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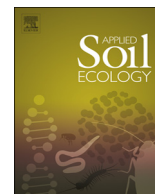
Differences in labile soil organic matter explain potential denitrification and denitrifying communities in a long-term fertilization experiment

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A B S T R A C T

Content and quality of organic matter (OM) may strongly affect the denitrification potential of soils. In particular, the impact of soil OM fractions of differing bioavailability (soluble, particulate, and mineral-associated OM) on denitrification remains unresolved. We determined the potential N_2O and N_2 as well as CO_2 production for samples of a Haplic Chernozem from six treatment plots (control, mineral N and NP, farmyard manure - FYM, and FYM + mineral N or NP) of the Static Fertilization Experiment Bad Lauchstädt (Germany) as related to OM properties and denitrifier gene abundances. Soil OM was analyzed for bulk chemical composition (^{13}C-CPMAS NMR spectroscopy) as well as water-extractable, particulate, and mineral-associated fractions. Soils receiving FYM had more total OM and larger portions of labile fractions such as particulate and water-extractable OM. Incubations were run under anoxic conditions without nitrate limitation for seven days at 25 °C in the dark to determine the denitrification potential (N_2O and N_2) using the acetylene inhibition technique. Abundances of $nirS$, $nirK$, and $nosZ$ (I + II) genes were analyzed before and after incubation. The denitrification potential, defined as the combined amount of N released as N_2O + N_2 over the experimental period, was larger for plots receiving FYM (25.9–27.2 mg N kg^{-1}) than pure mineral fertilization (17.1–19.2 mg N kg^{-1}) or no fertilization (12.6 mg N kg^{-1}). The CO_2 and N_2O production were well related and up to three-fold larger for FYM-receiving soils than under pure mineral fertilization. The N_2 production differed significantly only between all manured and non-manured soils. Nitrogenous gas emissions related most closely to water-extractable organic carbon (WEOC), which again related well to free particulate OM. The larger contribution of N_2 production in soils without FYM application, and thus, with less readily decomposable OM, coincided with decreasing abundances of $nirS$ genes (NO_2^- reductase) and increasing abundances of genes indicating complete denitrifying organisms ($nosZ I$) during anoxic conditions. Limited OM sources, thus, favored a microbial community more efficient in resource use. This study suggests that WEOC, representing readily bioavailable OM, is a straightforward indicator of the denitrification potential of soils.

1. Introduction

When oxygen (O_2) is absent and organic carbon (OC) sources are available denitrifying microorganisms reduce nitrate (NO_3^-) via a series of enzymatic steps to NO_2^-, NO, N_2O, and finally N_2 (Philippot et al., 2007). Therefore, denitrification can cause considerable N losses in form of nitrogenous gases from agricultural soils, resulting in limited crop production (Aulakh et al., 1992). In addition, N_2O exhibits the largest warming potential of all biogenic greenhouse gases (298 times that of CO_2) and accounts for about 6% of the current global greenhouse effect (Bouwman et al., 1995; IPCC, 2013). Its atmospheric concentration increased since pre-industrial times by approximately 20%, mainly in the wake of the increasing use of N fertilizers (WMO,

2017). Compared to N_2O, the N_2 production is rarely studied, due to the large background concentrations of N_2 in air and water, rendering it difficult to detect N_2 release by denitrification (Groffman et al., 2006). Complete denitrification to N_2 still results in a net loss of N but has no such effect on climate change as N_2O. Consequently, better understanding of factors controlling the N_2O/N_2 product ratio is of crucial importance for evaluating climatic effects by denitrification.

Denitrification in soil mainly occurs in anoxic microhabitats ('hot spots') where enough NO_3^- and carbon (C) are available (e.g., Groffman et al., 2009). Previous studies have shown that addition of plant biomass or well-defined low-molecular weight compounds, such as glucose or sucrose, affects denitrification rates, product ratios and denitrifier populations (e.g., Beauchamp et al., 1989; Miller et al., 2008;

Palmer et al., 2012). Much less information is available on effects of ecologically more relevant OM fractions, such as particulate and mineral-associated OM, on potential denitrification rates and resulting product ratios. Water-extractable organic C (WEOC) is considered to be readily decomposable and most effective in promoting denitrification (e.g., Bremner and Shaw, 1958; Burford and Bremner, 1975). Accordingly, the addition of plant-derived dissolved OM (water extracts of maize stalk) to repacked soil results in increased CO_2 and N_2O emissions (Qiu et al., 2015). Effects of sources, availability, and composition of WEOC on denitrification, however, have not been addressed so far. In agricultural soils, additional factors need to be taken into account when evaluating the relevance of different OM fractions, including rates and type of fertilizer application (organic versus mineral) and crop sequences (Janzen et al., 1992; Edmeades, 2003; Diacono and Montemurro, 2010). Mineral fertilization has controversial and at most indirect effects on the content and quality of OM (e.g., He et al., 2015; Dou et al., 2016). For example, sole addition of mineral N significantly accelerates the decomposition of OM with the decomposition products then becoming stabilized in mineral-organic associations (Neff et al., 2002). Manure application, by contrast, results in larger contents of plant-derived sugars (Xie et al., 2014), a generally higher proportion of labile OC, and an overall higher microbial activity (e.g., Aoyama et al., 1999; Hai et al., 2010; Wang et al., 2015). Randall et al. (1995) also observed that OM in a manured silty clay loam of the Broadbalk Experiment at Rothamsted (UK; monoculture of winter wheat since 1843) was slightly enriched in O/N-alkyl C and alkyl C components compared to soils under mineral NPK fertilization. Consequently, also the abundances of denitrifying organisms and the N_2O production can be higher in manured than in mineral fertilized soils (e.g., Sun et al., 2015; Cui et al., 2016). So far, the relationship between fertilization-induced changes in functional OM fractions and the respective potential denitrification as well as gene abundances have only been rarely addressed; with most denitrification studies neglecting the emission of N_2 relative to N_2O.

The objective of this study was, therefore, to test the effect of soil OM composition as caused by different fertilization regimes on (i) potential denitrification, (ii) the $N_2O/(N_2O + N_2)$ product ratio, and (iii) respective gene abundances. We used soil samples from six plots of the long-term Static Fertilization Experiment Bad Lauchstädt (Germany) to obtain a wide range of organic matter composition under similar textural properties. Based on the assumption that the long-term application of different fertilizers changed the amount and composition of soil OM, we hypothesize that there are specific and measurable OM fractions that allow for explaining and predicting denitrification rates and product ratios. We assume that treatments causing stronger accumulation of readily decomposable OM, indicated by larger portions of water-extractable and particulate OM, and (O/N-)alkyl C components, result in increased denitrification with increased proportions of N_2O. In addition, we surmise that the denitrifier community (abundances of NO_2^- and N_2O reductase genes) is directly linked to the amount and composition of OM fractions or their bioavailability.

2. Materials and methods

2.1. Soil sampling

Soil samples (four field replicates) were randomly collected at 0–30 cm depth from six treatment plots of the Static Fertilization Experiment Bad Lauchstädt, Germany (51°23′ N, 11°52′ E), in October 2016. The site is characterized by an exceptional homogenous soil, with very little variation in basic properties, such as soil texture, but providing a wide range of different compositions of soil organic matter (e.g., Ludwig et al., 2007). The loamy soil is classified as Haplic Chernozem with the topsoil (Ap horizon) having about 70% silt, 20% clay, and 10% sand on all experimental plots (Altermann et al., 2005; Ludwig et al., 2007). The mean annual precipitation and temperature at the site

is 486 mm and 8.8 °C, respectively. The Static Fertilization Experiment was established in 1902 and consists of eight strips, each divided into 18 treatment plots (except for strip number 4 and 5). In this study, only the following six treatments of strip number 2 were used: control, i.e., without any fertilization; mineral N (N) and N + P fertilization (NP); application of farmyard manure (FYM), also combined with mineral N and P (FYM + N and FYM + NP). The study is focused on the relevance of organic matter fractions for potential denitrification and less on the effect of fertilization. Nevertheless, in accordance with previous work, the sampled soils are designated according to the respective fertilization treatment. Calcium ammonium nitrate (27% N) and superphosphate were used as mineral N and P sources. The amount of mineral fertilizers depended on the crop and amount of applied FYM (Supplementary Table S1). Farmyard manure (30 t ha^{-1}) has been applied every second year with root crops (sugar beets, potatoes). In 2015, the original crop rotation (sugar beet – spring barley – potatoes – winter wheat) has been modified to: silage maize – spring barley – silage maize – winter wheat. FYM is now applied in years with maize cropping. Additional information is given by Körschens et al. (1994), Merbach and Körschens (2002), and Merbach and Schulz (2013).

2.2. Basic characterization of bulk soils

Soil samples were stored at 4 °C in the dark for a maximum of four days after collection. Large plant particles and stones were removed by sieving to < 2 mm. To estimate available P (Olsen, 1954), 2.5 g of field-fresh soil suspended in 50 ml 0.5 M NaHCO$_3$ (pH 8.5) solution were shaken for 30 min. After centrifugation (3000 $\times g$) for 10 min (Heraeus™ Cryofuge 8500i, Thermo Fisher Scientific, Waltham, MA, USA), the supernatant was passed through a 0.45-µm membrane filter (Supor®-450, Pall Cooperation, New York, NY, USA). Concentrations of P in the extracts were analyzed using ICP-OES (Ultima 2, Horiba Jobin-Yvon, Longjumeau, France). N_{min} (NO_3^- and NH_4^+) was extracted from field-fresh soils into 1 M KCl solution at a soil-to-solution ratio of 1:5 (wt./v), with centrifugation and filtration as described above, and determined using a Continuous-Flow Analyzer (ScanPlus, Skalar Analytical B.V., Breda, The Netherlands). Soil reaction was estimated by potentiometric measurement of pH in the supernatant of a soil suspension in 0.01 M CaCl$_2$ (1:5 wt./v). Air-dried and sieved bulk soils were analyzed for total C und N (TN) with a Vario Max Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). Inorganic C was not detectable (Vario Max Cube, Elementar), therefore, total C was assumed to represent OC. The difference TN – N_{min} is an estimate of organic N (ON). All values were normalized to the respective total dry matter, determined gravimetrically after oven-drying at 105 °C.

2.3. Solid-state ^{13}C NMR spectroscopy

Air-dried and sieved bulk soils ($n = 24$, including field replicates) were ground in an agate mortar, combined into a composite sample for each fertilization treatment ($n = 6$), and analyzed by solid-state ^{13}C cross-polarization magic angle spinning NMR spectroscopy (^{13}C-CPMAS NMR spectroscopy) with an Avance III 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). Samples were placed into a 7-mm zirconia rotor that was spun at 6.8 kHz around a 'magic angle' of 54.74°. Contact time was 1 ms and the recycle delay time was set to 0.4 s. The spectra were processed with 100 Hz line broadening, phase adjusted, and baseline corrected; no spinning side bands appeared in the spectra. Peaks were assigned to four integration areas: −10–45 ppm (alkyl C), 45–110 ppm (O/N-alkyl C), 110–160 ppm (aromatic C), and 160–220 ppm (carboxylic/carbonyl C). All spectra were well resolved, indicating no interfering effects of paramagnetic materials, such as iron oxides, on the measurements (Supplementary Fig. S1).

2.4. Characterization of functional OM fractions

Soil samples were fractionated according to density using a modified version of the procedure described by Christensen (1992). The procedure provides three fractions: particulate OM (POM) not or only weakly associated with mineral particles (i.e., free POM = fPOM), POM occluded within water-stable aggregates (oPOM), and OM strongly bound to mineral phases (mineral-bound OM = MOM). In brief, 25 g of air-dried soil (< 2 mm) were gently suspended in 125 ml of sodium polytungstate (SPT; 1.6 g cm^{-3}) in a 500-ml centrifuge beaker. After 1 h, the suspension was centrifuged (6800 $\times g$) for 30 min at 20 °C (Cryofuge 8500i). The supernatant with the floating fPOM material was aspirated and passed through a 0.45-µm membrane filter (Supor®-450, Pall). Soils were re-suspended in SPT solution and the centrifugation–filtration procedure was repeated once again. The fPOM on the filter was washed with distilled water until the electrical conductivity in washing solution was < 50 µS cm^{-1}, and then air-dried at 40 °C, and weighed. The soil was then re-suspended in SPT solution and sonicated at 60 J ml^{-1} (Sonoplus UW 2200, Bandelin electronic GmbH, Berlin, Germany) to release oPOM from aggregates. The selected sonication energy was shown to be sufficient to disrupt all aggregates in a preparatory test according to Cerli et al. (2012). Centrifugation, filtration, and washing were carried out as for the fPOM. The remaining soil material, representing the MOM fraction, was subjected to several washing–centrifugation cycles until the conductivity of the washing solution was < 50 µS cm^{-1}. Subsequently, the MOM fraction was freeze-dried and weighed. The MOM fraction was analyzed for OC and TN using a Vario Max Cube; analyses of fPOM and oPOM were carried out with a Vario EL analyzer (Elementar Analysensysteme GmbH). The total contents of OC and TN with the fractions in soil were calculated by multiplying the respective bulk soil OC and TN contents with the proportional contribution of the individual fractions to the sum of OC and TN in all fractions.

Water-extractable organic C (WEOC) and N (WEON) were determined on fresh soil samples. Briefly, 20 g of soil suspended in 100 ml deionized water were shaken for 1 h. After centrifugation (3000 $\times g$) for 10 min (Cryofuge 8500i), the supernatant was passed through a 0.45-µm membrane filter (Supor®-450) and analyzed for OC and total N, using a DIMATOC® 100 (Dimatec Analysetechnik GmbH, Essen, Germany).

2.5. Incubation and gas measurements

Bulk soils were anoxically incubated at 25 °C in the dark for seven days, as most N_2O production in soil occurs at timescales of less than two weeks (Kuzyakov and Blagodatskaya, 2015). To reactivate the microbial community, subsamples (110 g dry mass) of each soil were wetted to 40% water holding capacity and aerobically pre-incubated for seven days at 25 °C in the dark. Then, 100 g (dry mass) of each soil were packed into 500-ml glass infusion bottles to 1.3 g cm^{-3} bulk density, using a plunger. A KNO_3 solution (50 mg NO_3^--N kg^{-1} dry soil) was added to achieve 80% water-filled pore space and avoid nitrate limitation during the incubation period. The latter ensured that differences in denitrification related primarily to differences in OM content and quality. All bottles were sealed with a bromine-butyl-rubber stopper and crimped with an aluminum cap (32 mm; Chroma Globe GbR, Kreuznau, Germany). An O_2-free atmosphere for anoxic incubations was obtained by evacuating (below 250 hPa), and then rinsing the bottles with He gas (99.999%, Air Liquide, Düsseldorf, Germany) for three times, reaching a final pressure of about 1025 hPa. After 1 h, the first (t_0) gas sample (18 ml) was taken with a gastight syringe (25 ml, 25MDR-LL-GT; SGE Analytical Science Pty. Ltd., Ringwood, VIC, Australia), equipped with a push button valve (Luer Lock; SGE Analytical Science) and a 0.7-mm ID cannula (Sterican G26, 25 mm; B. Braun AG, Melsungen, Germany), and transferred into pre-evacuated (10 hPa residual pressure, rinsed with He) 12-ml Exetainer® vials (IVA

Analysentechnik e.k., Meerbusch, Germany). This resulted in an overpressure of > 200 hPa in the Exetainer® vials, which was necessary to avoid contamination with air during storage and for measuring gas concentrations with the gas chromatography system described below. An additional septum (12 mm, silicone–PTFE, 1.5 mm; IVA) was placed in the screw caps above the chlorobutyl rubber septum to achieve gas tightness. To avoid low pressure in the incubation bottles, 18 ml He at ~1000 hPa were injected after daily gas sampling, resulting in constant absolute pressure of ~1025 hPa during incubation. The absolute pressure in the bottles was measured before and after gas sampling as well as after He injection, using a GMSD 2 BA-K31-L01 pressure sensor coupled with a Greisinger GMH 3151 reader (GSG Geologie-Service GmbH, Würzburg, Germany).

All gas samples were analyzed on a custom-tailored gas chromatography system by Chromtech (Bad Camberg, Germany), using an Agilent HP 7890B GC as basis. The samples were introduced into the injector by an autosampler (PAL GC-xt; CTC Analytics AG, Zwingen, Switzerland), using an open needle syringe. Calibration was done online, using standard gas cups connected to bottles with certified calibration gases with known concentrations of CO_2 and N_2O in He (Linde Gas AG, Pullach, Germany). After injecting the sample at a liner temperature of 150 °C, it was transferred to a Shin Carbon chromatographic column (2 m, 0.53 mm ID, Restek GmbH, Bad Homburg, Germany) using He as carrier gas (purity 99.9999%) and directly transferred to a He ionization detector (Vici AG International, Schenkon, Switzerland) run at 180 °C. The GC oven was set to a starting temperature of 60 °C, kept constant for 3 min, increased to 110 °C at a rate of 10 °C min^{-1}, kept for 1 min, and finally increased to 220 °C at a rate of 50 °C min^{-1}, kept for 3 min. The limit of detection for both gases was calculated using 10 blank samples that were drawn and measured in the same way as all other samples. The limit of detection was calculated as 0.1 ppb and 11.76 ppm for N_2O and CO_2, respectively. Precision and accuracy were analyzed injecting a reference standard every ten samples. On average, precision was 4.8% and 0.5% relative standard error for N_2O and CO_2, respectively, while accuracy was 3.3% and 1.5% offset from the specified concentration for N_2O and CO_2, respectively.

Cumulative emissions of gases represent the sum of daily produced amounts, i.e., the detected gas mass in the headspace at time point t_x minus the gas mass at time point t_{x-1}. For setting up the acetylene inhibition technique (Yoshinari and Knowles, 1976), the entire incubation procedure described above was repeated with injection of 60 ml of C_2H_2 (99.6%; Air Liquide) in exchange for 60 ml He, resulting in an initial C_2H_2 concentration of ~10% (v/v) directly after flushing with He and 1 h before the first gas sampling. Considering the dilution by the He addition after gas sampling and assuming a microbial metabolism of $< 2.5\%$ of added C_2H_2 over seven days (Terry and Duxbury, 1985), the C_2H_2 concentration was high enough ($> 5\%$ v/v) to prevent the reduction of N_2O to N_2 over the entire incubation period (Yeomans and Beauchamp, 1978). To estimate the N_2 production, the produced amount of N_2O was subtracted from the respective amount of N_2O in presence of C_2H_2 (representing N_2O + N_2). The ratio of the two N_2O amounts was used to determine the molar N_2O-N/(N_2O + N_2)-N ratio for each incubation day. Proportional NO_3^--N losses as N_2O-N and N_2 were calculated based on cumulative gas emissions within seven days and the initial (natural + added) NO_3^--N content. The portion of mineralized OC was derived by relating the cumulative CO_2-C produced within seven days to the initial bulk soil OC as well as the WEOC content. Changes in soil pH in response to the incubations were little (± 0.3 pH units) and revealed no consistent patterns between differently fertilized soils.

2.6. DNA extraction and qPCR assay

Pre-incubated and incubated soil samples were frozen and stored at -20 °C prior to analyses of abundances of NO_2^- and N_2O reductase genes. While gene abundances in pre-incubated soils were mainly

Table 1

Soil pH, total organic C (OC), OC/ON ratio, KCl-extractable NO_3^--N, and $NaHCO_3$-extractable P of soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP). Since no ammonium was detected in any sample, NO_3^--N represented total mineral N. Values represent means ($n = 4$) ± standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

Fertilization treatment	pH[a] (CaCl$_2$)	Total OC [g kg^{-1}]	OC/ON ratio	NO$_3$-N [mg kg^{-1}]	Olsen P [mg kg^{-1}]
Control	6.9–7.4	15.5 ± 0.4b	14.3 ± 0.4a	8.0 ± 1.2b	11.0 ± 5.2d
N	7.1–7.4	16.0 ± 0.3b	13.6 ± 0.6ab	14.7 ± 3.3ab	4.4 ± 0.9d
NP	5.5–7.4	16.5 ± 0.5b	13.6 ± 0.5ab	9.8 ± 2.6b	35.4 ± 3.6c
FYM	6.4–6.9	22.3 ± 0.5a	12.8 ± 0.3bc	13.9 ± 4.6ab	48.1 ± 3.4b
FYM + N	6.2–6.7	23.3 ± 1.6a	12.6 ± 0.3c	17.8 ± 4.6a	39.3 ± 4.1c
FYM + NP	6.1–6.7	22.3 ± 0.5a	12.7 ± 0.1c	18.9 ± 3.6a	57.5 ± 4.8a

[a] Range of replicated samples ($n = 4$).

determined by the different long-term fertilization, changes over the incubation time represent short-time effects. The core reaction of denitrification is the conversion of soluble NO_2^- into gaseous NO, catalyzed by NO_2^- reductases. Canonical denitrifiers have either a copper-containing NO_2^- reductase or a cytochrome *cd*1 NO_2^- reductase, encoded by *nirK* or *nirS* genes, respectively (Gao et al., 2016). The last step, the reduction of N_2O to N_2, is catalyzed by the N_2O reductase encoded by *nosZ* genes, which can be divided into clade I and II (Hallin et al., 2017). Most organisms with *nosZ I* genes are complete denitrifiers, i.e., they also have *nirS* or *nirK* genes, whereas many organisms with *nosZ II* are non-denitrifying N_2O reducers (Graf et al., 2014). DNA was extracted from 0.3 g of soil, using the DNeasy Soil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration was measured spectrophotometrically (DS-11 FX, DeNovix, Wilmington, NC, USA) and *nirK*, *nirS*, as well as *nosZ* (clade I and II) were amplified by quantitative polymerase chain reaction (PCR) in a CFX Connect thermocycler (Bio-Rad Laboratories, Hercules, CA, USA), using the primer pairs F1aCu (ATCATGGTSCTGCCGCG)/R3Cu (GCCTCGATCAGRTTGTGGTT; Hallin and Lindgren, 1999), cd3aF (GTSAACGTSAAGGARACSGG)/R3cd (GASTTCGGRTGSGTCTTG; Throbäck et al., 2004), nosZ1840F (CGCRACGGCAASAAGGTSMSSGT)/ nosZ2090R (CAKRTGCAKSGCRTGGCAGAA; Henry et al., 2006), and nosZ-II-F (CTIGGICCIYTKCAYAC)/nosZ-II-R (GCIGARCARAAITCBG-TRC; Jones et al., 2013), respectively. Standard curves for each qPCR assay were derived from plasmids containing the respective cloned gene from either a soil sample (*nirK*, *nirS*) or pure culture (*Bradyrhizobium japonicum*, *nosZ I*; *Bacillus azotoformans*, *nosZ II*), where the inserts were amplified with primers specific for the multiple cloning site (Zaprasis et al., 2010; Palmer and Horn, 2015). The gene copy numbers were related to dry mass of soil. Details on the composition of the PCR mastermixes and thermocycling protocols are given in the Supplementary Table S2. The amplification of standards and samples was done in triplicate and the specificity of the reaction was tested via gel electrophoresis. The absence of PCR-inhibiting substances, such as humic acids, was shown by spiking a number of the soil DNA extracts with a known amount of standard DNA before subjecting them to qPCR and comparing the resulting Ct-values to those of the same standard DNA in pure water.

2.7. Statistical evaluation

Basic statistical analyses were performed using Sigma Plot 11.0 (Systat Software Inc., Erkrath, Germany). One-way ANOVA (fertilization treatment as independent variable, $n = 6$) followed by the Tukey HSD test was used for testing for differences in biochemical soil and OM properties, abundances of denitrification genes, as well as initial, maximum, and cumulative emissions of N_2O, N_2, and CO_2. Data not normally distributed were log-transformed to achieve normality, or the non-parametric Kruskal-Wallis test followed by the Tukey HSD test was used. Linear regression analyses were used to test for relationships between gas emissions and soil properties of different fertilization treatments after confirming the normal distribution of data by the

Shapiro-Wilk test ($n = 24$). One-way ANOVA followed by the Tukey HSD test was also used for testing for differences between cumulative N_2 emissions from all manured and non-manured soils (for each $n = 12$). For ^{13}C NMR spectroscopy data, linear regression analyses with mean values of gas emissions and gene abundances were performed using values of the six composite samples of each fertilization treatment ($n = 6$). Correlation coefficients were determined and considered statistically significant at $p < 0.05$.

3. Results

3.1. Chemical soil properties under different long-term fertilization

Different fertilization over 114 years altered the chemical soil properties (Table 1). Except for one replicate of the NP treatment with a pH of only 5.5, the pH values of soils with FYM application were lower (6.1–6.9) than those of soils without (6.9–7.4). The mean OC content of the FYM treatments was almost 44% larger than under the other three fertilization regimes (control, N, NP). Within each of the two groups, the OC contents varied only slightly (Table 1). The average OC/ON ratio was slightly less in soils with FYM application (12.6–12.8) than under mineral fertilization (13.6) and in the control (14.3). FYM application increased the contents of available P (Olsen P); sole calcium ammonium nitrate (N treatment) application resulted in decreased contents of available P (Table 1). The largest contents of KCl-extractable NO_3^- occurred in the manured (FYM, FYM + N, FYM + NP) and N-fertilized soils (13.8–18.9 mg N kg^{-1} dry soil); the control and NP treatments had 8.0 and 9.8 mg NO_3^--N kg^{-1} dry soil, respectively. Extractable ammonium (NH_4^+) was not detectable in any treatment.

3.2. Organic matter composition

The overall contribution of fPOM-OC and oPOM-OC to total soil OC was 1%–3% and 2%–7%, respectively (Table 2). Soils receiving FYM had about twice the content of fPOM (1.8–2.4 g kg^{-1} soil) as compared to mineral fertilized soils and the control (0.8–1.0 g kg^{-1}). N-fertilized soils and manured soils receiving additional mineral fertilizers (N and especially NP) had the largest oPOM contents (2.7–4.0 g kg^{-1} soil). In all soils, most OC resided within the MOM fraction (91%–97%). The OC/TN ratios of fPOM and oPOM in soils with FYM application were about 30% smaller than those of the other treatments (Table 3). The OC/TN ratio of MOM was 5% and 9% less for manured soils than for soils under mineral fertilization and for the control, respectively.

^{13}C-CPMAS NMR spectroscopy revealed that the contribution of O/N-alkyl C to total soil OC ranged from 34% to 38% in the order control < N, NP < FYM < FYM + N, FYM + NP; smaller proportions of aryl C and carboxyl/carbonyl C were measured for soils under mineral fertilization, with or without FYM (Fig. 1). The application of mineral N and P, especially without FYM, resulted in slightly more aliphatic C. Soils with FYM application showed about 13% smaller alkyl C-to-O/N-alkyl C ratios (0.5–0.6) than in those of the other three treatments. For all treatments, the proportion of WEOC and WEON

Table 2
Contents of water-extractable organic C (WEOC) and N (WEON), contribution of free and occluded particulate OC (fPOM, oPOM) and mineral-associated OC (MOM) to total soil OC as well as OC/TN ratios of the three fractions in soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP). Values represent means ($n = 4$) $\pm$ standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

Fertilization treatment	WEOC [mg kg^{-1}]	WEON [mg kg^{-1}]	Proportion in total OC [%]			OC/TN ratio		
			fPOM-OC	oPOM-OC	MOM-OC	fPOM	oPOM	MOM
Control	23.0 ± 4.7c	4.4 ± 0.7b	2.1 ± 0.1ab	3.4 ± 1.0bc	94.5 ± 1.1ac	30.8 ± 5.1a	21.7 ± 2.0ab	13.7 ± 0.4a
N	28.5 ± 1.9bc	6.9 ± 1.5ab	1.8 ± 0.7ab	6.6 ± 1.4a	91.6 ± 2.1b	29.5 ± 4.7a	20.1 ± 1.9ab	13.0 ± 0.6ab
NP	28.8 ± 7.1bc	5.6 ± 0.9ab	1.3 ± 0.3b	2.1 ± 0.5c	96.6 ± 0.3a	29.6 ± 5.1a	27.1 ± 8.4a	13.1 ± 0.3ab
FYM	37.9 ± 2.4ab	8.6 ± 1.7a	3.3 ± 1.1a	4.2 ± 1.1abc	92.5 ± 2.1bc	20.8 ± 5.2ab	15.7 ± 1.5b	12.5 ± 0.3b
FYM + N	38.6 ± 4.1ab	7.6 ± 2.3ab	3.3 ± 1.3a	5.5 ± 1.1ab	91.3 ± 2.4b	18.3 ± 3.0b	16.3 ± 1.3b	12.4 ± 0.2b
FYM + NP	39.8 ± 5.4a	8.7 ± 2.1a	3.2 ± 0.6a	6.0 ± 2.1ab	90.8 ± 1.6b	20.4 ± 3.3ab	15.2 ± 2.0b	12.4 ± 0.3b

varied between 0.1%–0.2% of total OC and between 0.3%–0.7% of total ON. In accordance with their larger OC and ON contents, the manured soils had larger amounts of WEOC and WEON of than the mineral fertilized soils and the control soil (Table 2). The WEOC/WEON ratios were almost similar for all fertilization treatments (4.3–5.5). WEOC contents were positively correlated with the fPOM-OC/soil OC ratio ($r = 0.63$, $p < 0.01$, $n = 24$) and, in turn, negatively to the MOM-OC/soil OC ratio ($r = -0.63$, $p < 0.01$, $n = 24$) and OC/TN ratio of fPOM ($r = -0.85$, $p < 0.001$, $n = 24$).

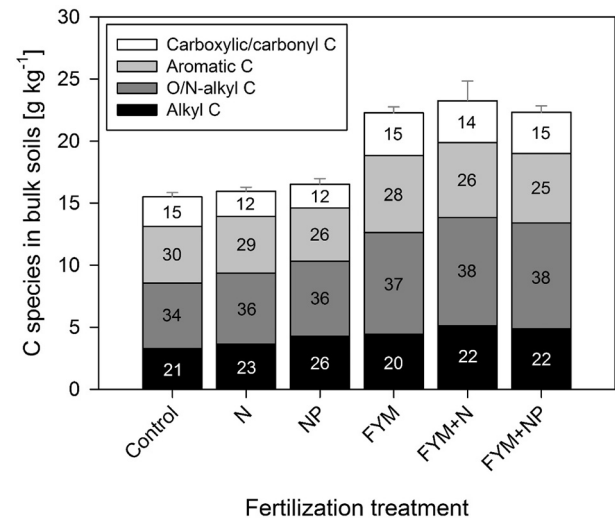### 3.3. Initial and cumulative production of CO₂, N₂O, and N₂

Initial (after one day) as well as cumulative N$_2$O and CO$_2$ production within seven days were significantly higher for soils receiving FYM than for soils under pure mineral fertilization and fertilizer deprivation (Figs. 2 and 3). Additional N and P input (FYM + N, FYM + NP) resulted in the highest average cumulative N$_2$O and CO$_2$ production (7.3–7.4 mg N kg^{-1} and 30.6–31.7 mg C kg^{-1}, respectively). The cumulative N$_2$O production of the control and the pure mineral N fertilization treatment reached its maximum within the first two days and dropped close to zero after seven days (Fig. 2). The maximum cumulative N$_2$O production for soils with FYM application was reached after five days and subsequently stagnated (FYM + N, FYM + NP) or decreased slightly (FYM). The NP treatment showed a similar N$_2$O production trend as the FYM treatments but on a lower level (Fig. 2). The initial N$_2$ production was about 25% higher for mineral fertilization than for FYM application but the manured soils released almost 18% more N$_2$ over the entire seven days than soils receiving mineral fertilization (Fig. 4). There was no significant difference in the initial and cumulative N$_2$ production among all fertilization treatments, but the average cumulative N$_2$ production was significantly larger for all manured soils ($n = 12$) than for soils receiving no FYM application ($n = 12$). The cumulative amount of N$_2$ emitted from the control (unfertilized) was substantially less than from the fertilized soils. After seven incubation days, about 40% of the initial (natural + added) NO$_3^-$-N was lost as N$_2$O and N$_2$ in soils with FYM application, while mineral fertilized soils (N, NP) and the unfertilized control soil emitted

Fig. 1. Distributions of C species in bulk soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP). Bars represent the soil's bulk mean OC contents ($n = 4$); error bars indicate standard deviation in one direction only. Stacks within bars represent the percentage contribution of the different integrated chemical shift regions.

only 26%, 32%, and 22% of the initial NO$_3^-$-N, respectively. The cumulative CO$_2$-C production accounted for 23%–49% (non-manured soils) and 64%–82% (manured soils) of the initial WEOC content.

When pooling all fertilization treatments, we found a number of statistically significant relations between gas emissions with soil and OM properties: The initial N$_2$O and cumulative N$_2$ emissions over seven days correlated positively with the content of WEOC ($r = 0.82$ and 0.69, respectively, $p < 0.001$, $n = 24$). In accordance with the narrow range of WEOC/WEON ratios this held also true for WEON ($r = 0.70$ and 0.77, respectively, $p < 0.001$, $n = 24$). In addition, the cumulative N$_2$O emissions were highly correlated to the cumulative release of CO$_2$

Table 3
Total release of nitrogenous gases (N$_2$O + N$_2$) and molar N$_2$O/(N$_2$O + N$_2$) ratios of cumulative gas emissions after one and seven days of incubation of soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP). Values represent means ($n = 4$) $\pm$ standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

Fertilization treatment	Cumulative N$_2$O + N$_2$ [mg N kg^{-1}]		N$_2$O/(N$_2$O + N$_2$) ratio	
	After 1 day	After 7 days	After 1 day	After 7 days
Control	1.7 ± 0.4c	12.6 ± 0.3c	0.14 ± 0.09c	0.00 ± 0.00b
N	3.9 ± 0.5b	17.1 ± 1.7bc	0.29 ± 0.06bc	0.01 ± 0.00ab
NP	3.9 ± 0.2b	19.2 ± 3.3b	0.43 ± 0.05b	0.11 ± 0.15ab
FYM	5.9 ± 1.3a	25.9 ± 3.5a	0.68 ± 0.14a	0.20 ± 0.09ab
FYM + N	6.0 ± 0.7a	27.2 ± 2.4a	0.64 ± 0.03a	0.27 ± 0.03ab
FYM + NP	5.5 ± 0.5a	26.1 ± 1.5a	0.68 ± 0.05a	0.28 ± 0.01a

Fig. 2. Cumulative N_2O emission during anoxic incubation under excess nitrate (50 mg NO_3^--N kg^{-1}) at 25 °C for soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP). Error bars show standard deviation of mean values ($n = 4$) per day in one direction only.
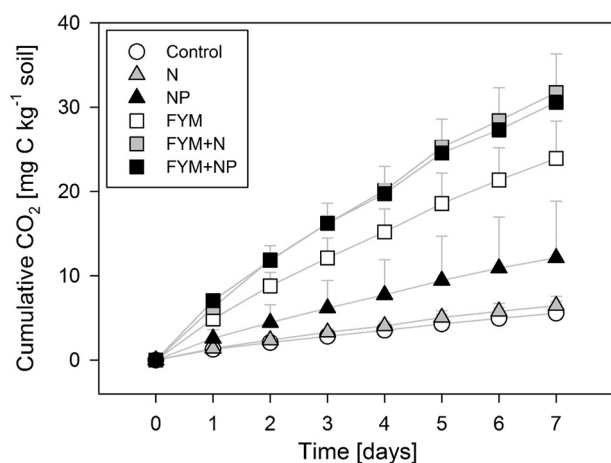

Fig. 4. Cumulative N_2 emission during anoxic incubation under excess nitrate (50 mg NO_3^--N kg^{-1}) at 25 °C for soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP). Error bars show standard deviation of mean values ($n = 4$) per day in one direction only.

Fig. 3. Cumulative CO_2 emission during anoxic incubation under excess nitrate (50 mg NO_3^--N kg^{-1}) at 25 °C for soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP). Error bars show standard deviation of mean values ($n = 4$) per day in one direction only.

over the seven incubation days ($r = 0.96$, $p < 0.001$, $n = 24$). The maximum N_2O production was significantly correlated with the proportion of O/N-alkyl C of bulk soils ($r = 0.92$, $p < 0.01$, $n = 6$) and the CO_2-C/WOEC ratio ($r = 0.94$, $p < 0.001$, $n = 24$; Fig. 5a). No statistical relations were found between the absolute contents of particulate organic material (fPOM, oPOM) or their proportions in bulk soil OC (POM-C/soil OC) as well as of contents of MOM-C and respective CO_2, N_2O, and N_2 emissions. Likewise, pH (before and after incubation), soil OC/ON ratio, and content of available P were not significantly related to measured gas emissions.

3.4. Potential denitrification and product ratios

After one day of incubation, N_2O was the dominant denitrification product of all manured soils (64%–68%); for soils receiving mineral or no fertilization, N_2O contributed merely to 14%–43% (Table 3). The initial molar $N_2O/(N_2O + N_2)$ product ratio was negatively correlated to the soil OC/ON ratio and positively to the WEOC content ($r = -0.87$, $r = 0.75$, respectively, $p < 0.001$, $n = 24$). The initial total denitrified N ($N_2O + N_2$ after one day of incubation) of manured

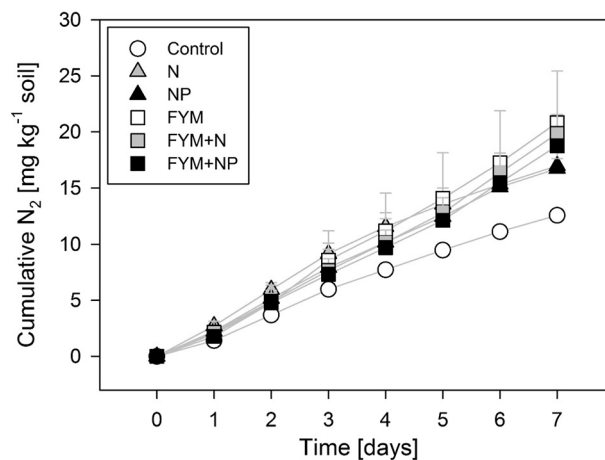soils was significantly higher (by 49%) than for soils under mineral fertilization (Table 3). Similar to N_2O production, the initial total denitrified N was well correlated to WEOC (Fig. 5b) and WEON ($r = 0.78$ and 0.76, respectively, $p < 0.001$, $n = 24$). During seven days of anoxic incubation, the molar $N_2O/(N_2O + N_2)$ ratio decreased for all treatments to 0.2–0.3 (FYM treatments), 0.01–0.1 (mineral fertilization), and 0.00 (control) (Table 3). At the end of incubation, the total release of nitrogenous gases by denitrification from the soils receiving only mineral fertilizers were still significantly lower (31%) than of those with FYM application but still 44% larger than of the control soil (Table 3). There was no significant difference in denitrified N (25.9–27.2 mg kg^{-1}) within the seven incubation days between manured soils receiving no or additional mineral fertilizers. The cumulative $N_2O + N_2$ production over the seven days was again positively correlated with the WEOC content ($r = 0.75$, $p < 0.001$, $n = 24$).

3.5. Abundance of denitrifier genes

For pre-incubated soils, the different fertilization regimes had no distinct effect on the abundance of NO_2^- reductase genes (*nirS* and *nirK*) and the N_2O reductase gene *nosZ II* (Fig. 6a–d). However, *nosZ I* (N_2O reductase) genes were significantly more abundant in soils receiving FYM than in soils under other fertilization regimes, even before anoxic incubation (Fig. 6c). In non-manured soils, abundances of *nirS* and *nosZ II* genes decreased during anoxic incubation by 35%–45% and about 50%, respectively, while the abundances of *nosZ I* genes increased by 130% (Fig. 6a, c, d). In soils under FYM application, *nosZ I* genes increased as well but the increases were by 58%–92% lower than in non-manured soils. Overall, soils receiving FYM showed significantly higher absolute abundances of NO_2^- reductase genes (*nirS* + *nirK*) and N_2O reductase genes (*nosZ I* + *nosZ II*) after anoxic incubation than soils receiving either no or only mineral fertilizers (Fig. 6a–d). The abundances of *nirS* genes were substantially higher than those of *nirK* for all treatments. While *nosZ I* gene copy numbers were mostly smaller than *nosZ II* before anoxic incubation, except for the FYM + N treatment, *nosZ I* was the dominant N_2O reductase gene at the end of incubation.

No significant relation between pH (before and after incubation) and abundance of denitrifier genes were observed. However, the initial *nosZ I* gene abundances (after aerobic pre-incubation) were positively correlated to Olsen P ($r = 0.80$, $p < 0.001$, $n = 24$). The initial abundances of *nirK* and *nosZ I* as well as the gene copy numbers of *nirS* and *nosZ I* after anoxic incubation correlated positively with the WEOC
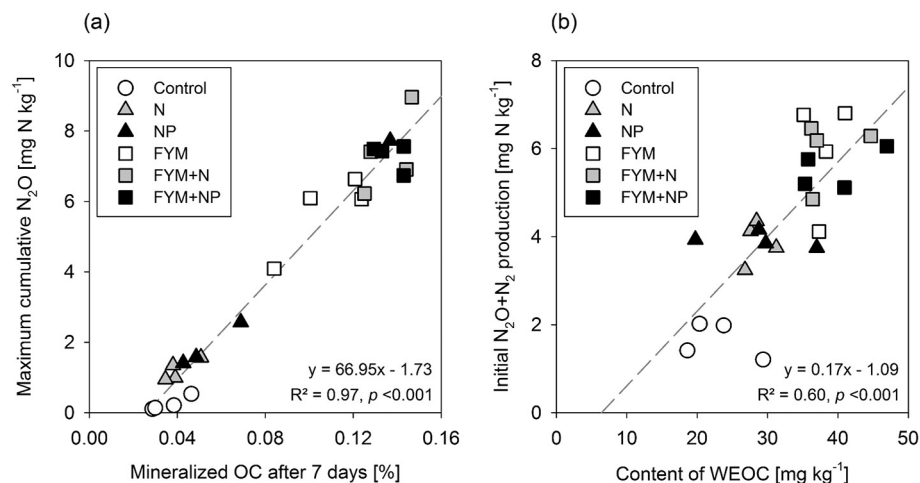
Fig. 5. Relationship between (a) mineralized OC (CO_2-C/OC) after seven days of anoxic incubation and maximum cumulative N_2O production as well as (b) between the content of water-extractable OC (WEOC) and initial $N_2O + N_2$ production for soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP).

content (r = 0.76, 0.86, 0.81, and 0.79, respectively, $p < 0.001$, n = 24; Fig. 7a, b). The abundance of NO_2^- reductase genes showed no relationship to the N_2O production. Also, the abundances of N_2O reduction genes were not significantly correlated to the production of N_2. In contrast, the initial abundances of $nosZ\ I$ were highly correlated with the initial N_2O production (r = 0.90, $p < 0.001$, n = 24).

4. Discussion

In accordance with our initial assumption, we found that different fertilization regimes affected not only chemical soil properties and total OC contents but also amounts of readily decomposable OM, as indicated by higher proportions of water-extractable and particulate OM as well as O/N-alkyl C components. In the following we relate these

changes to the denitrification potential, gas product ratios, and gene abundances.

4.1. Potential denitrification and product ratios as related to OM functional fractions

Denitrification potential and product ratio are both controlled by soil reaction (e.g., Bremner and Shaw, 1958; Saggar et al., 2013). Lower pH values tend to cause decreased denitrification rates but larger molar $N_2O/(N_2O + N_2)$ product ratios (Čuhel et al., 2010). However, except for NP, the soil acidity (pH) varied only slightly between fertilization treatments (Table 1). Accordingly, we observed no distinct relations between soil reaction and CO_2, N_2O, and N_2 emissions. In contrast to soil reaction, the fertilizer-induced variation in OM appeared as major
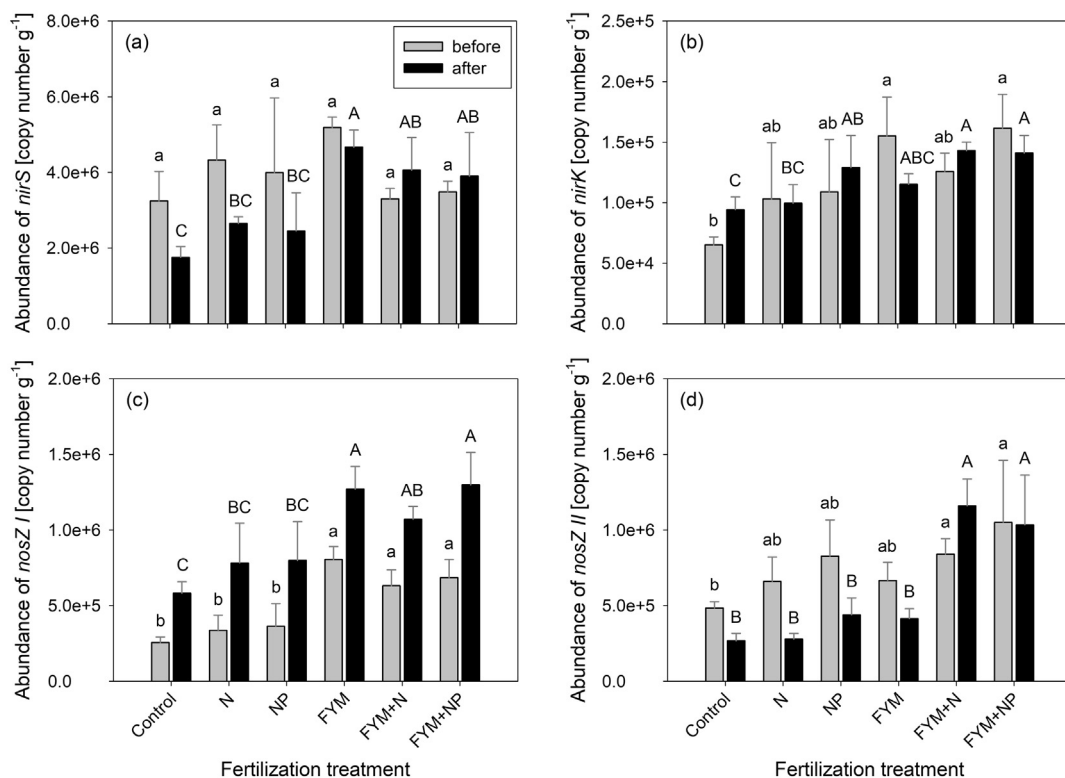

Fig. 6. Abundance of (a) *nirS*, (b) *nirK*, (c) *nosZ I*, and (d) *nosZ II* genes before (aerobically pre-incubated) and after anoxic incubation under nitrate excess for seven days at 25 °C. Different letters indicate significant differences ($p < 0.05$) between different fertilization treatments (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP) before (small letters) and after (capital letters) anoxic incubation. Error bars show standard deviation of mean values (n = 4) in one direction only.
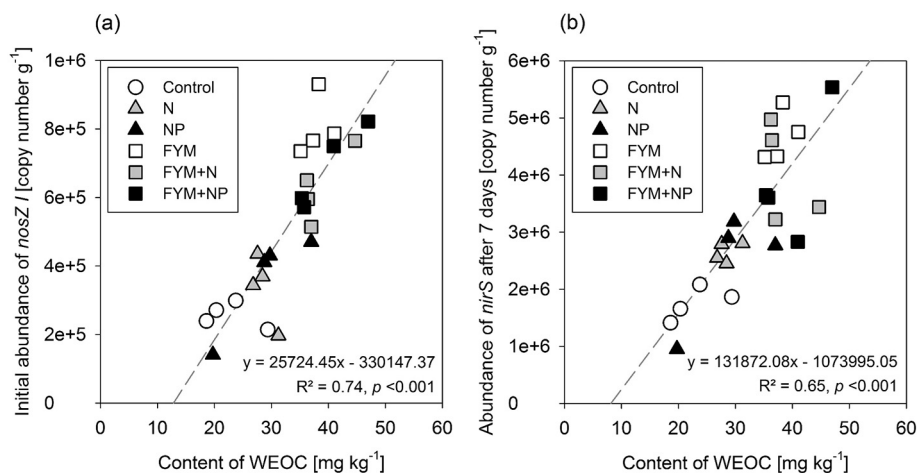
Fig. 7. Relationship between (a) the water-extractable OC (WEOC) content and the initial abundance of *nosZ I* genes and (b) abundance of *nirS* genes after seven days of anoxic incubation for soils from the Static Fertilization Experiment (control, mineral N and NP, farmyard manure – FYM, and FYM + mineral N or NP).

control on denitrification. Despite POM with adhering microorganisms being considered to have substantial effects on soil denitrification (Parkin, 1987; Parry et al., 2000), we observed no direct relations between POM-C and the N₂O and N₂ production. Gaillard et al. (2003) showed that soluble OC from POM enters the adjacent soil (several mm) and fuels microbial processes. Consequently, POM-derived WEOC could be a major factor in denitrification. In accordance, we found a positive relationship between the content of WEOC and the share and quality (OC/TN ratio) of fPOM as well as between the WEOC content and the production of N₂O and N₂ (Fig. 5b). These findings suggest that POM facilitates denitrification rather due to its large fraction of leachable C than because of being an easily accessible C source.

The positive correlation between WEOC contents and the contribution of the ratio of fPOM-OC to bulk soil OC suggests that the main part of soluble OC derived from fPOM, despite MOM-C the main portion of bulk soil OC (91%–97%). The observed negative correlation between WEOC and the OC/TN ratio of fPOM is consistent with the fact that plant residues with low C/N ratio decompose more rapidly than residues with higher C/N ratio (Aulakh et al., 1991; Lynch et al., 2016), and thus, releasing more soluble OC and causing higher CO₂ and N₂O emissions (Huang et al., 2004). Gaillard et al. (2003) showed that water-soluble OC in residues (young rye leaves) can comprise up to 23% of the total residue-C. Thus, plant residues are a major source of leachable OC in surface soils (McCarty and Bremner, 1993). The low and strikingly invariable WEOC/WEON ratios (4.3–5.5) suggest that those leachable components contained a large proportion of proteinaceous material, possibly originating from POM-associated microbial biomass. The C/N ratio of microbial biomass usually varies in the range of 6 to 9 (e.g., Cleveland and Liptzin, 2007).

Our results suggest, therefore, that the amount of fPOM and the related production of water-soluble OM determine the denitrification potential. This is in line with the notion that dissolved OM is the most important substrate and electron donor in denitrification reactions (Bremner and Shaw, 1958; Ottow, 2011). When pooling all fertilization treatments, the anoxic OC mineralization (CO₂ production) was well related to the N₂O production (Fig. 5a). This again supports the idea that denitrification is fueled by soluble OM and that a high bioavailability of C sources promotes incomplete denitrification (high N₂O/N₂ product ratio) in situations where oxygen is absent and nitrate not limited ('hot spots').

We also assume that the declining bioavailability of WEOC over the incubation time caused the decrease or leveling off in cumulative N₂O over the course of the incubation (Fig. 2). Since manured soils contained more labile OM than mineral fertilized and unfertilized soils, readily available C was longer available to denitrifying organisms,

resulting in larger overall gas emissions and continued gross N₂O production over the entire incubation period. In contrast, N₂O was no longer produced in N-fertilized soils and the control after only a few days, along with the complete depletion of previously accumulated N₂O at the end of incubation. The decreasing molar N₂O/(N₂O + N₂) ratio over time was mainly due to continuous production of N₂, while the gross N₂O formation remained at low level and previously accumulated N₂O was gradually reduced to N₂. Although the average N₂ production after seven days was significantly lower for all non-manured samples than for the FYM treatments, the differences in N₂ emission between fertilization regimes were small (Fig. 4). This indicates that the N₂O production was more strongly affected by the OM bioavailability than the N₂ emission or the total denitrification rate. The loss of 22%–40% of the initial (natural + added) NO₃⁻-N as N₂O or N₂ during incubation and the CO₂-C production representing 23%–82% of the initial WEOC may indicate that the microbial use efficiency of C and nitrate differed between fertilization regimes. Under exclusion of plant effects and related N limitation, the total emission of nitrogenous gases (N₂O + N₂) was, therefore, significantly higher in soils with FYM application than in soils with mineral and no fertilization (Table 3). As hypothesized, the accumulation of readily decomposable OM in manured soils, reflected by higher portions of components rich in O/N-alkyl C, fPOM, and WEOC, resulted in increased denitrification potential with increased proportions of N₂O. Our results show that not only the potential denitrification rate in total but also the product ratio was strongly affected by the content of WEOC that mostly derived from easily degradable POM sources.

4.2. Responses of denitrifier gene abundances to fertilization-induced changes in soil organic matter

Manured soils had significantly higher abundances of *nosZ I* genes than other soils, even before anoxic incubation (Fig. 6c). This observation may be ascribed to the fact that the higher C availability in manured soils results in faster C mineralization and O₂ consumption, and thus, − over long periods of time – supports larger abundances of complete denitrifiers, especially within anoxic microsites ('hot spots') where nitrate becomes limited. This could also explain why the initial gene abundances of *nosZ I* were related to initial WEOC contents (Fig. 7a). During anoxic incubation, *nosZ I* gene abundances increased for all treatments, especially in those under mineral fertilization, whereas abundances of *nosZ II* genes either decreased or remained roughly constant (Fig. 6c, d). One explanation for this observation could be that N₂O reduction kinetics of organisms having *nosZ II* genes differ from those with *nosZ I* genes. For example, Conthe et al. (2018)

reported that non-denitrifying N_2O reducers with *nosZ II* genes have a lower affinity for N_2O than canonical denitrifiers (bacteria with *nosZ I*) under N_2O- and C-limiting conditions. In addition, most organisms with *nosZ I* genes also possess *nirS* or *nirK* genes, thus, are able to reduce NO_2^- (Graf et al., 2014), while organisms with *nosZ II* often respire N_2O alone. This might explain why the initial N_2O emissions were only related to *nosZ I* and not to *nosZ II* gene abundances.

Changes in *nirK* gene abundances during incubation were only small and irregular across treatments (Fig. 6b). Considering the low gene copy numbers, *nirK* might play a minor role in N_2O production compared to *nirS*. The abundances of *nirS* genes decreased within seven incubation days only in soils without FYM application (Fig. 6a). Since not only the abundances of *nosZ I* but also of *nirS* genes after incubation were positively related to initial WEOC contents, this was probably due to limitation of suitable C substrates. This could also explain why the linear relationship between *nirS* gene abundance after incubation and WEOC contents was closer for non-manured soils than for manured soils (Fig. 7b). Henderson et al. (2010) found higher *nosZ I* gene abundances in soils (coarse loamy till; pH 6) amended with different POM materials than in soil amended with glucose, while the abundance of $nirS_p$ gene-bearing denitrifiers (*P. mandelii* and related species) was only increased by glucose addition. This is in line with our assumption that NO_2^--reducing organisms (having *nirS* genes) were more dependent on readily available C substrates than N_2O-reducing organisms with *nosZ I* genes. Accordingly, large abundances of *nirS* genes occurred in manured soils even at the end of the incubation (Fig. 6a). This suggests that the availability of WEOC not only determined the amount of denitrified N but also the $N_2O/(N_2O + N_2)$ product ratio. Correspondingly, gross N_2O production still continued after seven days, resulting in higher molar $N_2O/(N_2O + N_2)$ ratios for manured soils (0.2–0.3) than for soils under mineral fertilization (0.01–0.1; Table 3). Based on our results, we assume that small amounts of bioavailable OM favored complete denitrifiers (i.e., N_2O-reducing organisms) with *nosZ I* genes when NO_3^- was not limiting. Those complete denitrifiers are generally in advantageous position, since the maximum energy production of the complete reduction to N_2 is 10% higher than for the incomplete denitrification (release of N_2O) (Ottow, 2011). Consequently, N_2 was the dominant product in mineral or unfertilized soils, even at the beginning of the incubation (Table 3). In turn, larger amounts of WEOC favored increased denitrification with larger shares of N_2O. The fact that abundances of *nosZ I* and *nirS* genes after incubation as well as the production of $N_2O + N_2$ increased upon FYM application and both, gene abundance and denitrification potential, were well related to the content of WEOC underpins the relevance of WEOC for denitrification in agricultural soils.

5. Conclusions

As hypothesized, fertilization treatments causing stronger accumulation of labile OM resulted in increased denitrification with larger proportions of N_2O, while treatments causing smaller portions of readily decomposable OM favored complete denitrifying organisms. Therefore, this study highlights the close link between soil OM and denitrification potential, with larger portions of labile C substrates promoting denitrification reactions. In particular, we found that water-soluble OC readily available to denitrifiers shapes their community composition on a short-term, and thus, determines the overall denitrification and the $N_2O/(N_2O + N_2)$ product ratio in situations where oxygen is absent and nitrate not limited ('hot spots'). Despite soil OC was mainly present in the MOM fraction, water-soluble OC itself appears to largely derive from fPOM (i.e., undecomposed organic debris, especially enriched in manured plots); its source strength for water-soluble OC seemingly increases with decreasing C/N ratio of the fPOM. Consistent with our hypotheses, readily decomposable OM, especially water-soluble OC, seems to be a general and easily measurable indicator of a soil's immediate denitrification potential. The additional

determination and characterization of fPOM might offer a possible estimate for the production potential of water-soluble OC, and consequently, for the denitrification potential along longer time scales. The observed control of water-soluble OC on the potential denitrification also prompts investigating the possible effects of other, similar easily decomposable organic substrates, such as root exudates.

Declaration of competing 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103630.

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