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Impact of Land Use Management and Soil Properties on Denitrifier Communities of Namibian Savannas

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Abstract We studied potential denitrification activity and the underlying denitrifier communities in soils from a semiarid savanna ecosystem of the Kavango region in NE Namibia to help in predicting future changes in N_2O emissions due to continuing changes of land use in this region. Soil type and land use (pristine, fallow, and cultivated soils) influenced physicochemical characteristics of the soils that are relevant to denitrification activity and N_2O fluxes from soils and affected potential denitrification activity. Potential denitrification activity was assessed by using the denitrifier enzyme activity (DEA) assay as a proxy for denitrification activity in the soil. Soil type and land use influenced C and N contents of the soils. Pristine soils that had never been cultivated had a particularly high C content. Cultivation reduced soil C content and the abundance of denitrifiers and changed the composition of the denitrifier communities. DEA was strongly and positively correlated with soil C content and was higher in pristine than in fallow or recently cultivated soils. Soil type and the composition of both the *nirK*- and *nirS*-type denitrifier communities also influenced DEA. In contrast, other soil characteristics like N content, C:N ratio, and pH did not predict DEA. These findings suggest that due to greater availability of soil organic matter, and hence a more effective N cycling, the natural semiarid grasslands emit more N_2O than managed lands in Namibia.

Keywords Denitrifiers · Community composition · Abundance · Activity · Land use · Soil type

Introduction

Emissions of N_2O are a global concern, because the gas contributes to the degradation of ozone in the stratosphere [1]. Moreover, because each N_2O molecule traps 300 times more radiant energy per century than a CO_2 molecule, N_2O contributes considerably to global climate change [2]. In temperate ecosystems, managed soils are the major sources of atmospheric N_2O [3–5], because the greater availability of organic substrates in soils under agriculture drives relatively high N transformation rates [6]. Temperate soils and their responses to changes in land use, for instance the conversion of grasslands into crop fields or pastures grazed by animals, are well-studied [4, 7].

In contrast, far less is known about how soils in semiarid ecosystems may respond to changes in land use. This makes predicting how the continuing conversion of pristine savannas into managed lands will affect the contribution of these regions to global greenhouse gas emissions difficult. On a global scale, savannas, and seasonally dry ecosystems in warm climatic regions, cover a land surface of more than

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30×10^6 km^2. Hence, despite low average daily fluxes of 0.32 mg N_2O m^{-2} [8], these regions represent a significant source of atmospheric N_2O (4.4 Tg N_2O year^{-1}) and savanna soils contribute 25 % of N_2O fluxes from tropical soils [3]. In Africa, savanna ecosystems cover a large part of the continent's land mass and host the majority of the population. In 2005, Africa contributed only 16 % to total anthropogenic N_2O emissions, with agriculture accounting for 42 % (71 % if burning is included) of the African N_2O emissions [9]. This low level was attributed to extremely low inorganic fertilizer inputs into most of the sub-Saharan African soils [10]. However, N_2O emissions have more than doubled in Africa from 1970 to 2005 and are predicted to double again until 2050 due to the prospected green revolution and the associated changes in land use and land management [9].

To study the effects of land use, climate variations, and other habitat influences on savanna biodiversity and ecosystem functioning and services, the Biota Southern Africa project was launched in 2001. This research project concentrates on studies in Namibia and the western parts of South Africa and aims at providing scientific support for conservation and sustainable use of biodiversity in Namibia and South Africa. Our research focused on soil ecosystems in the Kavango region in northern Namibia and the likely consequences of these systems' ongoing conversion to agricultural land. In the Kavango region, the cropping area increased by 3.9 % per year because of clearing of natural vegetation from 1943 to 1996 [11]. Previous studies of semiarid ecosystems explored the effects of changes in land use on changes in N_2O emissions from soils in response to biotic, abiotic, and seasonal influences [12, 13]. In woodland and managed savannas, similar fluxes of N_2O were observed [8], but converting natural systems to agricultural use generally increases greenhouse gas release. For instance, clearing of humid primary forest in Costa Rica increased N_2O production, probably due to an increase in N cycling and higher diffusion rates in drying soils [14]. Further, compaction due to trampling by cattle, and fertilizer use dramatically increased N_2O release from tropical grasslands [4, 15].

We hypothesized that land use change in the Kavango region would affect the microbial communities involved in denitrification in the soils with feedback effects on N_2O fluxes in situ. Denitrifiers and nitrifiers are the main producers of N_2O from soils [16]. Even in arid and semiarid soil ecosystems, where bulk soils are generally aerobic, denitrification accounts for a significant fraction of N lost from the soils and drives N_2O emissions [17–19]. We used the denitrifier enzyme activity (DEA) assay as a proxy for N_2O production by denitrification in the field and linked these data to the abundance and composition of denitrifier communities in different soil types subjected to different types of land use (pristine, currently under cultivation, fallow). Pristine, more C-rich soils showed a higher propensity to produce N_2O than nutrient-depleted, managed soils, which was related to the abundance and composition of the underlying denitrifier communities.

Material and Methods

Site Characteristics and Soil Sampling

For this study within the "BIOTA Biodiversity Observatories in Africa" framework [20], the Mile 46 Livestock Development Centre (LDC) in the Kavango region in northeast Namibia was chosen. The Kavango region is dominated by semiarid savannas with ferralic arenosol as the prevalent soil type. Many of these soils are poor, weakly developed with reduced soil horizonation, and generally not very productive due to limited fertilizer inputs [21]. The majority of the Namibian population (70 %) depends on subsistence farming by rain-fed and dryland cropping [22]. Soil samples were collected between March 19 and 24, 2007 in the neighborhood of Mutompo village (18.30 S; 19.25 E; 1180 m NN) in the Kavango region of northeastern Namibia (Fig. S1). The climate is semiarid with a mean temperature of about 22.6 °C and an average rainfall of 527 mm year^{-1} which occurs primarily from November to March [23]. The soil parent material is formed by degraded dune sands of the Kalahari basin, and soil types are mainly dystric to hypereutric arenosols [24]. The region is characterized by forest savanna with northern Kalahari dry woodlands as the main vegetation type [25]. Common plant species in Mutompo are *Combretum* species, *Terminalia sericea*, *Burkea africana*, *Eragrostis echinochloides*, and *Guibourtia coleosperma* [23, 26]. Detailed descriptions of the vegetation of the Kavango region can be found in Strohbach and Petersen [27]. Land use of the area started with the construction of drinking water wells in the 1970s. The communities use the land for rain-fed arable fields, for open-access cattle grazing with little control over stocking rates and grazing times, and for firewood collection [28].

Twenty-nine plots (Table 1, Table S2) were chosen representing combinations of land use (P: pristine, Fa: fallow for up to 10 years, and Fi: field) and topsoil type (LD=dark and loamy, BR=brown-reddish and slightly loamy, and PS=pale and pure sandy), according to the Munsell notation [29]. Due to the homogenous parent material, the darkness of the soil is an indicator for the nutritional value of the soils [30]. One sample (P/PS-15) was excluded from all statistical analyses, because repeated extractions did not yield PCR-amplifiable DNA. Soil in situ temperature upon sampling was 30–31 °C. After removing mulch from the surface, soil samples from the surface layer (0–10 cm; diameter 5 cm) were taken within a plot of 20 by 20 m. Nine soil cores each (i.e., 18 cores in total) were collected at regular distances along two

Table 1 Soil sampling in Namibia 2007

Soil texture	Land use		
	Pristine (P)	Field (Fi)	Fallow (Fa)
Loamy-dark (LD)	P/LD-16, P/LD-25	Fi/LD-7, Fi/LD-8, Fi/LD-9, Fi/LD-26, Fi/LD-27	Fa/LD-1, Fa/LD-2, Fa/LD-3, Fa/LD-10, Fa/LD-22
Brown-reddish (BR)	P/BR-11, P/BR-12, P/BR-24	Fi/BR-4, Fi/BR-5, Fi/BR-14, Fi/BR-18, Fi/BR-21, Fi/BR-29	Fa/BR-17, Fa/BR-19, Fa/BR-20, Fa/BR-23, Fa/BR-28
Pale-sandy (PS)	P/PS-6, P/PS-13, P/PS-15		

intersecting, rectangular sampling transects. Samples were pooled per plot, homogenized, and sieved through a 2-mm mesh to remove large roots and pebbles. In the field, each soil homogenate was divided into two fractions (2 g into cryovials and the remainder into sterile plastic bags) and transported to the laboratory at the nearby Mile 46 Livestock Development Centre within a few hours. The cryovials were frozen and transported under liquid nitrogen to the laboratory in Germany, whereas the bags containing the remaining soil were kept at 4 °C and also transported to Germany.

Determination of Soil Water Content, pH, and Nitrate Concentrations

Soil water content (weight %) was determined gravimetrically. Soil (5–10 g) was weighed, dried at 105 °C overnight, and weighed again. To determine pH, an amount of soil equivalent to 1 g dry weight was suspended in 5 ml 0.01 M $CaCl_2$ solution. The soil slurries were incubated for 2 h at room temperature before pH was measured with a pH meter (Sentix 41; WTW, Weilheim, Germany). Soil electrical conductivity was determined in the laboratory in Hamburg (Germany) in a slurry (10 g soil/25 ml water) with a conductivity sensor (WTW).

Nitrate concentrations in soil were determined after extraction from an amount of soil equivalent to 2 g dry weight of soil in 10 ml bi-distilled water and shaking the suspension at 150 rpm for 1 h at 4 °C. Afterwards, the soil suspension was filtered (round filter 2095, Schleicher & Schuell GmbH, Dassel, Germany) and nitrate was measured with an ion chromatograph (IC, Sykam, Fürstenfeldbruck). Data were evaluated with the PeakSimple software (version 3.54, SRI Instruments, Torrence, CA, USA).

Denitrifier Enzyme Activity Assay

The DEA assay [31] measures the abundance of denitrification enzymes and allows comparison of the potential denitrification rates across samples. Aliquots equal to 3 g dry weight of soil were placed in serum bottles to which 3 ml of a substrate solution containing glucose, potassium nitrate (both 10 mM), and chloramphenicol (0.1 g l^{-1}) were added. The serum bottles were capped with butyl stoppers and oxygen was removed by flushing the bottles with N_2. Reduction of N_2O to N_2 was blocked by adding acetylene to 10 % of the gas phase. All experiments were set up in triplicates from the homogenized material and were incubated at 25 °C on a horizontal shaker at 200 rpm. After flushing the syringe with N_2 to prevent contamination of the gas phase with oxygen, head-space samples of 0.5 ml were collected every 60 min. Nitrous oxide was analyzed by gas chromatography (GC) with a ^{63}Ni electron capture detector (ECD; Carlo Erba GC 8000; Fisons Instruments, Mainz-Kastel, Germany). N_2O production rates per hour were inferred from the period of linear increase in N_2O concentration.

DNA Extraction

Total DNA was extracted from 0.5 g frozen soil using the FAST DNA® Spin Kit for Soil Kit according to the manufacturer's instructions (MP Biomedicals Germany, Heidelberg, Germany). Additional washing (3×) of the DNA with 5.5 M guanidine thiocyanate (pH 7.0) was introduced as additional cleaning to reduce the amount of humic substances in the DNA preparation [32]. Subsequently, the purity and quantity of the DNA were determined by UV spectrophotometry at 260 and 280 nm (Biophotometer, Eppendorf, Hamburg, Germany) and using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen GmbH, Karlsruhe, Germany).

T-RFLP Analysis

PCR amplifications of *nirS* and *nirK* genes from total environmental DNA extracts were performed in a total volume of 25 μl using the primer pairs cd3aF-R3cd [33, 34] and nirK1F-nirK5R [35] and PCR conditions described previously [34, 36]. Both primer pairs exclude detection of ammonia oxidizer species. The reverse primers R3cd and nirK5R were 5′-end labeled with 6-carboxyfluorescein (Sigma-Aldrich GmbH, Taufkirchen, Germany). Replicate

PCRs (5) were combined and analyzed by electrophoresis on 1.5 % [*w*/*v* (weight in volume)] agarose gels followed by staining with ethidium bromide (0.5 mg l^{-1}). Bands were visualized by UV excitation. For purification, fragments of proper size were excised from the agarose gel and eluted using the Wizard® SV Gel and PCR Clean-Up System (Promega GmbH, Mannheim, Germany).

Aliquots of the PCR product (50–100 ng DNA) were cleaved with 3 U of restriction endonuclease (*MspI* for *nirS* and *HaeIII* for *nirK*; New England Biolabs, Frankfurt/Main, Germany). After cleavage, enzymes and excess salts were removed from the digest with Autoseq G-50 columns (Amersham-Pharmacia Biotech, Freiburg, Germany). Aliquots (2 μl) were mixed with 11 μl of deionized formamide (Applera, Darmstadt, Germany) and 0.3 μM of an internal DNA fragment length standard (X-Rhodamine MapMarker® 30–1000 bp; BioVentures, Murfreesboro, TN). Restriction fragments were separated on an automated DNA sequencer (ABI PRISM® 3130xl Genetic Analyzer, Applied Biosystems, Weiterstadt, Germany), and the lengths of fluorescently labeled terminal restriction fragments (T-RFs) were determined by comparison with the internal standard using GeneMapper software (Applied Biosystems). Peaks >30 bp were analyzed by aligning fragments to the size standard. Reproducibility of patterns was confirmed for repeated terminal restriction fragment length polymorphism (T-RFLP) analysis using the same DNA extracts. A difference of less than 2 bp in estimated length between different profiles was the basis for considering fragments identical. Patterns from different samples were normalized to identical total fluorescence units by an iterative normalization procedure [37].

Quantitative PCR

The abundance of functional marker genes was determined using primer pairs nirK1F-nirK5R [35] and nirS4QF-nirS6QR [38] which also exclude detection of ammonia oxidizers and cycling conditions given in Table S1. Cloning confirmed that despite different primers and cycling conditions used for *nirS*-based T-RFLP and qPCR analyses, the genetic diversity targeted was essentially the same (data not shown). Gene fragments were amplified in 25-μl reaction mixtures containing 0.5 mM of each primer, 12.5 μl of SYBR green PCR master mix (SYBR® Green JumpStart™ Taq ReadyMix™; Sigma-Aldrich GmbH, Taufkirchen, Germany), 3 μl DNA diluted template corresponding to 15 ng of total DNA, and 500 ng of T4gene32 protein (New England Biolabs). Thermal cycling, fluorescent data collection, and data analysis were done using an iCycler iQ5 (Bio-Rad Laboratories GmbH, Munich, Germany) and the iQ5 Optical System Software 2.0. Standard curves were obtained using serial dilutions of a known amount of plasmid DNA containing the respective fragment of the *nirK* and *nirS* gene. Lack of inhibitory effects of co-extracted substances in soil DNA was proven by diluting soil DNA extracts and by quantifying a known amount of plasmid DNA added to soil DNA extracts.

Statistical Analysis

Effects of soil type and land use on soil characteristics (pH, C:N ratio, C and N content, nitrate), denitrification activity (DEA), and denitrification gene abundance (*nirK* and *nirS*) were studied by hierarchical analyses of variance. According to the sampling design (Table 1), in a first step, the sites with pale-sandy soils (PS) were compared with the sites characterized by other soils (LD or BR), and then in a factorial submodel, the effects of soil type (BR, LD) and land use (pristine, field, fallow) and their interactions were studied. The effects of land use were further partitioned into two orthogonal contrasts: (1) pristine vs. cultivated (field or fallow) and (2) fields vs. fallows.

To obtain the main gradients in the composition of the *nirK*- and *nirS*-type denitrifier communities, correspondence analyses (CA) were carried out with the relative height of the T-RF peaks. λ_1 and λ_2 stands for the eigenvalues of the first and second axes in the ordination diagram. The scores of the sites along the first two axes were then used for further analyses. The effects of soil type and land use on the composition of the denitrifier community expressed as the first two axes of the CA ordination (Ax1 *nirK*, Ax2 *nirK*, Ax1 *nirS*, Ax2 *nirS*), and the effects of soil type, land use, and denitrifier community composition on DEA were studied by multiple regression analyses. Potential explanatory variables for the composition of the denitrifier communities were PS (yes/no), BR (yes/no), pristine (yes/no), currently cultivated as a field (yes/no), pH, C, and nitrate content. Potential explanatory variables for DEA were the same variables plus the four CA axes, i.e., denitrifier community composition. Soil N content was not included as a potential explanatory variable, because it was strongly intercorrelated with C content (r=0.92). Regressions with all possible combinations of explanatory variables were calculated and the Bayesian information criterion (BIC) used to measure the strength of evidence for each candidate model. The BIC was preferred over the Akaike information criterion, because it favors models with less parameters [39]. In the results, we present the models with the lowest BIC. Nitrate concentration, *nirK* and *nirS* copy numbers, and DEA were log transformed prior to analysis to satisfy the assumptions of homoscedasticity and normally distributed residuals. Most statistical analyses were carried out with IBM-SPSS 20, the correspondence analyses with

CANOCO 4.5 [40], and the multiple regression analyses with the leaps package in R 2.15.1.

Results

Effects of Soil Type and Land Use on the Physicochemical Characteristics of the Soils

While conductivity was essentially the same for all soils sampled (21.3±3.7 μS cm^{-1}; $P>0.22$), other soil characteristics were influenced by the soil type, land use, and the interaction between soil type and land use. Sites with pale and sandy soils differed strongly from all other sites in terms of pH (5.3 vs. 6.3; Table 2, Fig. 1a) and C:N ratio (15.3 vs. 10.4; Table 2, Fig. 1b). The pH and C:N ratio of all loamy sites were similar, but the type of land use influenced the total C and N content and NO_3-N concentration (Table 2). Total soil C content depended on soil type and was influenced by cultivation. C content was higher in pristine soils that had never been cultivated (0.66±0.07 %) than in other soils (0.42±0.02 %). In LD soils, C content at sites currently under cultivation was higher than at fallow sites (0.51 vs. 0.39 %), whereas for BR soils, the opposite (0.37 vs. 0.42 %) was the case (Fig. 1c). Total N concentration was higher in pristine soils than in those that were or had been under cultivation, and these differences were stronger in loamy-dark (0.075 vs. 0.042 %) than in brown-reddish soils (0.052 vs. 0.039 %) (Fig. 1d). Moreover, total N concentration was higher in loamy-dark soils currently under cultivation than in fallows (0.048 vs. 0.036 %), but was lower in brown-reddish field soils than in fallows (0.037 vs. 0.041 %) (Fig. 1e). The concentration of NO_3-N was much higher at cultivated than at fallow sites (1.03 vs. 0.14 μg gdw^{-1} soil) (Fig. 1f).

Effects on Denitrifying Enzyme Activity

DEA ranged from not detectable in samples generated from cultivated sites to >9 ng N_2O-N gdw^{-1} soil h^{-1} in samples from pristine plots (Fig. S2). Production of N_2O was influenced by soil type and differed between pale and sandy and loamy sites (Table 3) with higher levels for pale and sandy soils (3.10 ng N_2O-N gdw^{-1} soil h^{-1}) than for other soils (0.64 ng N_2O-N gdw^{-1} soil h^{-1}) (Fig. 2a). Land use also influenced DEA with more than threefold higher N_2O production rates for the pristine sites (3.73 ng N_2O-N gdw^{-1} soil h^{-1}) than for cultivated soils (0.28 ng N_2O-N gdw^{-1} soil h^{-1}) (Fig. 2b).

Effects on Denitrifier Abundance

Denitrifiers of the *nirK*-type were roughly one to two orders of magnitude more abundant than those of the *nirS*-type (Fig. S3a and b). Denitrifier abundance was determined by soil type, land use, and the interaction of soil type and land

Table 2 Analyses of variance of the effects of soil type and land use on soil characteristics. For details of the statistical analysis, see "Material and Methods"

Significance of	df	pH		C:N		C_{tot}		N_{tot}		NO_3-N (log)	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Pale-sandy vs. loamy	1	**21.62**	**<0.001**	**44.55**	**<0.001**	0.80	0.382	2.36	0.139	0.03	0.857
Loamy-dark vs. brown-reddish	1	0.83	0.374	1.98	0.174	**5.76**	**0.026**	4.15	0.055	0.30	0.592
Land use	2	2.30	0.125	0.65	0.535	**18.15**	**<0.001**	**16.21**	**<0.001**	**3.55**	**0.047**
Pristine vs. cultivated	1	4.04	0.058	0.76	0.394	**35.65**	**<0.001**	**31.15**	**<0.001**	0.06	0.937
Field vs. fallow	1	0.56	0.464	0.53	0.474	0.69	0.416	1.31	0.265	**7.36**	**0.013**
Loamy-dark vs. brown-reddish × land use	2	1.72	0.204	1.05	0.368	**4.40**	**0.025**	**6.27**	**0.007**	0.17	0.849
Loamy-dark vs. brown-reddish × pristine vs. cultivated	1	0.48	0.498	2.02	0.170	3.02	0.097	**6.79**	**0.017**	0.19	0.669
Loamy-dark vs. brown-reddish × field vs. fallow	1	2.96	0.100	0.07	0.791	**5.77**	**0.026**	**5.76**	**0.026**	0.14	0.711
Residual	21										

Bold indicates significant effects

df degrees of freedom, *F F* value, *P P* value, *C:N* C:N ratio, C_{tot} total C content, N_{tot} total N content, *NO_3-N (log)* log-transformed NO_3-N content

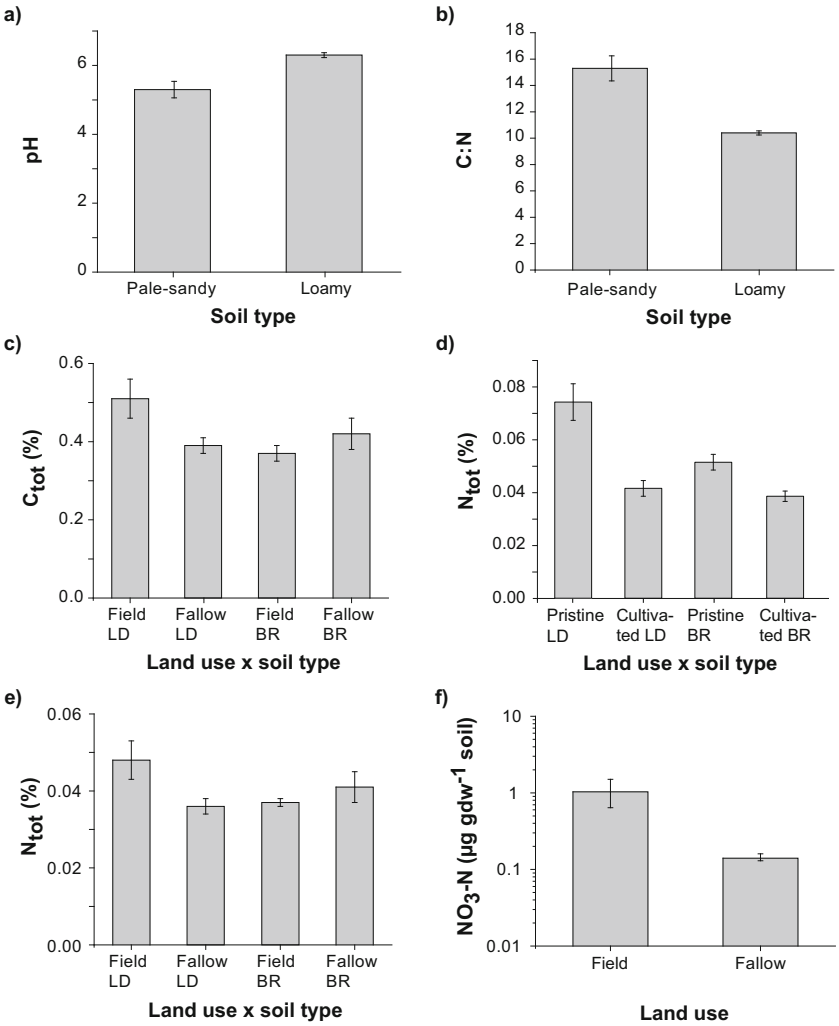

Fig. 1 Influence of land use and soil type on **a** pH; **b** C:N ratio; **c** total C content; **d**, **e** total N content; and **f** NO_3 content of Namibian savanna soils. Values are means±1 SE. For number of replicates, see Table 1

use, but community abundance depended on the nitrite reductase gene considered (Table 3). Copy numbers of *nirK* were similar for pale and sandy and loamy sites, but they were higher at sites with loamy-dark than brown-reddish soils (1.42×10^6 vs. 6.02×10^5 copies gdw^{-1} soil; Fig. 3a). In contrast, numbers of *nirS*-type denitrifiers were significantly lower in pale and sandy than in loamy soils (1.05×10^3 vs. 2.67×10^4 copies gdw^{-1} soil), but similar at all loamy sites (Fig. 3b). Land use influenced the abundance of both *nirK*-type and *nirS*-type denitrifiers (Table 3). Denitrifiers of the *nirK*-type were more abundant at pristine sites than at cultivated sites (2.05×10^6 vs. 7.35×10^5 copies gdw^{-1} soil; Fig. 3c). This was also the case for the *nirS*-type denitrifiers (9.9×10^4 vs. 2.27×10^4 copies gdw^{-1} soil; data not shown). In loamy-dark soils, their numbers differed between sites currently under cultivation and fallows (5.66×10^4 vs. 1.66×10^4 copies gdw^{-1} soil), but numbers were similar for brown-reddish soils (1.52×10^4 vs. 2.00×10^4 copies gdw^{-1} soil; Fig. 3d).

Effects on Denitrifier Community Composition

Correspondence analyses revealed strong gradients in the composition of both the *nirK*-type ($\lambda_1=0.43$, $\lambda_2=0.27$) and *nirS*-type ($\lambda_1=0.51$, $\lambda_2=0.29$) denitrifier communities. Overall, denitrifier community composition was best explained by soil type, soil characteristics, and cultivation, but the response of *nirK*- and *nirS*-type denitrifiers was community-specific (Table 4). The composition of *nirK*-type denitrifiers differed between pale-sandy and loamy soils (Fig. S4a) and between brown-reddish and loamy-dark soils, which largely explained differences in community composition along the first ordination axis and between fallow and currently farmed sites. Distinct *nirS*-type denitrifiers which were prevalent in the pale and sandy soils (Fig. S4b) and soil C content most strongly influenced community differences relative to those in the loamy soils.

Table 3 Analyses of variance of the effects of soil type and land use on denitrification activity and denitrification gene abundance. For details of the statistical analysis, see "Material and Methods"

Significance of	df	DEA (log)		*nirK* (log)		*nirS* (log)	
		F	*P*	*F*	*P*	*F*	*P*
Pale-sandy vs. loamy-dark/ brown-reddish	1	**8.83**	**0.007**	0.09	0.925	**68.88**	**<0.001**
Loamy-dark vs. brown-reddish	1	3.61	0.071	**4.69**	**0.042**	**4.74**	**0.041**
Land use	2	**21.17**	**<0.001**	2.74	0.087	**15.30**	**<0.001**
Pristine vs. cultivated	1	**40.93**	**<0.001**	**4.74**	**0.041**	**27.30**	**<0.001**
Field vs. fallow	1	1.41	0.248	0.75	0.396	3.29	0.084
Loamy-dark vs. brown-reddish × land use	2	2.30	0.125	0.94	0.408	**5.01**	**0.017**
Loamy-dark vs. brown-reddish × pristine vs. cultivated	1	1.82	0.192	1.34	0.261	0.13	0.727
Loamy-dark vs. brown-reddish × field vs. fallow	1	2.77	0.111	0.54	0.472	**9.89**	**0.005**
Residual	21						

Bold indicates significant effects

df degrees of freedom, *F F* value, *P P* value, *DEA (log)* log-transformed DEA values, *nirK (log)* log-transformed *nirK* gene abundance data, *nirS (log)* log-transformed *nirS* gene abundance data

Effects of Soil Factors and Denitrifier Communities on Denitrification Activity

Denitrification activity (DEA) increased strongly with soil C content (Fig. 4), but did not correlate significantly with other soil quality metrics. In the best model, land use and the composition of the denitrifier communities, independent of which type of nitrite reductase they had, significantly influenced DEA in addition to soil C (Table 4). Pristine soils sustained higher denitrification activity than managed soils.

Discussion

Influence of Land Use and Soil Type on Soil Characteristics

In this study, we explored soils of the Kavango region in northeast Namibia and the potential activity and communities of denitrifiers, which are a major source of N_2O emitted from soils [16]. We observed that land use may alter denitrifier communities and DEA. This suggests the potential for changes in N_2O flux, but N cycling in soils also depends strongly upon oxygen availability, soil texture, and soil porosity [41], which were not measured in this study. Intensive agriculture acidifies Namibian soils [24]; the lack of changes in soil pH with conversion of grasslands to agriculture may reflect the low intensity of agricultural practices in the region studied.

C:N ratios were higher in pale-sandy soils than in loamy soil, and the total C and N content of the soils was influenced by interacting effects of soil type and land use. In Namibia, the N and organic C pools of soils under cultivation are depleted [42]. Similarly, in semiarid grasslands in northeastern Colorado, soil organic matter, potentially mineralizable N, and potentially respirable C were generally lower in soils that had been cultivated than in pristine soils [43]. Our study also found higher N levels in pristine than in cultivated loamy soils. Furthermore, soil N and C contents were higher in loamy-dark soils currently used as fields than in those lying fallow. Land use, i.e., whether a site was field or fallow, also influenced the nitrate content of the soils, with higher nitrate levels in fields than in fallows. Higher nutrient levels in fields than in fallows are in line with the findings of Burke et al. who observed that fallow fields were depleted of nutrients [43].

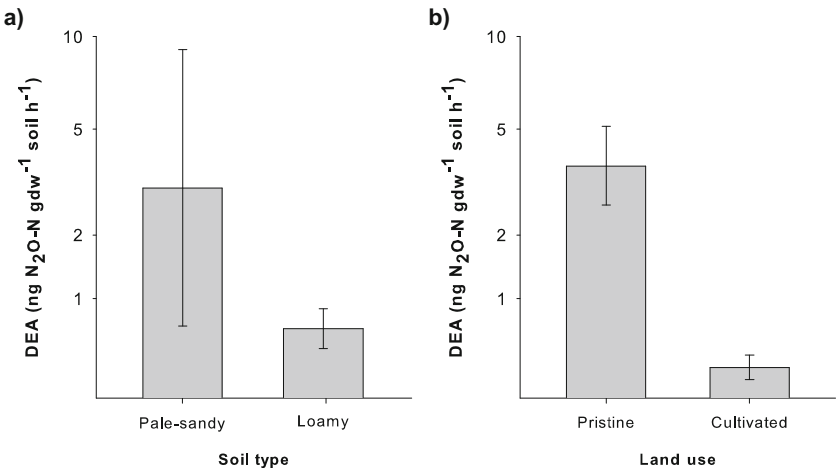

Fig. 2 Influence of land use and soil type on denitrifier enzyme activity (DEA) in Namibian savanna soils. **a** Influence of soil type and **b** influence of land use. Values are means±1 SE. For number of replicates, see Table 1

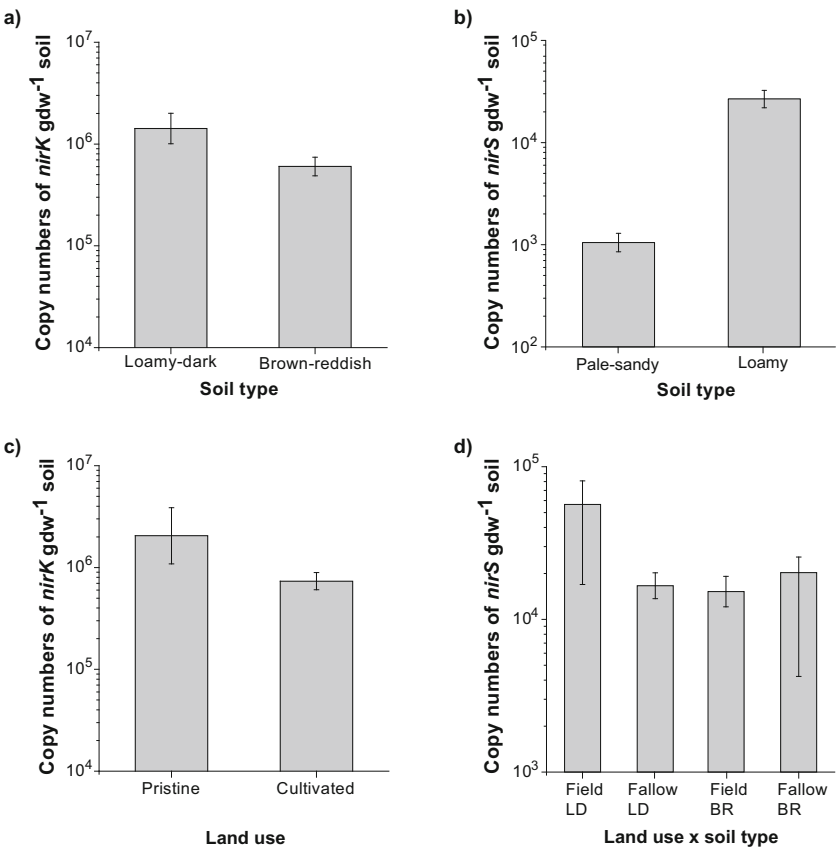

Fig. 3 Influence of land use and soil type on the abundance of *nirK*- and *nirS*-type denitrifiers in Namibian savanna soils. **a** *nirK* copy numbers in loamy-dark and brown-reddish soils, **b** *nirS* copy numbers in pale-sandy and loamy soils, **c** *nirK* copy numbers in pristine and cultivated soils, and **d** *nirS* copy numbers in loamy-dark and brown-reddish fields and fallows. Values are means±1 SE. For number of replicates, see Table 1. *LD* loamy-dark, *BR* brown-reddish soils

Burke et al. also found that recovery of active organic matter and nutrient availability requires a fallow period of 50 years [43]. The fact that the fallow sites in this study were abandoned for 10 years at most indicates that time was presumably not sufficient for recovery of at least the loamy-dark soils.

Influence of Land Use and Soil Type on Denitrifier Communities

In soils of the Kavango region, both the *nirK*- and *nirS*-type denitrifiers were influenced by soil type and land use, but environmental conditions also resulted in different responses of the two communities, presumably because they occupy distinct ecological niches [44, 45]. While the abundance of *nirK*-type denitrifiers depended on whether the soil was pristine or under cultivation, the assembly of their communities was strongly determined by the characteristics of the pale-sandy soils and whether it was currently cultivated or lying fallow. However, composition and abundance of denitrifier communities appear to be at least partly intertwined, because they responded to the same environmental stimuli and depended on whether the soil was brown-reddish or loamy-dark. Denitrifiers of the *nirS*-type were also strongly influenced by characteristics of the pale-sandy soils, which affected both the size and composition of their communities. In addition, their abundance also responded to the type of land use and depended on whether the soil was pristine or cultivated and whether loamy soils were fields or fallows. A further influence on their community composition was soil C content, which agrees with a previous report of the dependence of community composition on dissolved organic C [44]. Denitrifiers are mostly facultative aerobes that prefer a heterotrophic lifestyle. Hence, the availability of C may exert a much stronger selective pressure than availability of N oxides and oxygen.

Influence of Soil Characteristics on Denitrification Activity

Our findings that differences in land use and soil type determined a number of soil characteristics that are relevant for denitrification activity and also influenced the communities mediating the process suggest that denitrification activity and potential N_2O emissions of the Namibian savanna soils may indirectly depend on land management and soil type.

Table 4 The effects of soil type, soil characteristics, and cultivation on (A) the composition (first two axes of a correspondence analysis) of the *nirK*- and *nirS*-type denitrifier communities and (B) denitrifier enzyme activity (DEA)

Dependent variable	r^2	df	F	Explanatory variable	β	t
(A) Community composition						
Ax1 *nirK*	0.91	25	59.5***	Pale-sandy	0.94	10.9***
				Brown-reddish	0.20	2.3*
Ax2 *nirK*	0.27	26	9.4**	Field	0.52	3.1**
Ax1 *nirS*	0.50	26	25.6***	Pale-sandy	0.70	5.1***
Ax2 *nirS*	0.20	26	6.5*	C content	−0.45	−2.5*
(B) Denitrifier enzyme activity						
DEA	0.89	22	36.1***	C content	0.50	4.8***
				Pristine	0.36	3.4**
				Ax1 *nirK*	0.31	3.4**
				Ax2 *nirS*	−0.27	−3.3**
				Ax1 *nirS*	−0.28	−3.2**

Models are presented for which the Bayesian information criterion is minimal. Potential explanatory variables for the composition of the denitrifier communities were soil pale-sandy or not, soil brown-reddish or loamy-dark, sites pristine or cultivated, sites currently cultivated as a field or fallow, soil C content, soil pH, and soil nitrate content; for the DEA, the same variables plus denitrifier community composition and the abundance of the *nirK*- and *nirS*-type denitrifiers

r^2 coefficient of determination, *df* degrees of freedom, *F* *F* value, β standardized regression coefficient, *t* *t* value, *Ax1 nirK* first axis of a correspondence analysis of *nirK* T-RFLP data, *Ax2 nirK* second axis of a correspondence analysis of *nirK* T-RFLP data, *Ax1 nirS* first axis of a correspondence analysis of *nirS* T-RFLP data, *Ax1 nirS* second axis of a correspondence analysis of *nirS* T-RFLP data

*$P<0.05$; **$P<0.01$; ***$P<0.001$

Potential N_2O production from Namibian savanna soils in our study (range: from non-detectable levels to 9 ng N g^{-1} soil h^{-1}) was very low compared to that of temperate soils (1–3000 ng N g^{-1} soil h^{-1}; [45]) and even at the low end of DEA from semiarid grasslands in China (up to 339 ng N_2O-N g^{-1} soil h^{-1}; converted from [46]). Potential N_2O emissions estimated by the DEA method are assessed in laboratory studies under optimum conditions (oxygen limitation, optimal temperature, surplus of C and N) and inhibition of N_2O reductase by acetylene. Hence, N_2O production as estimated by DEA is generally much higher than in field measurements of N_2O production, because laboratory-like conditions rarely prevail in the field [47]. DEA reflects the long-term environmental conditions and the pool size of the persisting denitrifying enzymes and hence correlates to denitrification activity in the field [18, 48]. The low potential emissions of N_2O from semiarid soils in Namibia are in agreement with the results of a meta-analysis of fluxes from soils of savannas and other seasonally dry ecosystems [8]. At some sites, N_2O fluxes were low even during the wet season [49], but their magnitude was apparently influenced by soil organic matter and drainage [50]. Low N_2O fluxes were attributed to low soil organic matter, rapid drainage, and low nutrient levels [8]. In the Namibian savanna ecosystem studied here, DEA was higher at pristine and pale-sandy sites than at cultivated and loamy sites and depended primarily on C content and whether the soil was pristine or not. The concurrent availability of C and N and the C:N ratio are critical for denitrification in agricultural soils [51, 52], and the denitrification potential of semiarid grassland soils in China increased after addition of C and/or N [46]. We conclude that Namibian savannas resemble other semiarid grasslands where N and C are limiting denitrification in cultivated rather than in native semiarid

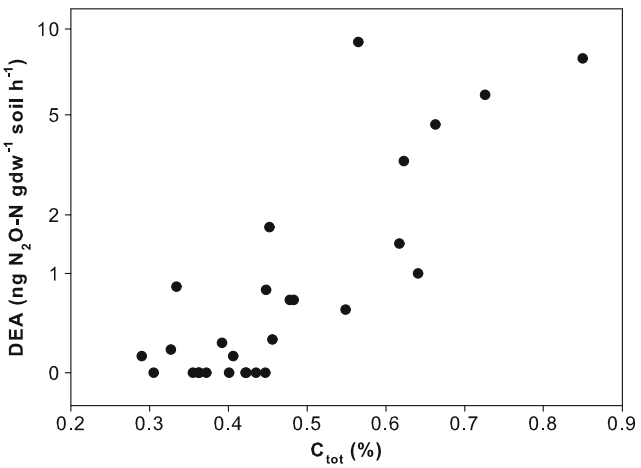

Fig. 4 The relationship between DEA and C content of soils

grasslands [43]. In these regions, the use of fertilizers is still uncommon and hence harvesting significantly depletes nutrients [10, 43]. Furthermore, changes in the use of Namibian savanna ecosystems such as logging by clear-cutting can alter soil bulk density and organic matter pools [53, 54], which may lead to increased N_2O emission from soils [4]. Trampling by grazing livestock also increases N_2O emissions from grassland soils [4]. Other management practices like tillage may affect the stability of aggregates, soil C content, and its dynamics [55]. Tillage destroys soil structures that occlude oxygen diffusion to organic materials and hence prevents enzymatic decomposition of C compounds [56, 57]. All this points to a greater availability of soil organic matter and hence a more effective N cycling and a higher potential for N_2O emissions in the pristine woodland savannas, which are not disturbed by management and receive higher organic matter input into the soil than the cultivated areas [58].

Influence of Denitrifier Communities on Potential Denitrification Activity

Denitrification activity may depend not only on soil characteristics but also on the size and the composition of the underlying denitrifier communities, although the literature is inconsistent in what matters most (e.g., [44, 59, 60]). In this study, characteristics of the soils determined by land use and soil type influenced both denitrifier abundance as well as community composition. However, DEA of the soils was related only to the composition of the *nirK*- and *nirS*-type denitrifier communities while the size of the communities was irrelevant. Although it is currently not possible to correlate differences in denitrification activity to the relative abundance of single terminal restriction fragments or nitrite reductase genotypes, it is known that specific denitrification activity differs among individual strains [61, 62]. This suggests that denitrification activity is determined by the taxonomic makeup of bacterial communities [63], whose development was influenced by the soil characteristics explicitly investigated in this study or by characteristics that were not specified [44] but were covered by the terms land use or soil type.

Conclusions

Overall, our results suggest that N_2O emissions in southern Africa may not increase by the conversion of pristine savanna to cultivated soils as long as fertilizers are not applied on a large scale. In pristine soils, N is conserved by microorganisms and plants via net primary production, thus limiting the availability of N for nitrification and denitrification [64, 65]. However, anthropogenic influences like the continuing conversion of native to agricultural lands together with the predicted inputs of N into African savanna soils by the use of fertilizers have the potential to disrupt the closed N cycle [65]. This may result in increased N_2O emissions from agricultural land [66–68], but denitrification activity and hence the size of the N_2O fluxes may be limited by the availability of soil C.

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