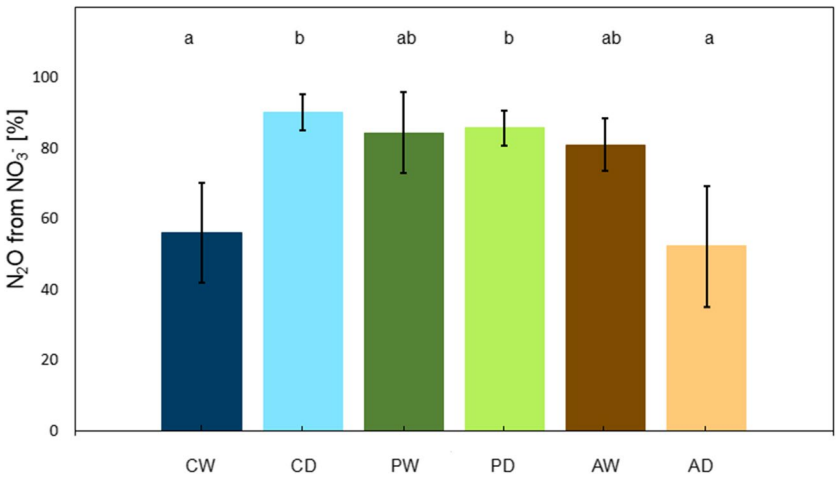

Fig. 4 N_2O produced from ^{15}N-$NO3^-$ (%) in soil incubations of the rewetted (CW) and drained (CD) coastal fen, the rewetted (PW) and drained (PD) percolation fen and the rewetted (AW) and drained (AD) alder forest. Shown are means and standard deviations

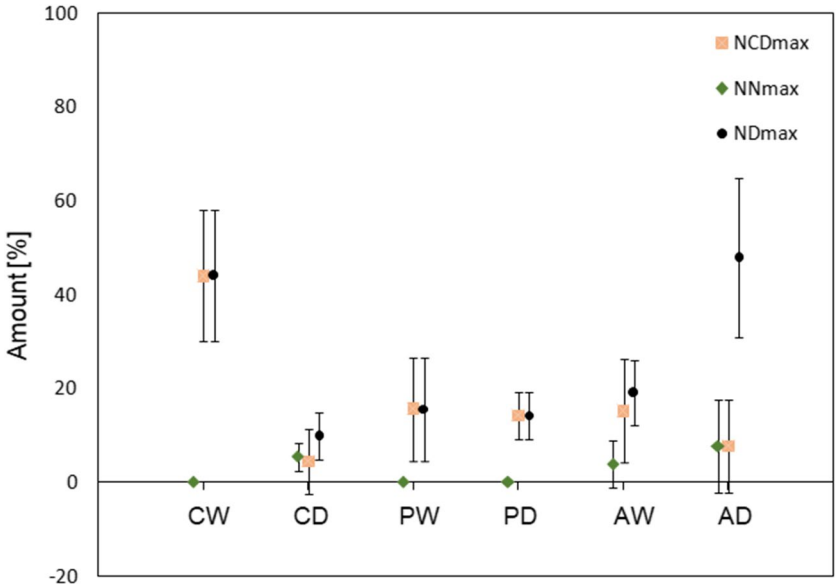

Fig. 5 Maximum N_2O production (%) from nitrification-coupled denitrification (NCDmax), nitrifier nitrification (NNmax) and nitrifier denitrification (NDmax) of the rewetted (CW) and drained (CD) coastal fen, the rewetted (PW) and drained (PD) percolation fen and the rewetted (AW) and drained (AD) alder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

also small (5.4 ± 3.6%, 3.8 ± 5.6%, 7.6 ± 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

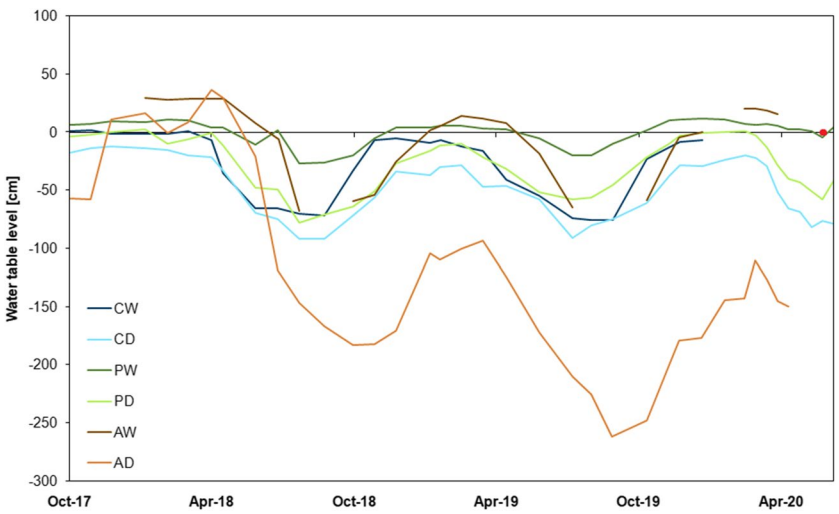

Fig. 6 Water table level of all six sites (rewetted (CW) and drained (CD) coastal fen, rewetted (PW) and drained (PD) percolation fen, rewetted (AW) and drained (AD) alder forest) from October 2017 to June 2020. The red dot marks the time of soil sampling for the incubation experiment. The interruptions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

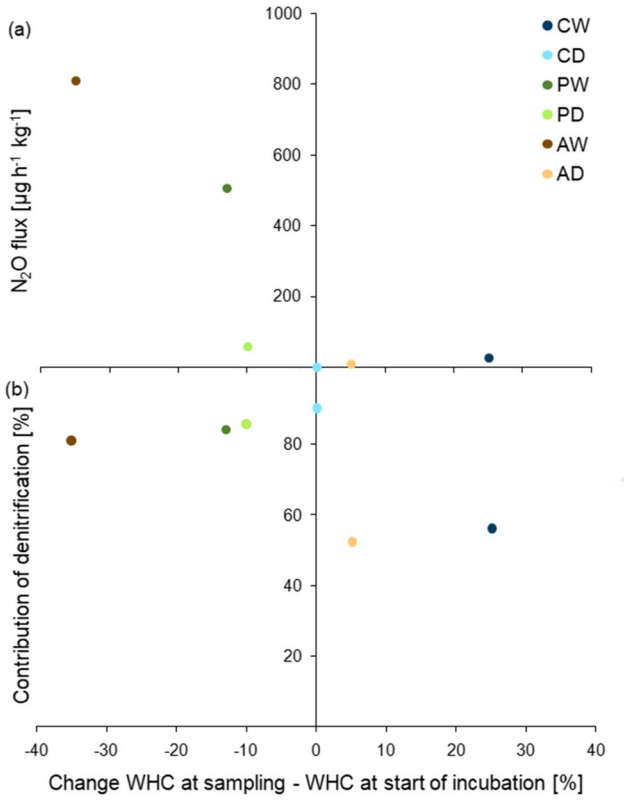

Fig. 7 Change of water contents between sampling time and start of the incubation experiment: shown are the changes in % water-holding capacity (WHC) against the N_2O flux (µg h^{-1} $kg.^{-1}$) (**a**) and changes in % WHC against the contribution of denitrification (%) (**b**)

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.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42729-023-01291-7.

Acknowledgements The European Social Fund (ESF) and the Ministry of Education, Science and Culture of Mecklenburg-Vorpommern funded this work within the scope of the project WETSCAPES (ESF/14-BM-A55-0027/16). We would like to thank Diana Werner for her excellent support in the lab.

Authors' Contribution Not applicable.

Funding Open Access funding enabled and organized by Projekt DEAL. The European Social Fund (ESF) and the Ministry of Education, Science and Culture of Mecklenburg-Vorpommern funded this work within the scope of the project WETSCAPES (ESF/14-BM-A55-0027/16).

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.

References

Augustin J, Merbach M, Rogasik J (1998) Factors influencing nitrous oxide and methane emissions from minerotrophic fens in northeast Germany. Biol Fertil Soils 28:1–4. https://doi.org/10.1007/s003740050455

Augustin J, Münchmeyer U, Russow R (2001) Nitrous oxide and dinitrogen losses from flooded fen peat with and without reed canarygrass (*Phalaris arundinacea* L.) and common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) plants. In: Horst WJ (ed) Plant nutrition: Food security and sustainability of agro-ecosystems through basic and applied research, vol 190. Kluwer Academic Publishers, Dordrecht, pp 958–959

Berendt J, Jurasinski G, Wrage-Mönnig N (2022) Influence of rewetting on N_2O emissions in three different fen types. Nutr Cycl Agroecosyst. https://doi.org/10.1007/s10705-022-10244-y

Brooks PD, Stark JM, McInteer BB, Preston T (1989) Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci Soc Am J 53:1707–1711. https://doi.org/10.2136/sssaj1989.03615995005300060016x

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos Trans R Soc Lond B Biol Sci 368:20130112. https://doi.org/10.1098/rstb.2013.0122

Canadell JG, Le Quéré C, Raupach MR, Field CB, Buitenhuis ET, Ciais P, Conway TJ, Gillett NP, Houghton RA, Marland G (2007) Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. Proc Natl Acad Sci USA 104:18866–18870. https://doi.org/10.1073/pnas.0702737104

Crutzen PJ (1991) Atmospheric chemical processes of the oxides of nitrogen including nitrous oxide. In: Delwiche CC (ed) Denitrification, nitrification and atmospheric nitrous oxide. Wiley, New York, pp 17–44

Davidsson TE, Trepel M, Schrautzer J (2002) Denitrification in drained and rewetted minerotrophic peat soils in Northern Germany (Pohnsdorfer Stauung). J Plant Nutr Soil Sci 165:199. https://doi.org/10.1002/1522-2624(200204)165:2%3c199:AID-JPLN199%3e3.0.CO;2-I

Dinsmore KJ, Skiba UM, Billett MF, Rees RM (2009) Effect of water table on greenhouse gas emissions from peatland mesocosms. Plant Soil 318:229–242. https://doi.org/10.1007/s11104-008-9832-9

Drösler M, Freibauer A, Christensen TR, Friborg T (2008) Observations and status of peatland greenhouse gas emissions in Europe. In: Caldwell MM, Heldmaier G, Jackson RB, Lange OL, Mooney HA, Schulze E-D, Sommer U, Dolman AJ, Valentini R, Freibauer A (eds) The Continental-Scale Greenhouse Gas Balance of Europe, vol 203. Springer. New York, New York, NY, pp 243–261

Eickenscheidt T, Heinichen J, Augustin J, Freibauer A, Drösler M (2014) Nitrogen mineralization and gaseous nitrogen losses from waterlogged and drained organic soils in a black alder (*Alnus glutinosa* (L.) Gaertn.) forest. Biogeosciences 11:2961–2976. https://doi.org/10.5194/bg-11-2961-2014

Gelbrecht J, Zak D, Augustin J (2008) Phosphor- und Kohlenstoff-Dynamik und Vegetationsentwicklung in wiedervernässten Mooren des Peenetals in Mecklenburg-Vorpommern. Status, Steuergrößen und Handlungsmöglichkeiten (German). Berichte des IGB Heft 26/2008

Hahn-Schöfl M (2015) Water table and organic substrate as triggers of greenhouse gas emissions from rewetted peatlands. University of Hannover

Heil J, Liu S, Vereecken H, Brüggemann N (2015) Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. Soil Biol Biochem 84:107–115. https://doi.org/10.1016/j.soilbio.2015.02.022

Höper H (2007) Freisetzung von Treibhausgasen aus deutschen Mooren (German). https://doi.org/10.23689/FIDGEO-3035

Ji C, Li S, Geng Y, Miao Y, Ding Y, Liu S, Zou J (2020) Differential responses of soil N_2O to biochar depend on the predominant microbial pathway. Appl Soil Ecol 145:103348. https://doi.org/10.1016/j.apsoil.2019.08.010

Jordan S, Strömgren M, Fiedler J, Lundin L, Lode E, Nilsson T (2016) Ecosystem respiration, methane and nitrous oxide fluxes from ecotopes in a rewetted extracted peatland in Sweden. Mires and Peat 17(7):1–23

Wrage-Mönnig N, Horn MA, Well R, Müller C, Velthof G, Oenema O (2018) The role of nitrifier denitrification in the production of nitrous oxide revisited. Soil Biol Biochem 123:A3–A16. https://doi.org/10.1016/j.soilbio.2018.03.020

Joosten H, Couwenberg J (2001) Bilanzen zum Moorverlust: Das Beispiel Europa (German). Schweitzerbart'sche Verlagshandlung

Jørgensen CJ, Elberling B (2012) Effects of flooding-induced N_2O production, consumption and emission dynamics on the annual N_2O emission budget in wetland soil. Soil Biol Biochem 53:9–17. https://doi.org/10.1016/j.soilbio.2012.05.005

Jurasinski G, Ahmad S, Anadon-Rosell A, Berendt J, Beyer F, Bill R, Blume-Werry G, Couwenberg J, Günther A, Joosten H, Köbsch F, Köhn D, Koldrack N, Kreyling J, Leinweber P, Lennartz B, Liu H, Michaelis D, Mrotzek A, Negassa W, Schenk S, Schmacka F, Schwieger S, Smiljanic M, Tanneberger F, Urich T, Wang H, Weil M, Wilmking M, Wrage-Mönnig N, Koebsch F, Smiljanić M, Teuber L, Zak D (2020) From understanding to sustainable use of peatlands: the WETSCAPES approach. Soil Syst 4:14. https://doi.org/10.3390/soilsystems4010014

Kool DM, Wrage N, Oenema O, Dolfing J, van Groenigen JW (2007) Oxygen exchange between (de)nitrification intermediates and H_2O and its implications for source determination of NO_3^- and N_2O: a review. Rapid Commun Mass Spectrom 21:3569–3578. https://doi.org/10.1002/rcm.3249

Kool DM, van Groenigen JW, Wrage N (2011) Source determination of nitrous oxide based on nitrogen and oxygen isotope tracing dealing with oxygen exchange. Meth Enzymol 496:139–160. https://doi.org/10.1016/B978-0-12-386489-5.00006-3

Kreyling J, Tanneberger F, Jansen F, van der Linden S, Aggenbach C, Blüml V, Couwenberg J, Emsens W-J, Joosten H, Klimkowska A, Kotowski W, Kozub L, Lennartz B, Liczner Y, Liu H, Michaelis D, Oehmke C, Parakenings K, Pleyl E, Poyda A, Raabe S, Röhl M, Rücker K, Schneider A, Schrautzer J, Schröder C, Schug F, Seeber E, Thiel F, Thiele S, Tiemeyer B, Timmermann T, Urich T, van Diggelen R, Vegelin K, Verbruggen E, Wilmking M, Wrage-Mönnig N, Wołejko L, Zak D, Jurasinski G (2021) Rewetting does not return drained fen peatlands to their old selves. Nat Commun 12:5693. https://doi.org/10.1038/s41467-021-25619-y

Lohila A, Aurela M, Hatakka J, Pihlatie M, Minkkinen K, Penttilä T, Laurila T (2010) Responses of N_2O fluxes to temperature, water table and N deposition in a northern boreal fen. Eur J Soil Sci 61:651–661. https://doi.org/10.1111/j.1365-2389.2010.01265.x

Martikainen PJ, Nykänen H, Crill P, Silvola J (1993) Effect of a lowered water table on nitrous oxide fluxes from northern peatlands. Nature 366:51–53. https://doi.org/10.1038/366051a0

Masta M, Espenberg M, Gadegaonkar SS, Pärn J, Sepp H, Kirsimäe K, Sgouridis F, Müller C, Mander Ü (2022) Integrated isotope and microbiome analysis indicates dominance of denitrification in N_2O production after rewetting of drained fen peat. Biogeochemistry 161:119–136. https://doi.org/10.1007/s10533-022-00971-3

Merbach W, Augustin J, Gans W (2001) Nitrous oxide emissions from fen mires in dependence of anthropogenic activities. J Appl Bot Food Qual 75(3–4):118–123

Mohn J, Biasi C, Bodé S, Boeckx P, Brewer PJ, Eggleston S, Geilmann H, Guillevic M, Kaiser J, Kantnerová K, Moossen H, Müller J, Nakagawa M, Pearce R, von Rein I, Steger D, Toyoda S, Wanek W, Wexler SK, Yoshida N, Yu L (2022) Isotopically characterised N_2O reference materials for use as community standards. Rapid Commun Mass Spectrom 36:e9296. https://doi.org/10.1002/rcm.9296

Page SE, Siegert F, Rieley JO, Boehm H-DV, Jaya A, Limin S (2002) The amount of carbon released from peat and forest fires in Indonesia during 1997. Nature 420:61–65. https://doi.org/10.1038/nature01131

Palmer K, Köpp J, Gebauer G, Horn MA (2016) Drying-rewetting and flooding impact denitrifier activity rather than community structure in a moderately acidic fen. Front Microbiol 7:727. https://doi.org/10.3389/fmicb.2016.00727

Pihlatie M, Syväsalo E, Simojoki A, Esala M, Regina K (2004) Contribution of nitrification and denitrification to N_2O production in peat, clay and loamy sand soils under different soil moisture conditions. Nutr Cycl Agroecosyst 70:135–141. https://doi.org/10.1023/B:FRES.0000048475.81211.3c

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125. https://doi.org/10.1126/science.1176985

Regina K, Nykänen H, Silvola J, Martikainen PJ (1996) Fluxes of nitrous oxide from boreal peatlands as affected by peatland type, water table level and nitrification capacity. Biogeochemistry 35:401–418. https://doi.org/10.1007/BF02183033

Rezanezhad F, Price JS, Quinton WL, Lennartz B, Milojevic T, van Cappellen P (2016) Structure of peat soils and implications for water storage, flow and solute transport: a review update for geochemists. Chem Geol 429:75–84. https://doi.org/10.1016/j.chemgeo.2016.03.010

Stein LY (2019) Insights into the physiology of ammonia-oxidizing microorganisms. Curr Opin Chem Biol 49:9–15. https://doi.org/10.1016/j.cbpa.2018.09.003

Tian H, Xu R, Canadell JG, Thompson RL, Winiwarter W, Suntharalingam P, Davidson EA, Ciais P, Jackson RB, Janssens-Maenhout G, Prather MJ, Regnier P, Pan N, Pan S, Peters GP, Shi H, Tubiello FN, Zaehle S, Zhou F, Arneth A, Battaglia G, Berthet S, Bopp L, Bouwman AF, Buitenhuis ET, Chang J, Chipperfield MP, Dangal SRS, Dlugokencky E, Elkins JW, Eyre BD, Fu B, Hall B, Ito A, Joos F, Krummel PB, Landolfi A, Laruelle GG, Lauerwald R, Li W, Lienert S, Maavara T, MacLeod M, Millet DB, Olin S, Patra PK, Prinn RG, Raymond PA, Ruiz DJ, van der Werf GR, Vuichard N, Wang J, Weiss RF, Wells KC, Wilson C, Yang J, Yao Y (2020) A comprehensive quantification of global nitrous oxide sources and sinks. Nature 586:248–256. https://doi.org/10.1038/s41586-020-2780-0

Timmermann T, Joosten H, Succow M (2016) Restaurierung von Mooren (German). In: Zerbe S, Wiegleb G (eds) Renaturierung von Ökosystemen in Mitteleuropa, 1st edn. Springer Spektrum, Berlin, Heidelberg, pp 55–93

Vengadaramana A, Ponipus Thairiyanathan JJ (2012) Effect of organic fertilizers on the water holding capacity of soil in different terrains of Jaffna peninsula in Sri Lanka. J Nat Prod 2:500–503

Verhoeven E, Barthel M, Yu L, Celi L, Said-Pullicino D, Sleutel S, Lewicka-Szczebak D, Six J, Decock C (2019) Early season N_2O emissions under variable water management in rice systems: source-partitioning emissions using isotope ratios along a depth profile. Biogeosciences 16:383–408. https://doi.org/10.5194/bg-16-383-2019

Vybornova O, van Asperen H, Pfeiffer E, Kutzbach L (2019) High N_2O and CO_2 emissions from bare peat dams reduce the climate mitigation potential of bog rewetting practices. Mires Peat 24(4):1–22. https://doi.org/10.19189/MaP.2017.SNPG.304

Wilson D, Farrell CA, Fallon D, Moser G, Müller C, Renou-Wilson F (2016) Multiyear greenhouse gas balances at a rewetted temperate peatland. Glob Chang Biol 22:4080–4095. https://doi.org/10.1111/gcb.13325

Wolf I (2000) Different pathways of formation of N_2O, N_2 and NO in black earth soil. Soil Biol Biochem 32:229–239. https://doi.org/10.1016/S0038-0717(99)00151-0

Wösten J, Ismail A, van Wijk A (1997) Peat subsidence and its practical implications: a case study in Malaysia. Geoderma 78:25–36. https://doi.org/10.1016/S0016-7061(97)00013-X

Wrage N, Velthof GL, Laanbroek HJ, Oenema O (2004) Nitrous oxide production in grassland soils: assessing the contribution of nitrifier denitrification. Soil Biol Biochem 36:229–236. https://doi.org/10.1016/j.soilbio.2003.09.009