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RESEARCH ARTICLE

Effects of warming and drought on potential N_2O emissions and denitrifying bacteria abundance in grasslands with different land-use

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One sentence summary: Microorganisms in grassland soils respond to climate change, showing enhanced denitrification potential after experimental warming and drought, but these effects seem to be overruled by site-specific land-use and geographic region.

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ABSTRACT

Increased warming in spring and prolonged summer drought may alter soil microbial denitrification. We measured potential denitrification activity and denitrifier marker gene abundances (*nirK*, *nirS*, *nosZ*) in grasslands soils in three geographic regions characterized by site-specific land-use indices (LUI) after warming in spring, at an intermediate sampling and after summer drought. Potential denitrification was significantly increased by warming, but did not persist over the intermediate sampling. At the intermediate sampling, the relevance of grassland land-use intensity was reflected by increased potential N_2O production at sites with higher LUI. Abundances of total bacteria did not respond to experimental warming or drought treatments, displaying resilience to minor and short-term effects of climate change. In contrast, *nirS*- and *nirK*-type denitrifiers were more influenced by drought in combination with LUI and pH, while the *nosZ* abundance responded to the summer drought manipulation. Land-use was a strong driver for potential denitrification as grasslands with higher LUI also had greater potentials for N_2O emissions. We conclude that both warming and drought affected the denitrifying communities and the potential denitrification in grassland soils. However, these effects are overruled by regional and site-specific differences in soil chemical and physical properties which are also related to grassland land-use intensity.

Keywords: climate change; microbial community; denitrification; grassland; land-use index; potential N_2O emissions; Biodiversity Exploratories

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INTRODUCTION

Soil microorganisms are key drivers of nutrient cycling and therefore essential for ecosystem functioning (Wardle *et al.* 2004; van der Heijden, Bardgett and van Straalen 2008). The factors controlling soil microbial-driven ecosystem function are not fully understood to date (Mooney *et al.* 2009; Gärdenäs *et al.* 2011); for example, many nitrogen cycling processes are described at the enzymatic level, but substantial uncertainty remains when predicting associated trace gas fluxes at the ecosystem level.

Soil microbial nitrification and denitrification are crucial processes for N-cycling in soil, e.g. controlling plant inorganic N availability, nitrate (NO_3^-) leaching and the production of nitrous oxide (N_2O) (Firestone, Firestone and Tiedje 1980; Wrage *et al.* 2004). N_2O has a warming potential 300 times higher than CO_2 and thus is an effective greenhouse gas despite its relatively low atmospheric concentrations (Galloway *et al.* 2004). N_2O also contributes to the destruction of the ozone layer (e.g. Forster *et al.* 2007).

A major source of N_2O is soil microbial denitrification, in that NO_3^- is sequentially reduced to NO, N_2O and N_2, all of which can escape to the atmosphere. Functional genes of denitrification are generally induced and only expressed under anaerobic conditions, as high soil water content (SWC) and low O_2 concentrations inhibit the complete denitrification and increase soil emissions of NO and especially of N_2O (Smith and Tiedje 1979; Bollmann and Conrad 1998). In denitrifying bacteria, the nitrite reductase enzyme is encoded by *nirK* or *nirS* genes. N_2O is reduced to N_2 by the nitrous oxide reductase (*nosZ* genes) (Philippot 2002), while some denitrifying soil microorganisms lack the *nosZ* fragment and release N_2O as final denitrification product (Zumft 1997; Philippot 2002). A recent study by Philippot *et al.* (2011) showed a relationship between denitrifiers lacking the *nosZ* gene and potential N_2O emissions, thus demonstrating the importance of the nature of the microorganisms involved in N-cycling for soil N_2O emissions.

In the soil environment, N_2O emissions strongly depend on the microbial potential for denitrification, but also on soil environmental conditions including temperature, SWC, oxygen potential, mineral N, organic carbon availability and acidity (Smith *et al.* 2003; Niklaus, Wardle and Tate 2006; Jones *et al.* 2007; Cuhel *et al.* 2010). More distal controls of N_2O emissions include land management (Flechard *et al.* 2007) and plant diversity (Niklaus, Wardle and Tate 2006), but again, the exact underlying mechanisms are not well understood.

Although the role of functional genes and processes that influence microbial denitrification is increasingly well understood, much less is known about the controls of gas fluxes in undisturbed ecosystems. Understanding these controls however is crucial when aiming at understanding climate-change effects on gas fluxes of microbial denitrification in soils.

N_2O emissions from grasslands depend on different management practices (Mosier *et al.* 1991; Velthof *et al.* 1996), e.g. the amount of fertilizer application, mowing practices and grazing intensity (Blüthgen *et al.* 2012), which can be expressed as site-specific land-use indices (LUI). Management strongly controls N input and output and thus nutrient status of the soils.

Beside the effects of land-use, the influence of climate change, with expected changes in temperature and precipitation and consequently SWC, is likely to affect soil N turnover (Vitousek *et al.* 1997; Melillo *et al.* 2002; Singh *et al.* 2010). Both soil temperature and moisture affect soil microbial activity and soil aeration, thus controlling the emission of N_2O (e.g. Skiba and Smith 2000; Dobbie and Smith 2001; Horváth *et al.* 2010).

In the present study, we investigate the influence of experimental soil warming in spring and summer drought on the N_2O emission potential from denitrification and relate it to the abundance of denitrifying bacteria. This study design was replicated in grassland soils differing in land-use, separately in three regions of Germany differing in soil characteristics ('Biodiversity Exploratories', for details see Fischer *et al.* 2010). The degree of land-use was defined individually for each grassland site through an LUI (Blüthgen *et al.* 2012), which integrates mowing, grazing and fertilization at the sites over the last three years before sampling the soil. We further tested whether these effects depended on soil organic carbon content, water holding capacity (WHC) and acidity to uncover effects of these more static site properties in interaction with grassland land-use, the region and climate-change treatment. In addition, we tested to which extent the N_2O emission potential was further influenced by more dynamic properties, e.g. the actual water content, the availability of organic carbon and nitrate or the size of the denitrifier community itself.

We hypothesized that warming would increase the denitrification potential due to increased activity of denitrifiers. In contrast, we expected drought to reduce microbial activity. We further anticipated that grassland sites with higher LUI should have greater denitrification potentials than low LUI grasslands due to e.g. a higher availability of nitrogen from fertilizer inputs. By investigating similar grassland land-use within each of three different regions, we aimed to identify the main explanatory factors for the effects of climate change on the denitrification potential in grassland soils.

MATERIALS AND METHODS

Study sites and experimental design

The studied grassland plots are located in three regions of Germany, namely (i) the UNESCO Biosphere Reserve *Schwäbische Alb* in southwestern Germany, (ii) the *Hainich* National Park and its surroundings in central Germany, and (iii) the UNESCO Biosphere Reserve *Schorfheide* in northeastern Germany. In the following, these regions are referred to as 'Exploratories' (Fischer *et al.* 2010) (Table 1). In each Exploratory, 15 grasslands were selected; each site was characterized by an individual LUI derived from fertilization, mowing and grazing impact on the sites (Blüthgen *et al.* 2012) (Table S1, Supporting Information). At each site, two 2 × 3 m subplots were established, one of which was subjected to a climate manipulation treatment (= experimental plot) whereas the other subplot served as undisturbed control (Bütof *et al.* 2011). The climate manipulation was based on regional climate-change predictions (Christensen and Christensen 2007), and therefore combined spring warming and summer drought.

All soil samples analyzed in this study were collected in 2009. The warming treatment started in spring 2009, followed by the summer drought experiment 2009. As a pre-experimental treatment, experimental plots had one additional drought that was performed for the first time in summer 2008.

The warming treatment was achieved by passively increasing air temperature with an open-top chamber, where the side walls were made of transparent plastic foil (2 × 3 m ground area × 1.4 m height, UV 5 coex-foil, folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany). The warming treatment in the *Schwäbische Alb* started at the end of March until the second week of May, the warming treatment in the *Hainich* was conducted from the second week of February until end of March and

Table 1. Study regions, climatic conditions, main soil types, pH, WHC and effects of warming and rain exclusion for the grassland sites in the three investigated Exploratories. Experimental warming in spring was set up 44 days in *Schwäbische Alb* and *Schorfheide* (*Hainich*: 45 days). Rain exclusion was set up 45 days in summer in *Schwäbische Alb* and *Schorfheide*, and 43 days in *Hainich*. Values in brackets indicate standard deviation.

	Schwäbische Alb	Hainich	Schorfheide
Mean annual temperature (°C)[1)]	6°C–7°C	6.5°C–8°C	8°C–8.5°C
Mean annual precipitation (mm)[1)]	700–1000 mm	500–800 mm	500–600 mm
Elevation (m a.s.l.)	460–860 m	285–550 m	3–140 m
Main soil types	Leptosol, Cambisol	Cambisol, Stagnosol, Vertisol	Histosol, Gleysol
pH (0.01 M $CaCl_2$)[2)]	6.8 (0.4)	7.2 (0.3)	7.2 (0.5)
WHC (%)	78.1 (12.6)	44.9 (5.3)	85.7 (11.1)
Warming effect (°K)[3)]	+0.45 (0.82)	+0.30 (0.58)	+0.19 (0.46)
Drought effect (Δ SWC%)[4)]	−32.4 (9.7)	−10.1. (22.8)	−17.8 (47.1)

[1)] Fischer *et al.* (2010).
[2)] Determined at the intermediate sampling in 2009.
[3)] Mean temperature difference of experimental vs. control plots in 10 cm soil depth during experiment.
[4)] Average Δ SWC in% of max. WHC at the soil sampling after rain exclusion between experimental and control plot.

the warming treatment in the *Schorfheide* was conducted from the beginning of March until the middle of April in 2009 (see Table S5, Supporting Information, for exact dates).

The summer drought treatment that was performed for the first time in summer 2008 was continued in June 2009 by covering the roof of the open-top chamber with transparent plastic foil and leaving the side walls open. The effectiveness of this passive warming was monitored by recording soil temperatures at 10 cm depth every 30 min (Thermochron iButton logger, Maxim Integrated Products, Inc., Sunnyvale, USA). The drought treatment started in the second or third week of June and lasted until the end of July or the first week of August 2009, depending on Exploratory (see Table S5, Supporting Information, for exact dates).

Soil sampling

Soils were sampled three times in 2009. A first sampling took place after the experimental warming period in spring 2009 (= in the following named warming sampling) to test for effects of warming alone (plus potential carryover effects from the drought experiment already conducted in summer 2008, see Bütof *et al.* 2011). Soils were sampled a second time immediately before installing the rain exclusion roofs (= in the following named intermediate sampling), potentially reflecting longer term or continuing effects of the warming treatment in 2009, and possibly also effects of the drought applied in 2008. A third sample was collected after the drought treatment 2009, reflecting effects of rain exclusion plus the cumulated long-term effects of all previous treatments (= in the following named drought sampling). The sampling dates are summarized in Table S5 (Supporting Information).

At each sampling, five soil cores (5 cm diameter × 10 cm depth) were collected at random locations within the subplots, pooled and transported to the lab where they were kept frozen (−20°C) until further analysis. Prior to analyses, roots, stones and soil macrofauna were removed, and soils were sieved (<5 mm).

SWC, pH, total C and N content, extractable organic carbon and nitrogen, and mineral N content

The soil samples were allowed to thaw overnight in a refrigerator before the following analyses were performed. Gravimetric SWC was determined by drying soil at 60°C for 3 days and calculated as a percentage of the maximum WHC of the soils. Total soil C and N were analyzed from a dried and ball mill ground 5 mg soil subsample and measured with an elemental analyzer (LECO TruSpec CHN, LECO Corporation, St. Joseph, MI, USA).

Extractable organic carbon (EOC) and nitrogen (EON) were determined in 2 g soil subsamples extracted with 8 mL 0.5 M K_2SO_4 on a horizontal shaker (30 min, 250 rpm) followed by centrifugation (30 min, 4400 g). EOC and EON were measured in the supernatants (Dimatoc 100 DOC/TN-analyzer, Dimatec, Essen, Germany).

Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted for 20 min from 5 g soil using 50 mL 1 M KCl. After filtering (Black Ribbon 589/1 filter paper, Whatman, Maidstone, UK), NH_4^+ and NO_3^- concentrations were determined colorimetrically using an Eppendorf EPOS 5060 spectrometer (Eppendorf AG, Hamburg, Germany).

Potential denitrification

Potential denitrification was determined using a modified assay of Smith and Tiedje (1979), with addition of chloramphenicol to inhibit the *de novo* synthesis of proteins during incubation. In brief, two replica of 2 g of the frozen soil samples were gently thawed at 4°C for 3 days and incubated in air-tight bottles (inner volume of 118 mL) with 5 mL solution containing 1.1 mM KNO_3, 1 mM glucose and 0.7 mM chloramphenicol. Anaerobic conditions were established by evacuating and flushing the headspace with N_2 gas three times. One replicate remained untreated, while for the second one, 10 mL N_2 was removed and replaced by 10 mL acetylene (C_2H_2) to inhibit nitrous oxide reductase activity. Bottles were incubated at 25°C on a horizontal shaker (150 rpm) and 1 mL headspace samples were taken after 30, 60, 90 and 120 min from each sample and transferred into evacuated 5.9 mL septum-capped exetainers (Labco Ltd, UK). These samples were diluted with 10 mL N_2 before gas chromatic analysis (Agilent 7890 gas chromatograph equipped with an ECD detector, Agilent, Santa Clara, CA, USA). Potential N_2O release (ng N_2O g^{-1} dry soil h^{-1}) from soil was calculated from the linear regression of N_2O concentration against time.

The sum of N_2O + N_2 is referred to the potential denitrification activity, when C_2H_2 is added to the assay. N_2O is referred to the potential N_2O production, when all enzymes of

denitrification are active, and includes both the production and consumption of N_2O.

DNA extraction

Soil subsamples (0.2 g) were agitated briefly on a Fast Prep FP 120 shaker (Qbiogene, Illkirch, France) before DNA extraction (FastDNA® SPIN for Soil Kit, MP Biomedicals, LLC, Solon, OH, USA). Amounts and quality of DNA were determined with a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

16S rRNA gene and denitrifier functional gene abundances

The abundances of 16S rRNA genes and the denitrifier functional genes *nirK, nirS* and *nosZ* were determined by quantitative PCR according to Keil *et al.* (2011) (Table S4, Supporting Information). Prior to all assays, optimal template dilutions were determined to minimize inhibitory effects of coextracted soil compounds.

Statistical analyses

All data were analyzed by analysis of variance based on mixed-effects models reflecting the design of the study. Models included Exploratory as fixed effects (similar to block term in standard block designs). LUI, warming/drought treatment and their interaction were also fitted as fixed effects. Random effects were plot and plot × treatment (i.e. the subplot, which was equivalent to the residual for most data sets). Depending on analysis, we further included covariates as fixed effects, e.g. soil pH, soil C content and soil WHC. These were fitted after LUI but before treatment.

When testing for effects of acetylene inhibition on potential N_2O production, data sets included two entries for each subplot, depending on assay type (with or without acetylene inhibition). In this case, we included the random effects plot, plot × type and plot × treatment in the model (plot × type × treatment equaled the residual). These terms were required to ascertain proper replication for significance testing of the respective fixed effects (LUI tested against plot, LUI × type against plot × type, LUI × treatment against plot × treatment and LUI × type × treatment against the residual).

Functional gene abundances, potential denitrification, and soil NH_4^+ and NO_3^- data were log-transformed prior to analysis. The different sampling dates were analyzed separately. All analyses were performed in R (2012), using ASReml 3.0 (VSNI International, Hempstead, UK). We also analyzed the data using classical linear models with sequential sum of squares, fitting the 'random effects' at the appropriate position. This analysis is virtually equivalent to the mixed model given that the design is balanced; we estimated the relative amount of variance related to the respective fixed effects (or groups of fixed effects) from the percentage of total sum of squares associated with these terms.

RESULTS

Experimental treatments

Experimental warming in spring increased average soil temperatures by +0.45°C in the *Schwäbische Alb*, by +0.30°C in the *Hainich* and by +0.19°C in the *Schorfheide* (Table 1).

Experimental drought significantly reduced average SWC relative to control plots, with −32.4% in the *Schwäbische Alb*, −10.1% in the *Hainich* and −17.8% in the *Schorfheide* (average ΔSWC in % of max. WHC at the soil sampling after rain exclusion between experimental and control plot) (Table 1).

Effects of site-specific properties

Site-specific properties are considered to represent rather persistent conditions, not already altered by changing environmental conditions. The potential of the grassland soils for N_2O production was significantly increased by 15.0% in the elevated soil temperature treatment at the end of the warming experiment ($P = 0.008$, Table S3, Supporting Information), explaining 1.2% (percent sum of squares, SS) of the total variation. At the intermediate sampling, no effect of the spring warming treatment was detectable anymore, but the potential for N_2O production strongly increased with increasing LUI ($P < 0.001$; Fig. 1A) and slightly with an increasing C content of the soils ($P = 0.04$) at the intermediate sampling, explaining 20.9 and 3.5% of the variation, respectively. Similar to the intermediate sampling, LUI positively influenced N_2O production also after the drought treatment ($P = 0.003$), explaining 16.7% of the variation without any significant influence of the drought treatment itself (data not shown). However, after drought the WHC of the soils also tended to affect the N_2O emission with slightly higher rates in soils with lower WHC ($P = 0.07$), accounting for 4.9% of the variation.

The total denitrification potential of the grassland soils was increased with LUI at all three sampling dates after warming (Fig. 1B), at the intermediate sampling (Fig. 1C) and after drought (Fig. 1D). The effect of LUI was most pronounced at the intermediate sampling in comparison to after warming and after drought, explaining 21.1, 11.6 and 17.8% of the variation, respectively. In addition, the highest potentials for gaseous N losses from the soils were found in soils with low soil C contents ($P = 0.023$) at the intermediate sampling, accounting for 4.3% of the variation.

Total abundances of bacteria (16S rRNA genes) significantly differed between the three Exploratories at all samplings ($P < 0.001$) and this effect accounted for > 89% variation in all Exploratories. Highest abundances were detected in the *Schorfheide*, followed by *Schwäbische Alb* and *Hainich*. At all samplings, bacterial abundances were positively affected by LUI (Fig. S1A–C), and the effect of LUI was more pronounced at the intermediate (1.5% SS) and after drought sampling (1.0% SS) than after warming (0.7% SS). At all dates 16S rRNA gene abundances were also positively related to the soil C content and the WHC of the soils. Yet, only after warming the explained variance of soil C was higher than that of LUI (1.2 and 0.7% SS, respectively).

Similar to total bacteria, abundances of all three denitrifier functional genes (*nirK*, *nirS* and *nosZ*) depended on Exploratory ($P < 0.001$), explaining > 61.8% of the total variance. *Highest* gene copy numbers were detected in the *Schorfheide*, and lowest in the Hainich (Table S2, Supporting Information). The abundance of the *nirS* genes was additionally negatively affected by soil pH at the intermediate sampling ($P = 0.04$), explaining 1.9% of the variance. Abundances of *nirK* genes showed significant and positive relationships with LUI at all sampling occasions, explaining between 4.4 and 6.5% of the variance ($P < 0.01$). This effect was most pronounced in the *Schwäbische Alb* and *Hainich* sites (exemplarily shown for the intermediate sampling, Fig. 2A). Similar to *nirK*, gene abundances of *nosZ* were significantly positively related to LUI after warming ($P = 0.005$) in the *Schwäbische Alb* and *Hainich* but not at the later samplings, explaining 2.8% of the variance (Fig. 2B). The drought treatment tended to decrease

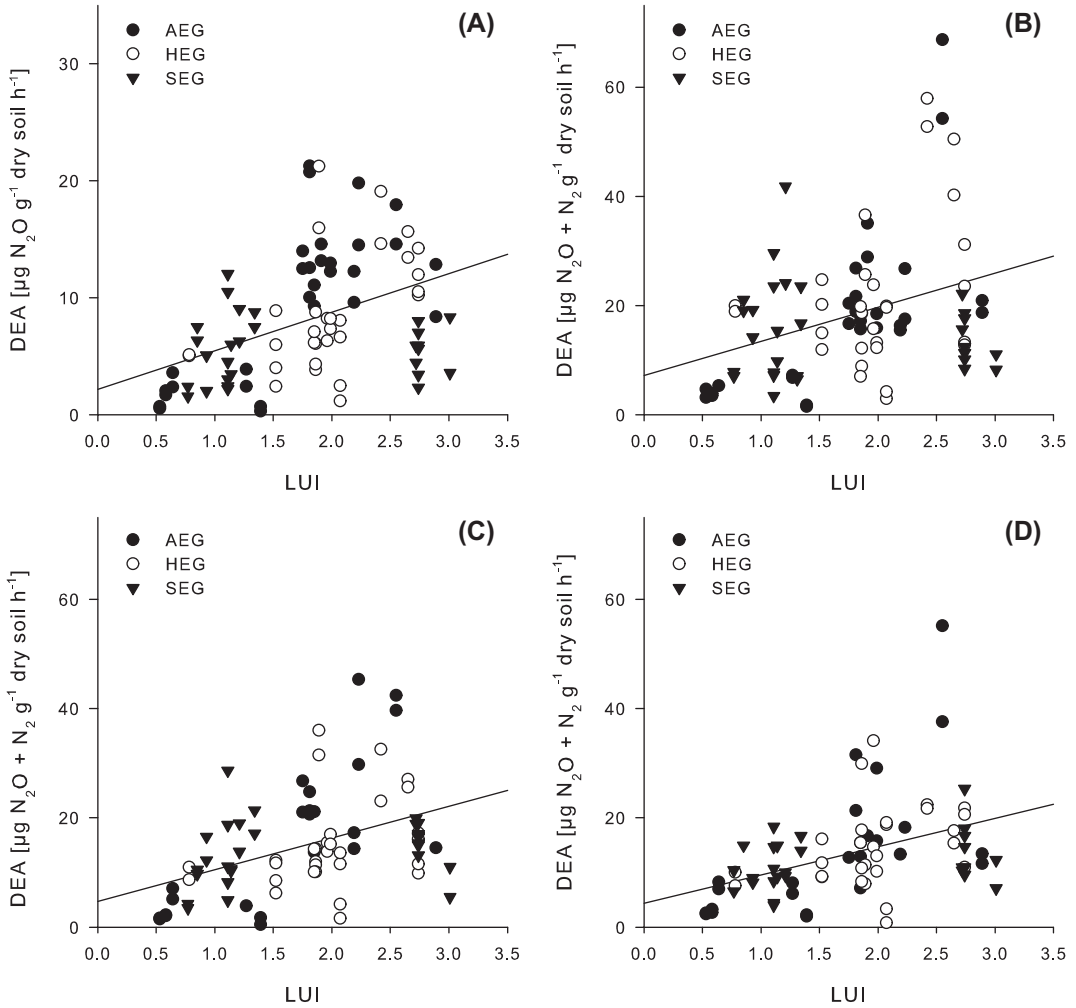

Figure 1. Potential denitrification activity (DEA) at the *Schwäbische Alb*: AEG, *Hainich*: HEG and *Schorfheide*: SEG. **(A)** Potential N_2O emissions at intermediate sampling in relation to LUI. **(B–D)** Potential denitrification ($N_2O + N_2$) at the three samplings: after warming **(B)**, at intermediate sampling **(C)** and after drought **(D)** in relation to LUI.

($P = 0.062$) the abundance of *nosZ* genes by 3.1%, explaining 0.2% of the variance.

Effects of dynamic variables

In contrast to the more static and constant site-specific properties, dynamic variables (SWC, EOC, NO_3^-) are subject to relatively fast changes in the environment, probably resulting in short-term effects of the potential denitrification activity of the soils. After warming, the potential N_2O production was negatively related to the EOC concentration ($P = 0.03$, Table S2, Supporting Information), explaining 1.2% of the variation. At the intermediate sampling, N_2O was negatively ($P = 0.015$ and total denitrification potential was positively ($P = 0.03$) affected by the soil NO_3^- concentration (Table S2, Supporting Information), accounting for 19.2 and 22.4% of the variation, respectively. After drought, a positive effect of NO_3^- on total denitrification potential was observed ($P = 0.017$) that explained 9.8% of the variation. After drought, the SWC positively influenced both the N_2O and the $N_2O + N_2$ production potentials (Table S2, Supporting Information).

At all samplings, the potential N_2O production and the total denitrification potential were significantly and positively related with the *nosZ* gene abundance ($P < 0.05$; Fig. 3A and B), explaining between 7.6–16.6% (N_2O) and 12.4–27.3% ($N_2O + N_2$), respectively. In addition, N_2O was positively affected by the *nirS* abundances ($P = 0.02$), accounting for 20.7% and 16S rRNA abundance ($P = 0.01$), accounting for 40.8% of the variance. $N_2O + N_2$ was further positively affected by the gene abundances of *nirK* ($P = 0.03$) and 16S rRNA ($P < 0.001$) at the intermediate sampling (Table S2, Supporting Information), explaining 26.4 and 48.5% of the variance, respectively.

DISCUSSION

Experimental warming effects differed between the Exploratories and were much weaker than those projected for Europe within the 21st century (IPCC 2013). Increasing temperature by open-top chambers further typically leads to even stronger manipulation artifacts, such as lower precipitation and extreme temperature spikes (Aronson and McNulty 2009). Nevertheless, changes in soil temperatures achieved by the experimental setup seem to have an overall stimulating effect on potential N_2O production, as well as on potential activity of the denitrifying community and functional gene abundances.

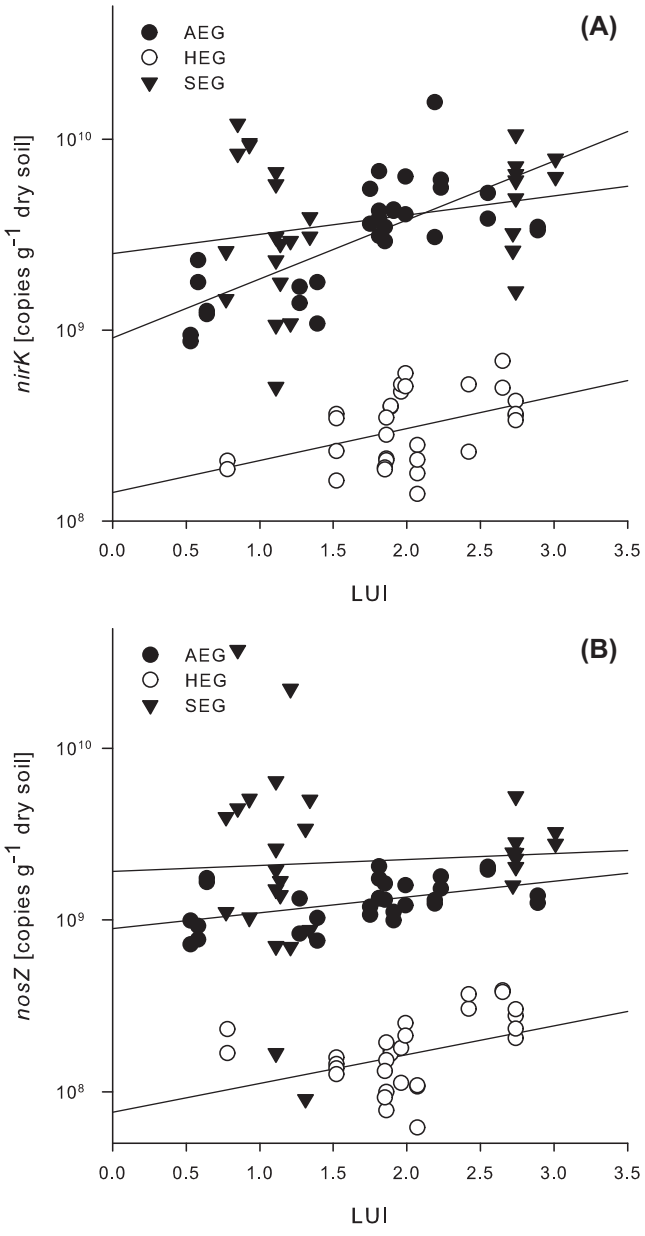

Figure 2. Abundance of *nirK*-type denitrifiers at the *Schwäbische Alb*: AEG, *Hainich*: HE, and *Schorfheide*: SEG at intermediate sampling in dependence of LUI (**A**) and abundance of *nosZ*-type denitrifiers at the *Schwäbische Alb*: AEG, *Hainich*: HEG and *Schorfheide*: SEG after warming in dependence of LUI (**B**).

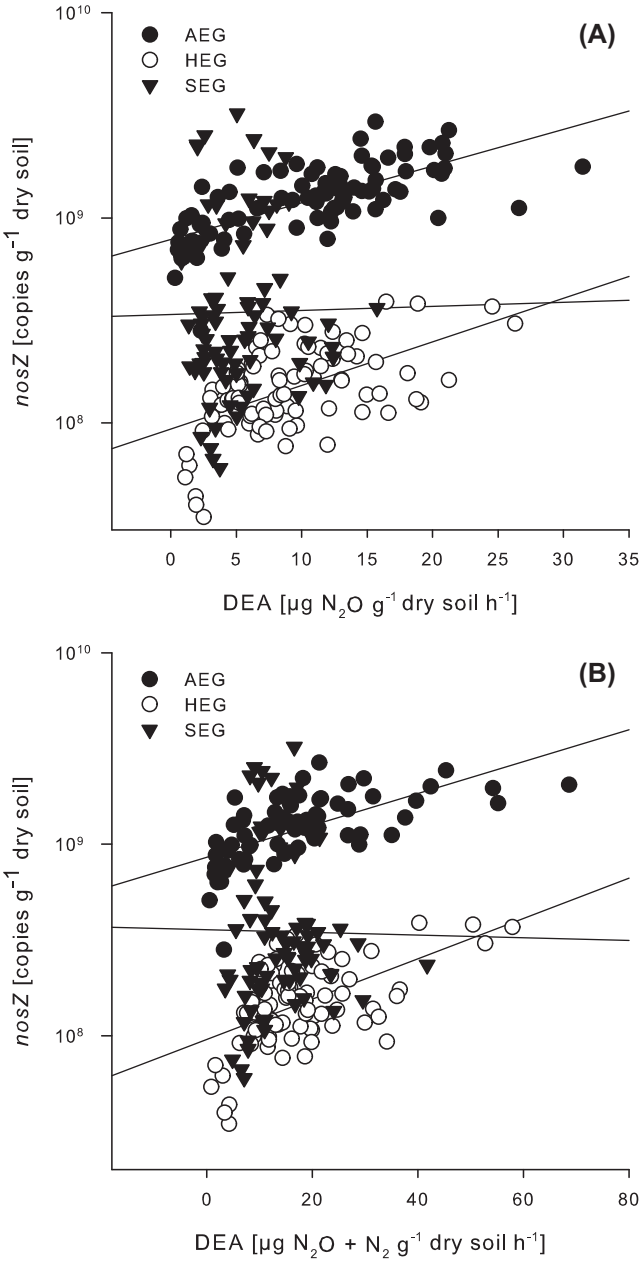

Figure 3. Abundance of *nosZ*-type denitrifiers in relation to potential denitrification activity (DEA) at the *Schwäbische Alb*: AEG, *Hainich*: HEG and *Schorfheide*: SEG expressed as potential N_2O production (**A**) and potential denitrification (N_2O + N_2) (**B**) over all three samplings.

Effects of warming on N_2O emissions seemed to be independent of LUI (as measured by the LUI) but were influenced by study region (effect of the Exploratories). In contrast, the potential denitrification activity was exclusively influenced by LUI, and neither by warming nor Exploratory. In a review, Oenema *et al.* (1997) concluded that grazing effects (dung and urine input, soil compaction) contribute up to 10% of the global N_2O budget. Potential denitrification increased after warming, with effect sizes that depended on Exploratory, climate-change treatment and land-use intensity. Various studies have shown a positive relationship between soil temperature and N_2O emissions (e.g. Gödde and Conrad 1999), but also nutrient supply (mineral N) (Skiba and Smith 2000; Jones *et al.* 2007), acidity (Cuhel *et al.* 2010) and water filled pore space of between 60 and 90% (Flechard *et al.* 2007) are important factors for N_2O emissions from soils.

It is likely that soil warming increased microbial activity in our study. For example, Sheik *et al.* (2011) found that warming and reduced soil water budgets strongly influenced bacterial population size and diversity, and warming significantly increased microbial population size by 40–150%. Earlier, Maag and Vinther (1996) and Mosier (1998) reported an increased denitrification activity as a response to increased soil moisture and warming. Maag and Vinther (1996) also demonstrated that the N_2/N_2O ratio increased exponentially with increasing temperature, implying a linear relationship between the log (N_2/N_2O ratio) and warming. After experimental warming in spring, the

abundance of denitrifiers responsible for the production of nitric oxide (*nirS*) as a precursor of N_2O tended to increase.

At the intermediate sampling, effects of both N_2O emissions and denitrification responded to management practices and were influenced by regional differences of the study sites, while the climate-change manipulations of the warming did not persist. After drought, potential N_2O emissions tended to respond to the treatment, as indicated by a detectable effect of the treatment on both SWC and *nosZ* gene abundances. Furthermore, the plot-specific LUI was a stronger driver for both N_2O emissions and denitrification than site-depended variables such as Exploratory.

As previously demonstrated by Berner *et al.* (2011) and Keil *et al.* (2011), soil microorganisms profited from the additional nutrient supply in intensely managed grasslands of the *Schwäbische Alb*, which are managed by a combination of grazing, mowing and fertilization. Both potential N_2O emissions and denitrification potential were influenced by land-use after the drought experiment. Sites receiving additional fertilizer input and mowing had higher potential denitrification enzyme activities than those being less intensely managed, which indicates the influence of fertilizer input on potential N_2O emissions, as for example discussed by Flechard *et al.* 2007. Overall, we believe that warming induces decreasing O_2 concentrations in the soil, resulting in enhanced denitrification, and thus being an indirect effect of the increased heterotrophic microbial activity.

The effects of the drought experiment varied among Exploratories and were most pronounced in the *Schwäbische Alb*. Soil mineral N was closely linked to soil aeration and SWC (Stres *et al.* 2008), and also affected by the climate-change treatments. In all three Exploratories, drier plots had lower NH_4^+ concentrations than plots with ambient SWC. Soils in the *Schwäbische Alb* and *Hainich* were probably too dry to promote nitrification and likely denitrification was completely inhibited under these conditions (Flechard *et al.* 2007), resulting in increased ammonium concentrations in soils.

One major prerequisite for microbial denitrification in soil is anaerobic microsites created by high soil moisture (Abbasi and Adams 2000) or by e.g. high rates of heterotrophic respiration, as described in a model for N_2 and N_2O production from nitrification and denitrification by Parton *et al.* (1996). Experimental summer drought was performed to simulate climatic model predictions of reduced precipitation under climate change. As for warming in spring, effects depended mostly on Exploratory and LUI. We speculate that differences among study regions and of land-use intensity may have a strong influence on the potential denitrifying enzyme performance. In this study, potential N_2O production (N_2O) and potential denitrifying enzyme activity ($N_2O + N_2$) were significantly influenced by LUI at the intermediate sampling and after drought. Recently, Attard *et al.* (2011) proposed that soil environmental conditions, rather than the denitrifier abundance and diversity, control potential denitrification after a change in land use from crop to grassland or from tilling to non-tilling in grasslands. This expectation is not supported by our measurements, where experimental plots with high LUI in the *Schwäbische Alb* were about 22% drier than controls, but had the highest potential DEA of all treatments. In contrast, effects of experimental drought in the *Schorfheide* were less pronounced, and potential denitrification activity was lowered in drier experimental subplots. Further controls of SWC (i.e. water table) controlling N_2O emissions in grasslands were recently demonstrated by Regan *et al.* (2011). They provided evidence for a relationship between increased N_2O emissions under elevated atmospheric CO_2 and wet soil conditions. In addition, these findings correlated with a decreased *nosZ/nirK* ratio, indicating the influence of soil water status on the denitrifying community, probably resulting in elevated N_2O emissions through a higher proportion of N_2O producers than N_2O consumers under these conditions. In contrast, Cantarel *et al.* (2011) found that only warming but not a combination of summer drought, warming and elevated CO_2 had stimulating effects on mean annual N_2O fluxes in upland temperate grassland.

CONCLUSION

Warming effects in grasslands influenced the performance of denitrifying microorganisms towards enhanced potential denitrification. While differences among the study regions were mainly related to soil chemical and physical properties, land-use was a stronger driver for potential denitrification, and grasslands with higher LUI also had greater potentials for N_2O emissions. The total bacterial community (16S rRNA gene abundance) did not respond to experimental warming or drought treatments, displaying resilience to minor and short-term effects of climate change. In contrast, the denitrifier community composition tended to be influenced by the experimental treatments: *nirS*- and *nirK*-type denitrifiers were more influenced by drought in combination with LUI and pH, while the *nosZ* abundance was responding to the summer drought manipulation. We conclude that both warming and drought affected both the denitrifying communities and the potential denitrification in grassland soils, but these effects are overruled by Exploratory and site-specific LUI.

SUPPLEMENTARY DATA

Supplementary data is available at FEMSEC online.

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