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Review Paper

Contrasting effects of grasses and trees on microbial N-cycling in an African humid savanna

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ABSTRACT

African humid savannas are highly productive ecosystems, despite very low soil fertility, where grasses and trees coexist. Earlier results showed that some perennial grass species are capable of biological nitrification inhibition (BNI) while trees likely influence differently on nitrogen cycling. Here we assessed the impact of the dominant grass and tree species of the Lamto savanna (Ivory Coast) on soil nitrifying and denitrifying enzyme activities (NEA and DEA, respectively) and on the abundances of archaeal and bacterial ammonia oxidizers (AOA and AOB, respectively) and nitrite reducers. This is one of the first studies linking nitrifying and denitrifying activities and the abundances of the involved groups of microorganisms in savanna soils. NEA was 72-times lower under grasses than under trees while AOA and AOB abundances were 34- and 3-times lower. This strongly suggests that all dominant grasses inhibit nitrification while trees stimulate nitrification, and that archaea are probably more involved in nitrification than bacteria in this savanna. While nitrite reducer abundances were similar between locations and dominated by *nirS* genes, DEA was 9-times lower under grasses than trees, which is likely explained by BNI decreasing nitrate availability under grasses. The *nirS* dominance could be due to the ferruginous characteristics of these soils as *nirS* and *nirK* genes require different metallic co-enzymes (Fe or Cu). Our results show that the coexistence of grasses and trees in this savanna creates a strong heterogeneity in soil nitrogen cycling that must be considered to understand savanna dynamics and functioning. These results will have to be taken into account to predict the feedbacks between climate changes, nitrogen cycling and tree/grass dynamics at a time when savannas face worldwide threats.

1. Introduction

Savannas are characterized by the coexistence of two contrasting plant types, trees and grasses, and cover 12–13% of global terrestrial areas (Rutten et al., 2016). This coexistence is traditionally explained by disturbances or resource partitioning (Barot and Gignoux, 2004; Sankaran et al., 2004): in nutrient-limited ecosystems, tree-grass coexistence could also be explained by positive plant-soil feedbacks (Bonanomi et al., 2008) creating heterogeneity in soil resources (Brandt et al., 2013). Some perennial grass species of savannas (e.g. *Brachiaria* spp., *Sorghum bicolor, Hyparrhenia diplandra)* are known to inhibit nitrification (Lata et al., 1999, 2000, 2004; Subbarao et al., 2009). In particular, *Hyparrhenia diplandra*, the dominant species in the Lamto savanna in Ivory Coast, is the first species for which such an ability has been documented (Lata et al., 2004, 1999, 2000). The mechanism allowing nitrification inhibition has been described for *Brachiaria humidicola* (Subbarao et al., 2009): roots release exudates into the soil that can block the bacterial ammonia oxidation pathway (Subbarao et al.,

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2009). The ability of grass to inhibit nitrification has probably a strong influence on savanna functioning as it decreases the availability of nitrate and the subsequent possible losses of nitrogen (N) by denitrification and nitrate leaching, which is likely to increase primary production through a plant-soil feedback (Boudsocq et al., 2009). This has also broad implications for tropical pastures as African grasses have been exported worldwide and are widely used in pastures e.g. in Brazil, the largest beef exporter in the world (Del' Alamo Guarda and Del' Alamo Guarda, 2014). However, it is not yet known whether biological nitrification inhibition (BNI) or at least very low nitrification rates in rhizospheric soils, is a common trait among African tropical perennial grasses.

Concerning trees, previous results obtained in Lamto savanna showed that mineralization rates and soil organic matter content were higher under tree canopies than under grasses (Mordelet et al., 1993). This suggests that nitrification could also be enhanced under trees if all microbial activities are enhanced by the higher availability in organic matter. However, tree canopy effect on soil functioning is complex and likely depends on tree species characteristics (Mordelet et al., 1993). Modelling results (Boudsocq et al., 2012) suggest that such differences in nitrification rates and possible differences between trees and grasses in their preference for the absorption of ammonium vs. nitrate could contribute to their coexistence. Our first objective was thus to compare nitrification rates under dominant tree species and grasses in Lamto savanna to test whether these two plant types can lead to contrasting types of N cycling.

The impact of grasses and trees on the various fluxes involved in N cycling are likely linked to variations in the abundance of the involved microbial groups. A first issue is the identification of the major microbial groups involved in N fluxes in savanna soils. Since the discovery of their role in nitrification, ammonia oxidizing archaea (AOA) have been found to be more abundant than ammonia oxidizing bacteria (AOB) in most temperate soils (Prosser and Nicol, 2012; Sterngren et al., 2015). However, the relative importance of AOA and AOB for nitrification likely depends on soil characteristics (Hatzenpichler, 2012). Therefore, a growing number of articles have documented the actual importance of archaea in the realization of ammonia oxidation. Significant AOA activities have been found in acidic soils and soils with low ammonium concentration (Nicol et al., 2008; Prosser and Nicol, 2012). On the contrary, AOB are important drivers of nitrification in soils with high ammonium concentrations and in response to N fertilization (Ma et al., 2016; Simonin et al., 2015; Verhamme et al., 2011). However, very few studies have been published on nitrification and nitrifiers in savanna soils (Catão et al., 2016; Rughöft et al., 2016; Wild, 2016; Assémien et al., 2017). Moreover, to our knowledge, no study has assessed the denitrifying activity and its link with denitrifying microorganisms in savanna soils.

A second issue is to determine the respective impacts of savanna trees and grasses on microbial communities, e.g. the abundances of nitrifiers and denitrifiers. These plants, by their likely different impacts on soil N functioning, could also modify the relative importance of different nitrifier and denitrifier groups. Indeed, nitrification is a key metabolic pathway for nitrifying bacteria and archaea with ammonia as the only source of energy, and BNI could modify the respective competitive abilities of nitrifying bacteria and archaea. BNI by decreasing the availability of nitrate under perennial grasses should also be detrimental to denitrifiers. Denitrification rates and denitrifier abundances should thus be lower under grasses than under trees. Taken together, by their impacts on organic and mineral N (nitrate and ammonium) trees and grasses likely impact all components of microbial communities. We expect these impacts to be particularly strong because perennial grasses inhibiting nitrification are long-lived tussock grasses, living as long as savanna trees (ca. 80 years; Abbadie et al., 2006).

We tested here whether the presence of grass *vs*. tree species, and possibly species identity, is associated to contrasting types of N cycling in their rhizospheric soil in the Lamto savanna. To do so, we compared soils under the influence of the four dominant perennial grass species and the four dominant tree species in terms of nitrifying and denitrifying enzyme activities (i.e. NEA and DEA), abundances of nitrifiers (i.e. AOA and AOB) and denitrifiers (*nirK*- and *nirS*-nitrite reducers), and soil physicochemical characteristics. The total abundances of bacteria and archaea were also quantified. The following hypotheses were tested: (1) The dominant savanna grass species are associated to very low soil NEA and low DEA due to direct and indirect impacts of BNI, respectively. (2) Contrary to grasses, dominant tree species are associated to higher soil NEA and DEA due to the absence of BNI activity under tree. (3) The differences in nitrifying and denitrifying activities between tree and grasses are linked to contrasting abundances in nitrifiers and denitrifiers due to the presence or absence of BNI. (4) The presence of trees and grasses modifies the relative abundance of the main groups involved in nitrification (i.e. AOA/AOB ratio) and denitrification (*nirS/nirK* ratio) in relation to the soil physicochemical characteristics (higher N, C contents and humidity under trees).

2. Material and methods

2.1. Study sites

The Lamto reserve is located in Ivory Coast, West Africa (6°13′N, 5°20′W). The vegetation is a mosaic of savannas with various tree densities, and gallery forests. Temperatures are relatively constant throughout the year (27 °C on average). Four seasons can be distinguished: (i) a long dry season from December to February; (ii) a long wet season from March to July; (iii) a short dry season in August; (iv) a short wet season from September to November. Annual precipitation in 2014 was 826 mm (data from the Geophysical Station of Lamto). The soils are composed of granites and derived sands and classified as tropical ferrugineous soils with a superficial gravelly horizon. The soils are sandy (ca. sands 77%; silts 14%; clays 9%) and with a bulk density of ca. 1.65 (Lata, 1999).

2.2. Soil sampling

Lamto savanna ecosystem is highly structured due to high environmental constraints (e.g. scarcity of nutrients, fire). Trees and grass individuals are spatially well separated (e.g. grass tussock individuals can be separated up to 70 cm distance). We therefore focused on the rhizospheric soil as the microbial processes are concentrated in the close vicinity of roots (Abbadie et al., 1992). Soil was sampled during the long wet season (April 2014) in the open shrub savanna under the tussocks of the four dominant perennial grass species: *Andropogon canaliculatus* (AC), *Andropogon schirensis* (AS), *Hyparrhenia diplandra* (HD) and *Loudetia simplex* (LS); and under the canopy of the four dominant tree species: *Bridelia ferruginea* (BF), *Cussonia barteri* (CB), *Crossopteryx febrifuga* (CF) and *Terminalia glaucescens* (TG). In total, these dominant species represent in this ecosystem a proportion of minimum 80% of individuals and biomasses for both grass and tree compartments (Abbadie et al., 2006).

Soil sampling below trees was achieved in patches of bare soil between grass tussocks. Grass tussocks were chosen to have similar basal diameter (ca. 20 cm) and trees were selected to have similar diameter at breast height (ca. 22.5 cm). The choice of plant individuals was made randomly on a surface of *ca*. 10 ha and local sources of heterogeneity (termite mounds, small depressions, rocks) were avoided. For each of the eight species, five replicated soil samples (about 1 kg, each of them composed of two pooled sub-samples) were collected from the top 15 cm with an auger (8 cm in diameter) and stored at 4 °C for a very short period during transport. Samples were subsequently sieved (2 mm), homogenised and 200 g of soil were stored at −20 °C for molecular biology analyses and the measurements of nitrifying/denitrifying enzyme activities. Freezing of soil samples was preferred to fresh or drying conservation methods because (i) the time between

sampling and analysis (including transportation) was too long to consider the soil fresh, (ii) previous studies on different soil matrices shown that for soil enzyme activity studies (e.g. denitrification), freezing was a preferable storage method (Wallenius et al., 2010), (iii) preliminary tests on fresh *vs*. frozen samples on our soils showed no discrepancy. The remaining soil was immediately air-dried in the shade and stored at ambient temperature for physicochemical analyses. Roots were collected from all soil samples through dry sieving. Fine root densities varied between 0.57 and 9.77 g dry root dm^{-3} dry soil under grass and between 0.77 and 3.68 g dry root dm^{-3} dry soil under trees. Grass aboveground biomass of each tussock was collected and dried at 50 °C before weighing. Depending on species, aboveground biomasses varied between 39.20 and 276.70 g dry matter $tussock^{-1}$. Soil moisture was measured with a ThetaProbe ML3 (Delta-T Devices) previously calibrated on oven-dried soils.

2.3. Microbial abundances and activities

2.3.1. Total nucleic acids extraction

Total nucleic acids were extracted from 0.5 g of soil (wet weight) with a Bio-101 FastDNA Spin kit in combination with the FastPrep FP120 bead beating system (Bio-101, Inc., Ca, USA) according to the manufacturer's instructions. Bulk total DNA was purified by elution through Geneclean Turbo columns (MP Biomedicals, CA, USA) according to the manufacturer's instructions. The concentration and purity of the resulting DNA was determined by measuring the absorbance at 260 and 280 nm using a spectrophotometer and calculation of the ratio A260/A280 (NanoDrop ND-1000 spectrophotometer). All extracted DNA samples were stored at −20 °C before being analysed.

2.3.2. Real-time PCR quantification

The archaeal nitrifying (*amoA-AOA* gene), bacterial nitrifying (*amoA-AOB* gene), bacterial denitrifying (*nirK* and *nirS* genes), total bacterial (16S rRNA) and archaeal (16S rRNA) communities were determined by Real-Time PCR (CFX96 Real-Time System, Bio-Rad, France) with specific primer sets (see qPCR conditions in Table S1). Quantification was based on the increasing fluorescence intensity of the SYBR Green dye during amplification. The real-time PCR assay was carried out in a 20 µL reaction volume containing SoAdvanced SYBR-Green Supermix (2×, Bio-Rad) and 1.25 µL of Bovine Albumine Serum (2 mg ml^{-1}). Standard curves were obtained using serial dilutions of linearized plasmids containing the studied genes. The amplification efficiencies were calculated as [10(-1/slope) - 1]. The average PCR efficiency for the different assays was 83.9%, 85.5%, 90.3%, 90.3%, 90.8% and 87.2% for *amoA*-AOA, *amoA*-AOB, *nirK* gene, *nirS* gene, bacterial 16S rRNA and archaeal 16S rRNA respectively. All DNA extractions were subsequently diluted 10-fold and 100-fold with nuclease-free water to reduce potential PCR inhibition. It was then tested whether these diluted samples led to the same amplification efficiency as the non-diluted ones. Two independent quantitative PCR assays were performed for each gene to test methodological repeatability. Results were expressed as gene copy numbers per gram of dry soil. After the qPCR reaction, a melting step allowed DNA to denaturate by elevating the temperature from 65 °C to 95 °C by 0.5 °C every 0.05 s. Then, the melting curve was integrated using the Dissociation Curve Analysis Software (Applied Biosystems). We used this curve to check the generation of specific amplicons and this was also checked by gel electrophoresis analysis.

2.3.3. Nitrifying and denitrifying enzyme activities

Potential nitrification and denitrification rates were measured through Nitrifying (NEA) and Denitrifying Enzyme Activities (DEA) assays, which corresponds to short-term laboratory incubations under non-limiting conditions focusing on the period of constant activity i.e. the incubations are too short to allow the multiplication of the involved organisms that could result in an increase in activity. NEA and DEA avoid the short-term variations observed in the field that are induced by climate or other environmental factors (Attard et al., 2011; Lata et al., 1999). Frozen soil samples were placed at ambient room temperature for 2 h before the analyses. DEA was measured according to Patra et al. (2006) as the linear rate of production of N_2O during a short time (here 2-h incubation) at 100% water holding capacity, 28 °C, and with no limiting N and C availabilities (here additions of 50 µg $N\text{-}NO_3^-$ g^{-1} and 1 mg C g^{-1}). A 90:10 He-C_2H_2 atmosphere provided anaerobic conditions and inhibited N_2O-reductase activity. Measurements were taken every 30min using a gas chromatograph (Agilent µGC R3000, Santa Clara, CA, USA). NEA was measured according to Dassonville et al. (2011) as the linear rate of production of nitrate during a 72 h incubation. Sub-samples of fresh soil (3 g equivalent dry soil) were incubated with 6 ml of a solution of $(NH_4)_2SO_4$ (22 µg $N\text{-}NH_4^+$ g^{-1} dry soil). Distilled water was added in each sample to reach 24 ml of total liquid volume in flasks. Soil nitrate content was measured after 5, 24, 48 and 72 h during the aerobic incubation under constant agitation (180 rpm) by ion chromatography (DX120, Dionex, Salt Lake City, USA).

2.4. Physicochemical soil characteristics analyses

Soil pH was measured in water (5:1 v/v water:soil) with a pH meter (Mettler Toledo SevenEasy™) according to the NF ISO 10390 standard. Mineral N was extracted from 2 g frozen soil by adding 2 M KCl solution (soil:solution = 1:4). Nitrate was reduced to nitrite, and then nitrite and ammonium concentrations (expressed as mg $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ kg^{-1} dry soil) were measured with a continuous-flow N analyzer (SKALAR, San Plus System, Breda, the Netherlands). Total C and N contents in soils (expressed as %) were measured using a CHN Elemental Analyzer (NA1500 Series 2, Fisons, Manchester, UK) after grinding at 10 µm.

2.5. Statistical analysis

All statistical analyses were performed by using R software (R Core Team 2016). For all measured variables, differences between grasses and trees (plant type) and between species within grasses and trees (the type of species being nested within the plant type), were tested using an ANOVA. In case of a significant effect of species within plant type (grasses or trees), species of trees or species of grasses were compared using post-hoc tests (Tukey Honestly Significant Difference test). The normality and homoscedasticity of the residuals of all linear models were tested with Shapiro-Wilk and Bartlett tests, respectively. Data was log-transformed in case of significant deviation from normality and homoscedasticity. Herein, all variables were log-transformed expect pH, NO_3^-, C:N ratio and AOA/(AOA + AOB) ratio. We tested the relation between NEA (or DEA) and the abundances of nitrifying (or denitrifying) communities using linear and non-linear saturating (using nls R function) models. For all tests, the null hypothesis was rejected for $p < 0.05$ and significance is represented as follows: *** when $p \leq 0.001$; ** for $0.001 < p \leq 0.01$; * when $0.01 < p \leq 0.05$.

3. Results

3.1. Soil physical and chemical characteristics

Except for pH, soil physical and chemical characteristics varied between grasses and trees (average mean 6.75 ± 0.18%). Soil under trees had significantly higher values than grasses in terms of water content (WC (19.26 ± 2.32%; 5.70 ± 2.05%)), mineral N content ($N\text{-}NH_4^+$ (9.64 ± 1.69 mg kg^{-1} dry soil; 6.50 ± 0.68 mg kg^{-1} dry soil) and $N\text{-}NO_3^-$ (2.32 ± 0.10 mg kg^{-1} dry soil; 1.93 ± 0.15 mg kg^{-1} dry soil)) and total N (0.0767 ± 0.0154%; 0.0600 ± 0.0062%) and C (1.10 ± 0.27%; 0.97 ± 0.13%) contents ($p < 0.001$ except for C content $p < 0.05$; Table 1 and Table S2). On the contrary, C:N ratio

Table 1
Statistical results (ANOVA) of the effects of plant type and plant species on different soil physicochemical characteristics.

		Df	F	p-value
pH	Plant type	1	0.05	0.83
	Grasses	3	1.57	0.23
	Trees	3	13.03	< 0.001
Water content	Plant type	1	304.06	< 0.001
	Grasses	3	3.0353	0.06
	Trees	3	0.9679	0.43
$[NH_4^+]$	Plant type	1	72.39	< 0.001
	Grasses	3	0.16	0.92
	Trees	3	0.71	0.56
$[NO_3^-]$	Plant type	1	87.91	< 0.001
	Grasses	3	0.49	0.70
	Trees	3	1.78	0.19
N content	Plant type	1	25.34	< 0.001
	Grasses	3	0.97	0.43
	Trees	3	2.25	0.12
C content	Plant type	1	4.29	< 0.05
	Grasses	3	0.92	0.45
	Trees	3	2.13	0.14
C:N ratio	Plant type	1	42.22	< 0.001
	Grasses	3	0.89	0.47
	Trees	3	0.32	0.81

was significantly lower under trees (14.32 ± 0.88) than grasses (16.09 ± 0.80) ($p < 0.001$). There was no significant effect of grass species on soil WC, mineral N contents (N-NH_4^+ and N-NO_3^-) and total N and C contents (Table 1 and Table S2). The only significant difference between tree species was for pH: TG (6.91 ± 0.09) higher than CB (6.73 ± 0.19; $p < 0.01$) and BF (6.61 ± 0.03, $p < 0.001$), and CF (6.78 ± 0.15) higher than BF ($p < 0.05$).

3.2. Nitrifying and denitrifying enzyme activities

Nitrifying enzyme activities (NEA) were 72 times higher under trees than under grasses and no significant difference was observed between species within each plant type (Fig. 1A and Table 2). Similarly, denitrifying enzyme activities (DEA) were 9 times higher under trees than under grasses, without any significant difference among grass species or among tree species (Fig. 1B and Table 2).

3.3. Archaea and bacteria abundances

Total archaeal abundances (16S rRNA) were lower under trees ($7.97 \pm 2.95 \times 10^8$ copies g^{-1} dry soil) than under grasses ($11.9 \pm 6.95 \times 10^8$ copies g^{-1} dry soil; Table 2). Within grass species, significant differences were found ($p < 0.05$), whereas within tree species no significant difference was found. Total bacterial abundances (16S rRNA) were lower under trees ($2.95 \pm 2.95 \times 10^9$ copies g^{-1} dry soil) than under grasses ($4.31 \pm 1.32 \times 10^9$ copies g^{-1} dry soil; Table 2). Within grass (not tree) species, significant differences were found ($p < 0.01$).

3.4. Nitrifier abundances

Archaeal *amoA*-AOA gene abundance was 34 times higher under trees ($6.84 \pm 3.47 \times 10^6$ copies g^{-1} dry soil) than under grasses ($2.00 \pm 1.57 \times 10^5$ copies g^{-1} dry soil; Fig. 2A and Table 2). Within grass species, significant differences were found: *Andropogon canaliculatus* (AC) higher than *Hyparrhenia diplandra* (HD, $p < 0.01$) and *Loudetia simplex* (LS, $p < 0.001$); *Andropogon schirensis* (AS) and HD higher than LS ($p < 0.01$ and $p < 0.05$ respectively) (Fig. 2A and Table S3). AOA abundance also varied significantly between tree species, and was significantly higher under BF and CB than TG ($p < 0.01$ and $p < 0.001$ respectively) (Fig. 2A and Table S3).

Bacterial *amoA*-AOB gene abundance was three times higher under trees ($7.57 \pm 0.73 \times 10^5$ copies g^{-1} dry soil) than under grasses ($2.75 \pm 0.89 \times 10^5$ copies g^{-1} dry soil; Fig. 2B and Table 2). Within grass species, AOB was more abundant under HD than LS ($p < 0.01$), while there was no significant difference between tree species (Table 2 and Table S3).

To investigate the relative proportion of AOA in the nitrifying community, the ratio AOA:(AOB + AOA) was calculated (Fig. 2C and Table S3). The ratio was significantly higher for trees (0.83 ± 0.10) than for grasses (0.37 ± 0.14). No difference was found between grass species, while within trees species the ratio was significantly higher under BF, CB and CF than TG ($p < 0.001$; $p < 0.001$; $p < 0.05$, respectively; Fig. 2C and Table S3).

Finally, AOA represented 0.82 ± 0.41% of the total archaea under trees and 0.02 ± 0.02% under grasses, a significant difference ($p < 0.001$). AOB represented 0.03 ± 0.01% of the total bacteria under trees and 0.01 ± 0.001% under grasses. This difference between trees and grasses was also significant ($p < 0.001$) (Table 2 and Table S3).

3.5. Denitrifier abundances

No difference was observed between grasses and trees for the *nirK* gene abundance (Fig. 3A – mean value of $1.38 \pm 0.20 \times 10^8$ copies g^{-1} dry soil). Within grasses, it was significantly higher under AS than HD ($p < 0.01$). Within trees, the *nirK* gene abundance was significantly higher under BF than under CF ($p < 0.05$; Fig. 3A and Table S3).

No difference was observed between grasses and trees for the *nirS* gene abundance (Fig. 3B – mean value of $1.49 \pm 0.27 \times 10^8$ copies g^{-1} dry soil). Within grasses, it was significantly higher under AC, AS and HD than LS ($p < 0.05$, $p < 0.05$ and $p < 0.01$ respectively, Fig. 3B and Table S3). No significant difference was observed between tree species (Fig. 3B).

To investigate the relative proportion of *nirS* gene within the nitrite reducing community, the ratio *nirS*:(*nirK* + *nirS*) was calculated (Fig. 3C and Table 2). It was similar under trees (0.69 ± 0.06%) and grasses (0.67 ± 0.09%). Within grass species, it was significantly higher under AC and HD than under LS ($p < 0.05$; $p < 0.001$). No difference was observed between tree species.

3.6. Links between nitrifier and denitrifier abundances and enzyme activities

Regressions between enzyme activities and microbial abundances were performed separately for trees and grasses (Fig. 4A and B). A significant positive non-linear relation was observed between the NEA and AOA abundance under trees (NEA = a × *amoA*-AOA/(b + *amoA*-AOA); a = 0.10, $p < 0.001$; b = 2.88×10^6, $p < 0.05$). A significant positive linear relation was observed between NEA and AOB abundance under trees (NEA = c × *amoA*-AOB + d; c = 6.76×10^{-8}, $p < 0.01$, d = 0, $p > 0.05$; $r^2 = 0.39$). With constant and very low activity values, no relation was found under grasses between NEA and AOA or AOB abundance. Finally, no relation was observed under trees and grasses between DEA and *nirK* or *nirS* abundances.

4. Discussion

4.1. Contrasting impacts of grasses and trees on nitrification and ammonia oxidizer abundances

In our study, NEA rates under the grass species HD, known to inhibit

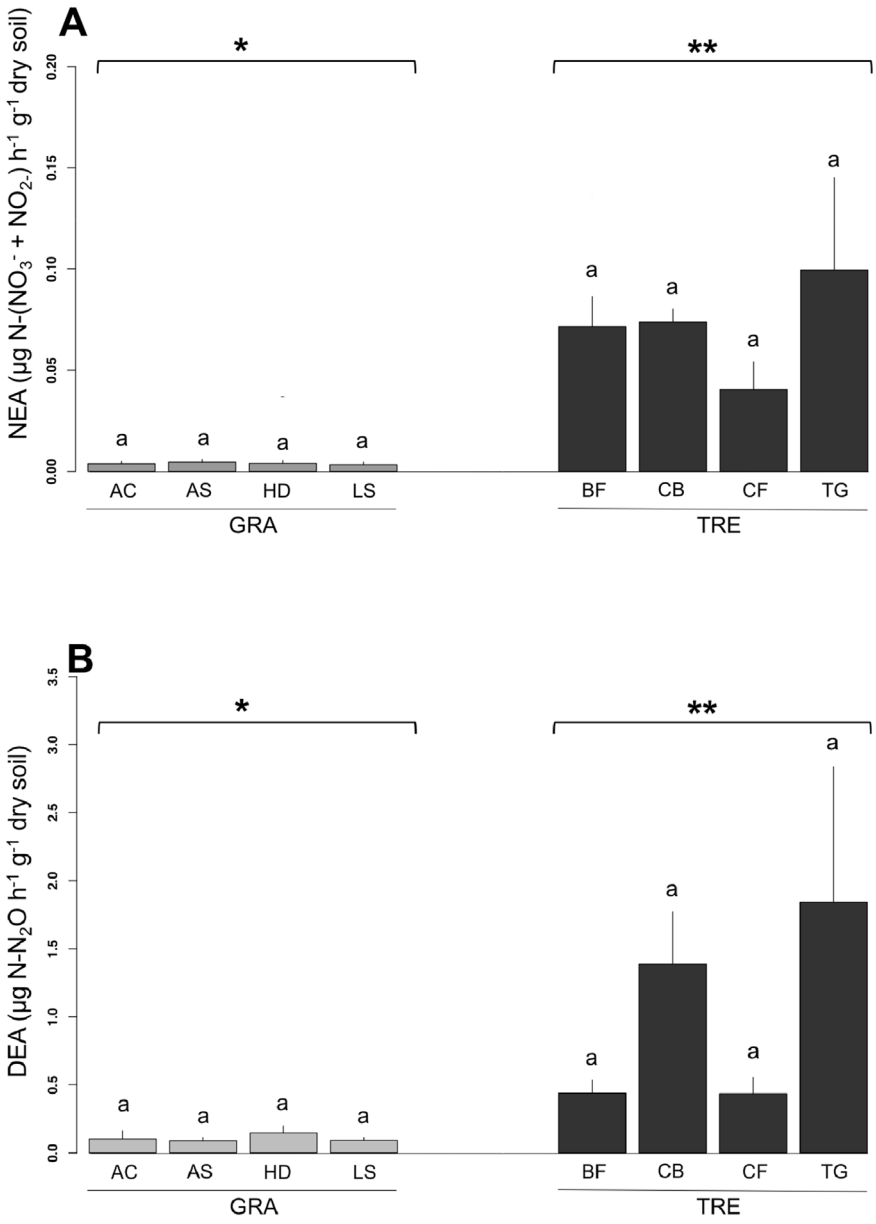

Fig. 1. Nitrifying (panel A) and denitrifying (panel B) enzyme activities according to plant species: grasses (grey bars) AC (*Andropogon canaliculatus*), AS (*Andropogon schirensis*), HD (*Hyparrhenia diplandra*), *LS* (*Loudetia simplex*), and trees (black bars): BF (*Bridelia ferruginea*), CB (*Cussonia barteri*), CF (*Crossopteryx febrifuga*), TG (*Terminalia glaucescens*), GRA (Grasses) and TRE (Trees). Means and SEs were calculated from five replicates. Different numbers of stars indicate statistically significant differences between tree and grass species. Different small letters indicate statistically significant differences between species within each plant type (either trees or grasses) ($p < 0.05$).

nitrification, are as low as previously reported. The BNI effect of HD was previously demonstrated in the field, with a negative correlation between nitrification and HD root density (Lata et al., 2004), and also verified in controlled hydroponic conditions where a nitrifying inhibitory activity has been directly measured (between 45 and 70 ATU (inhibitory activity of roots expressed in allylthiourea unit) plant^{-1} day^{-1}, unpublished data) under different HD individuals grown from seeds using Subbarao's method (Subbarao et al., 2009). We have found similar low NEA values under HD and the other three dominant grass species of Lamto savanna (the four species represent together 80% of grass tussocks in this savanna). Hence, our results strongly suggest that all the dominant grasses of Lamto savanna are capable of BNI through root exudates acting on microbial enzymatic pathways, as demonstrated on the two African grass species *B. humidicola* and *Sorghum bicolor* (Subbarao et al., 2009; Zakir et al., 2008).

The data presented here, based on nitrifying enzyme activity, NEA, only provide an indirect proof of the capacity of the three dominant grasses (in addition to HD) to inhibit nitrification. However, NEA is measured in optimal condition (temperature, humidity, NH_4^+ availability) thus expressing long-lasting changes in the expression of microbial communities rather than a response to a transient state of soil. This suggests that a long-lasting strong mechanism is at play. An alternative possibility to nitrification inhibition by the four dominant grass species would be that HD tussocks alone are able to inhibit nitrification on the whole savanna away from trees. This is however unlikely because (i) the grass cover is very discontinuous with ca. 90% of the soil surface being bare between tussocks (Abbadie et al., 2006; Lata, 1999; Lata et al., 2000); (ii) the root systems of neighbouring tussocks are not overlapping (Abbadie et al., 1992); (iii) these perennial grasses are very long-lived (several decades; Abbadie et al., 2006) and (iv) the collected soils under AC, AS and LS were often several meters away from any HD tussock. Besides, NEA values for patches of soils deprived of any grass root influence were found (within the first weeks after patch creation and later on) to be (Lata, 1999) 20-fold higher than the values found in the present study under the four grass species. This further supports the hypothesis that the four dominant grasses of Lamto savanna do inhibit nitrification.

We have observed much higher (72-fold higher) NEA under trees than under grasses. While for grasses an active and specific mechanism of BNI has been demonstrated (Lata et al., 2004; Subbarao et al., 2012),

Table 2
Statistical results (ANOVA) of the effects of plant type and plant species on the nitrification (NEA) and denitrification (DEA) enzymes activities, on the abundance of nitrifying and denitrifying communities, and on the total archaeal and bacterial abundances.

		Df	F	p-value	Effect
NEA	Plant type	1	123.86	< 0.001	***
	Grasses	3	0.10	0.96	NS
	Trees	3	1.59	0.23	NS
DEA	Plant type	1	43.30	< 0.001	***
	Grasses	3	0.40	0.76	NS
	Trees	3	2.20	0.13	NS
amoA-AOA	Plant type	1	228.643	< 0.001	***
	Grasses	3	17.347	< 0.001	***
	Trees	3	7.771	< 0.001	***
amoA-AOB	Plant type	1	118.305	< 0.001	***
	Grasses	3	4.2978	0.01048	*
	Trees	3	0.7096	0.5526	NS
nirK	Plant type	1	1.030	0.314	NS
	Grasses	3	5.1395	0.004415	**
	Trees	3	3.2848	0.03168	*
nirS	Plant type	1	3.701	0.058	NS
	Grasses	3	4.5961	0.007687	**
	Trees	3	1.8023	0.1642	NS
AOA/AOB + AOA	Plant type	1	214.32	< 0.001	***
	Grasses	3	2.22	0.15	NS
	Trees	3	10.40	< 0.001	***
nirS/nirS + nirK	Plant type	1	2.4208	0.1240004	NS
	Grasses	3	7.5892	< 0.001	***
	Trees	3	1.6755	0.1895	NS
Total archaeal abundances (16S rRNA)	Plant type	1	6.039	0.016	*
	Grasses	3	4.0837	0.01313	*
	Trees	3	1.8165	0.1616	NS
Total bacterial abundances (16S rRNA)	Plant type	1	15.732	< 0.001	***
	Grasses	3	4.5442	0.008111	**
	Trees	3	1.4158	0.254	NS
AOA/16S rRNA Archaea	Plant type	1	470.709	< 0.001	***
	Grasses	3	39.64	< 0.001	***
	Trees	3	10.118	< 0.001	***
AOB/16S rRNA Bacteria	Plant type	1	133.796	< 0.001	***
	Grasses	3	0.352	0.7879	NS
	Trees	3	0.6134	0.6108	NS

the way savanna trees impact N cycling remains unknown. The most simple hypothesis would be that the increase in organic matter, mineral N availability and water availability, maintains a higher and more active microbial biomass under tree canopies (Mordelet et al., 1993). However, it would be worth testing the existence of a more specific mechanism based on the release of particular exudates by trees (or leaf leachates) impacting directly the nitrification and denitrification pathways of microbial communities (Andrianarisoa et al., 2010). In any case, the observed NEA values for trees are higher (ca. 5-fold higher) than under bare soil patches (see above, Lata, 1999). This supports the hypothesis that the four dominant tree species increase nitrification, whatever the underlying mechanism.

We found that the AOA and AOB abundances were positively correlated to the NEA under trees, suggesting that both are involved in the NEA measured herein. However, the proportion of AOA is rather high both under trees (AOA abundance higher than AOB) and under grasses (same AOA and AOB abundances). Previous studies have highlighted the importance of AOA in soils with low NH_4^+ concentrations or low organic matter content and with low pH (Bates et al., 2010; Nicol et al., 2008; Prosser and Nicol, 2012; Assémien et al., 2017). Indeed, differences in transporters for NH_3/NH_4^+ between both AMO enzymes have been strongly suggested, leading to the hypothesis that AOA are better competitors than AOB for NH_4^+ (Hatzenpichler, 2012). Even if soil is N-richer under trees, Lamto savanna soils can be viewed as being extremely nutrient poor (Abbadie and Lata, 2006) when compared to other ecosystems. The low availability of mineral nutrients in these slightly acidic savanna soils could therefore reinforce the role of AOA for nitrification in this ecosystem (Stempfhuber et al., 2015; Taylor et al., 2012), which has also been suggested when comparing nitrification and nitrifier abundances in fertilized and non-fertilized soils from the same savanna area (Assémien et al., 2017). Recent studies in South African savanna soils have also documented the importance of AOA in correlation with soil nutrient availability (Rughöft et al., 2016).

The relative dominance of AOA and AOB was different between trees and grasses, as shown by the AOA/(AOB + AOA) ratio. AOA were much more abundant than AOB under tree canopies, while AOB were slightly more abundant than AOA under grasses. Under grasses and in contrast with soils under trees, no correlation was observed between NEA and AOA or AOB abundance. In comparison to trees, grasses induced a much stronger decrease in AOA abundances (6.84×10^6 and 2.00×10^5, respectively for trees and grasses) than in AOB abundances (7.57×10^5 and 2.75×10^5, respectively for trees and grasses). This is again clearly shown by results on the AOA/(AOB + AOA) ratio. Moreover, these decreases were not related to an overall decrease in total archaea and bacteria abundances, the total abundances of both archaea and bacteria being significantly higher under grasses than trees. This strongly suggests the existence of a specific mechanism impacting nitrifiers and further supports the strong influence of AOA on nitrification.

All these results thus support the possible role of BNI molecules released by grasses that impact AOA abundance and activity in a savanna where nitrification is largely due to archaea. The impact of BNI on the abundance of nitrifying microorganisms is supported by a field study of crops with different BNI capabilities (Subbarao et al., 2009) showing that AOB and AOA abundance decreases under crop species with high BNI capabilities. Currently the mechanism responsible for BNI has been described only for its effect on AOB (Subbarao et al., 2012). Further research is thus needed to confirm that African grasses can also inhibit archaeal nitrification through BNI.

Finally, considering all grasses and trees, the proportion of nitrifying archaea and bacteria within the whole bacteria and archaea communities (between 0.02 and 0.82% and between 0.01 and 0.03%, respectively) are slightly lower than found in the literature for sandy soils, likely reflecting the very low soil N availability and the climate constraints, i.e. two dry seasons, occurring on such savanna soils. Leininger et al. (2006), comparing 12 soils of 3 climatic zones, found that for sandy soils AOA and AOB were around 1% and 0.005%, respectively, while Banning et al. (2015) in a semi-arid acidic sandy soil found that around 1% of archaea and bacteria are nitrifying.

4.2. Contrasting impacts of grasses and trees on denitrifying enzyme activity but not denitrifier abundances

No difference in the abundances of nitrite reducers was observed between soil under grasses and trees. In parallel, a significant predominance of *nirS* gene within the denitrifying community was observed. Previous studies have demonstrated that the ratio *nirS:nirK* depends on soil physicochemical characteristics especially soil pH, soil nutrients, moisture, organic carbon content, soil N content, and physical properties (Dambreville et al., 2006; Bárta et al., 2010; Chen et al., 2010; Enwall et al., 2010; Ma et al., 2016) although functional diversity exists within each broad group in terms of response to environmental drivers (Xie et al., 2014). The ratio *nirS:nirK* may also influence soil N_2O sink capacity (Jones et al., 2014). Overall, high copper concentrations tend to increase *nirK* gene abundances (Enwall et al., 2010) while low

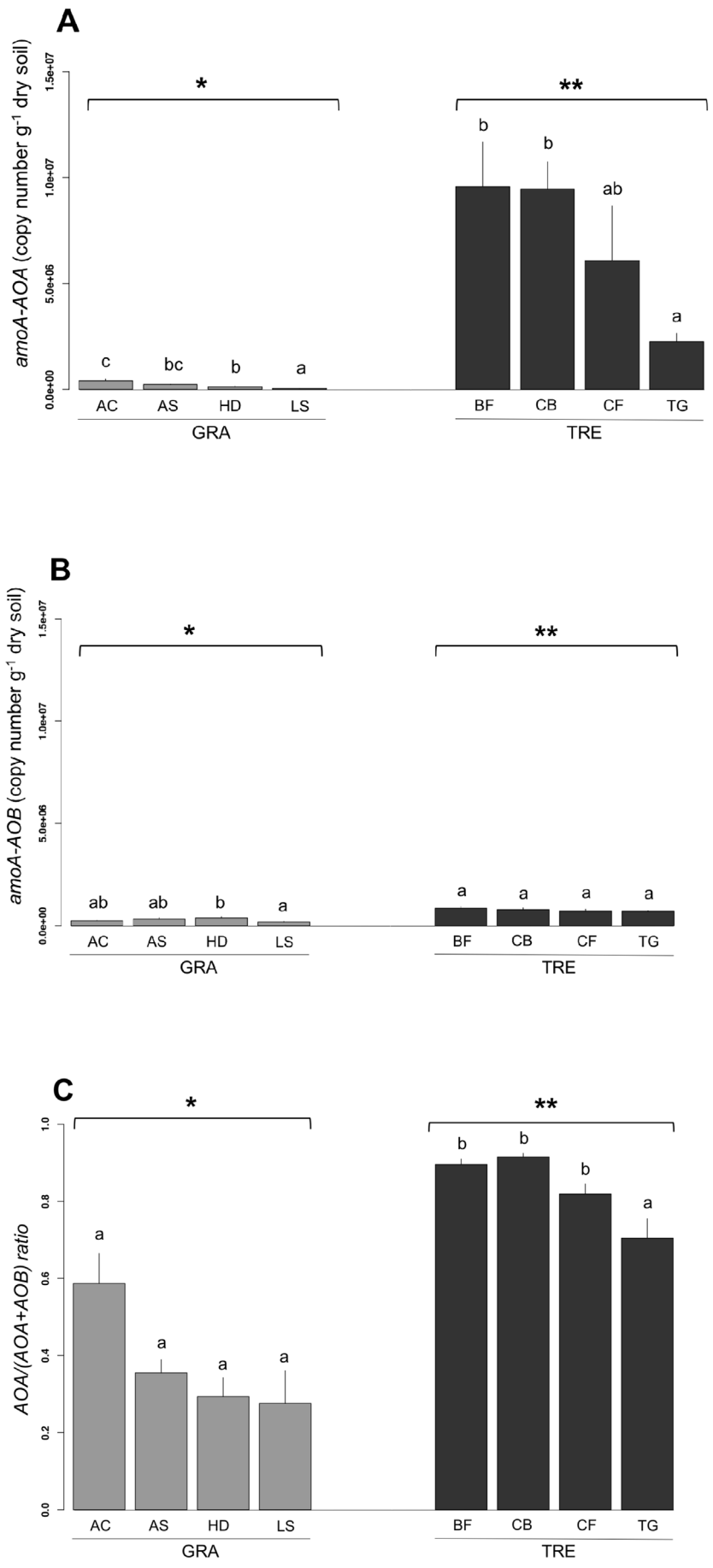

Fig. 2. Abundances of archaeal-AOA (panel A) and bacterial-AOB (panel B) *amoA* genes according to plant species and the AOA/AOA + AOB ratio (panel C): grasses (grey bars) AC (*Andropogon canaliculatus*), AS (*Andropogon schirensis*), HD (*Hyparrhenia diplandra*), LS (*Loudetia simplex*), and trees (black bars): BF (*Bridelia ferruginea*), CB (*Cussonia barteri*), CF (*Crossopteryx febrifuga*), TG (*Terminalia glaucescens*), GRA (Grasses) and TRE (Trees). Means and SEs were calculated from five replicates. Different numbers of stars indicate statistically significant differences between tree and grass species. Different small letters indicate statistically significant differences between species within each plant type (either trees or grasses) ($p < 0.05$).

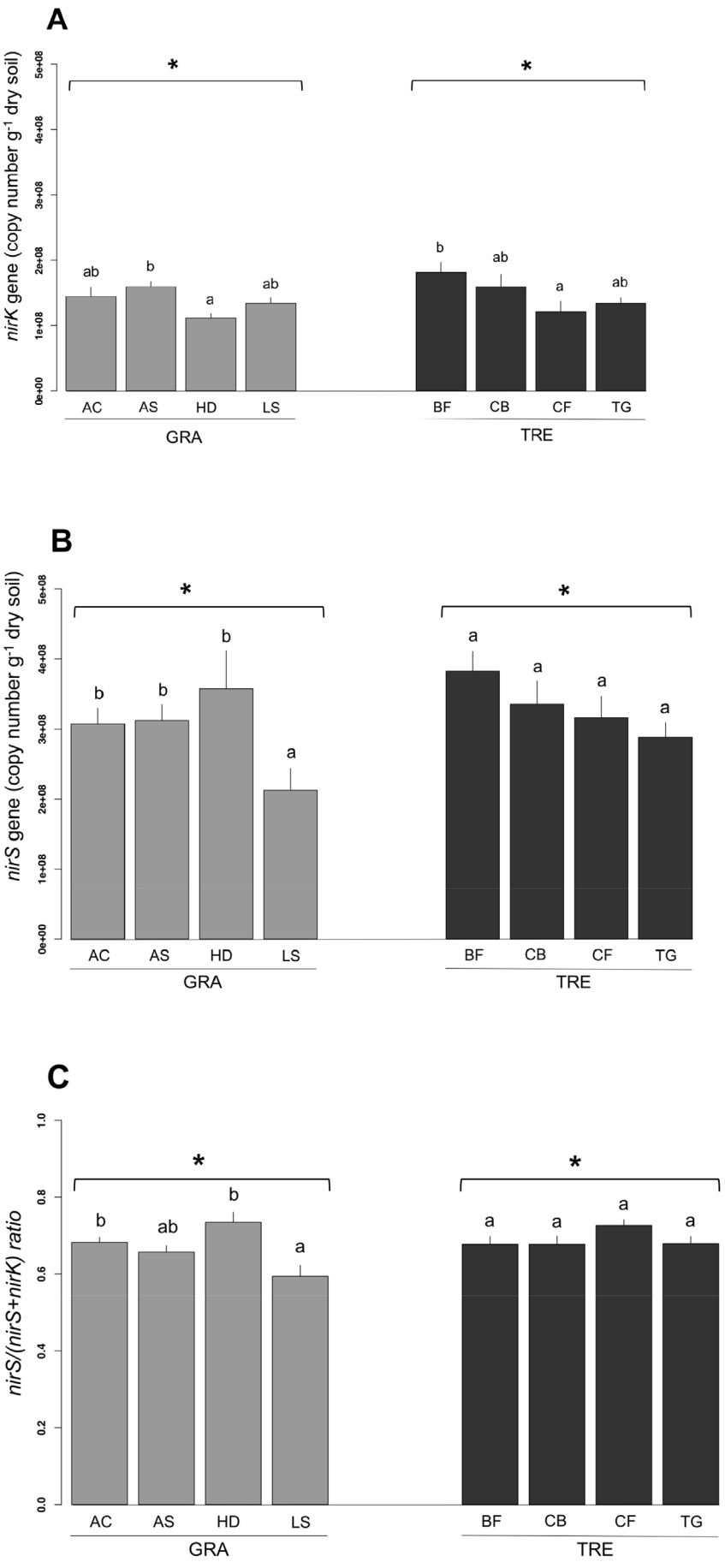

Fig. 3. Abundances of denitrifying *nirK* genes (panel A) and *nirS* genes (panel B) according to plant species and the *nirS/nirS + nirK* ratio (panel C): grasses (grey bars) AC (*Andropogon canaliculatus*), AS (*Andropogon schirensis*), HD (*Hyparrhenia diplandra*), *LS* (*Loudetia simplex*), and trees (black bars): BF (*Bridelia ferruginea*), CB (*Cussonia barteri*), CF (*Crossopteryx febrifuga*), TG (*Terminalia glaucescens*), GRA (Grasses) and TRE (Trees). Means and SEs were calculated from five replicates. Same number of stars indicates that there is no statistically significant difference between tree and grass species. Different small letters indicate statistically significant differences between species within each plant type (either trees or grasses) ($p < 0.05$).

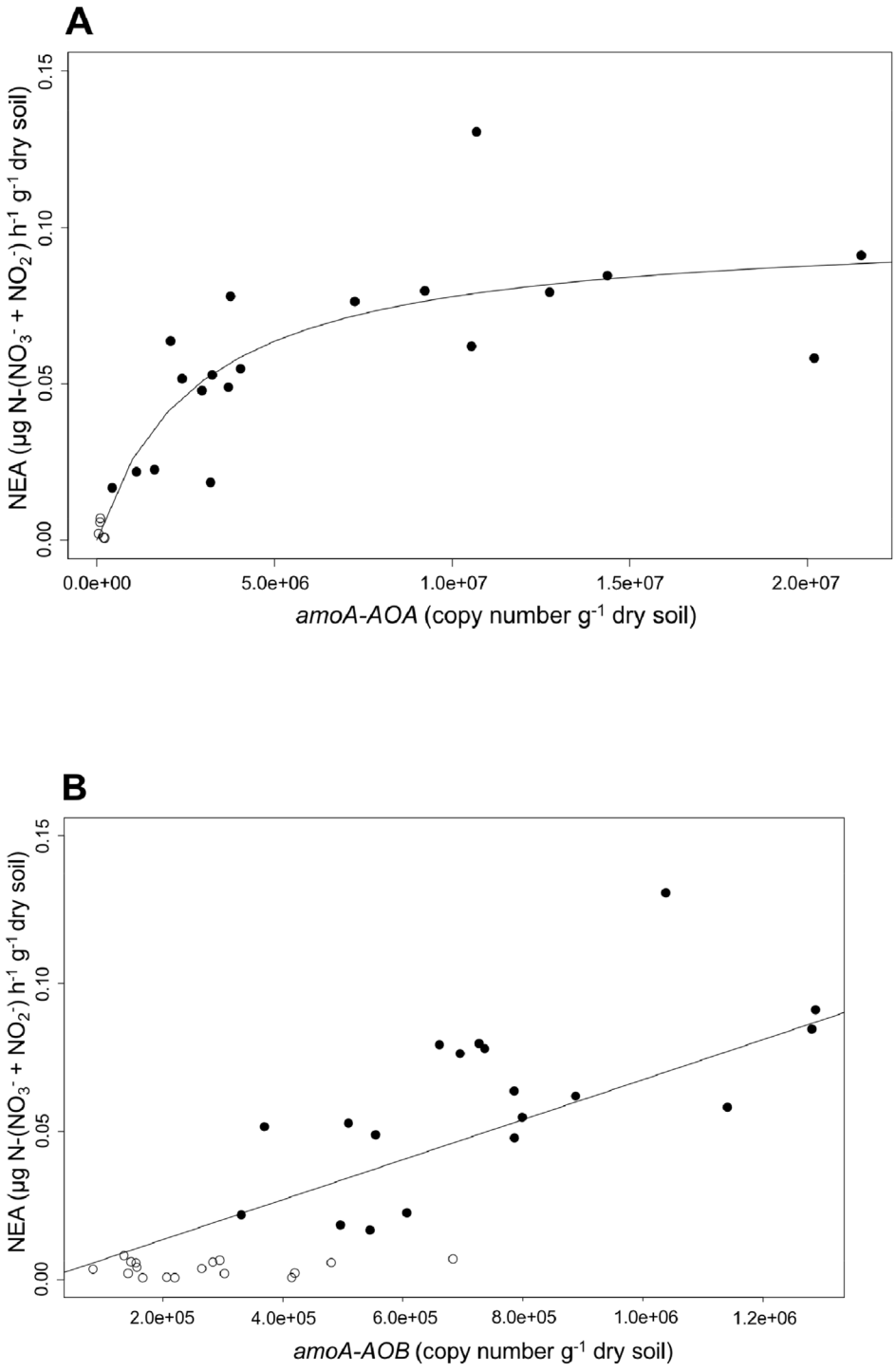

Fig. 4. Regressions between nitrifying enzyme activities (NEA) and AOA (panel A) or AOB (panel B) *amoA* gene abundances. Regressions were tested separately for trees and grasses. The regression was not significant for grasses (white dots) but was significant for trees (black dots). In panel A, we used for trees the following formula for the non-linear regression: NEA = a × *amoA*-AOA/(b + *amoA*-AOA) (a = 0.10, $p < 0.001$; b = 2.88 × 106, $p < 0.05$). And in panel B, we used for trees a linear regression (NEA = c × *amoA*-AOB + d (c = 264 6.76 × 10^{-8}, $p < 0.01$, d = 0, $p > 0.05$; $r^2 = 0.39$).

oxygen availability might favour *nirS* gene that are thus more abundant in anoxic environment (Desnues et al., 2007). Thus, the predominance of *nirS* gene could be explained by the slightly acidic soil pH, or the poor availability of oxygen associated to the proprieties of sandy soil with high water content (Bartholomeus et al., 2008). Moreover, the NirS enzyme needs a Fe co-factor while the NirK enzyme needs a Cu co-factor (Lammel et al., 2015; Qiu et al., 2004). The high Fe content (Abbadie et al., 2006) of Lamto soils could thus explain the predominance of *nirS* denitrifiers whatever the plant type. Moreover, the values of the *nirS/(nirS + nirK)* ratio (*ca.* 0.68) are in the upper range when compared to published values. For example, when calculating *nirS/(nirS + nirK)* ratios from a survey on 47 different soils across Europe (Jones et al., 2014), the values ranged between 0.21 and 0.63, with *nirK* gene > *nirS* gene in 35 out of the 47 soils. Lammel et al. (2015) have found a ratio of 0.535 when investigating a tropical pasture, to our knowledge the only article on this ecosystem type. However, values outside this range have been reported, *e.g.* 0.001 for a riparian temperate ecosystem (Dandie et al., 2011) and 0.99 for a subalpine Tibetan meadow and a temperate forest soil (Ribbons et al., 2016; Xie et al., 2014). So far, no value for the *nirS/(nirS + nirK)* has been published for a savanna so that new studies are required to test the generality of our result.

No correlation between DEA and denitrifier abundances was observed: DEA was much higher (9 fold) under trees than under grasses,

while the abundances of *nirK*- and *nirS*-nitrite reducers were similar. Several studies have already reported lack of correlation between changes in DEA and in nitrite reducer abundances (Le Roux et al., 2013; Philippot et al., 2009). This is not surprising because denitrification is a facultative process mainly realized by heterotrophs when they are under anaerobic conditions and with sufficient NO_3^- availability: under aerobic conditions that prevail in the sandy and well-drained soils studied here, denitrifiers are likely selected for different reasons than their denitrification capacity. More generally, such a mismatch between the measured activity and abundance of the responsible microorganisms is possible if denitrifiers are present but dormant, if denitrifiers are active but do not produce denitrification enzymes, which seems here the most likely case, or if denitrification enzymes are produced but their activity is inhibited. Concerning the contrasting DEA values observed between grasses and trees, at least three reasons could explain the observed pattern: (1) The lower DEA values observed under grasses than under trees could be explained by the lower soil organic matter content (lower C and N concentrations) and/or lower WC observed under grasses in our study and in a previous study (Mordelet et al., 1993). (2) The decrease in NO_3^- availability (Mordelet et al., 1993 and this study) induced by BNI under grasses could on the long term decrease DEA. (3) It cannot be excluded that savanna grasses could also inhibit denitrification. Demonstrated for the first time for a forb (*Fallopia* spp.) that inhibits denitrification through a mechanism similar to BNI (involving specific root exudates), Biological Denitrification Inhibition (BDI; Bardon et al., 2015, 2014) is a process which may be widespread in plants. However, both BNI and BDI are costly to plants (due to the required production of specific root exudates), and BDI is useless if nitrate is already hardly available due to BNI. Thus a plant species is unlikely to have evolved both BNI and BDI. New experiments manipulating soil properties in controlled conditions and combining enzymatic measurements and quantification of the expression of denitrification genes could help deciding between these alternative hypotheses.

5. Conclusions

Our study shows that the dominant grass and tree species of the Lamto savanna have contrasting impacts on N cycle. BNI is known to lead to a tighter N recycling because limiting nitrification decreases NO_3^- availability, and subsequently decreases ecosystem N losses through denitrification and leaching (Boudsocq et al., 2009). Under the four dominant grass species and as compared to trees, the very low rates of NEA, DEA and nitrifier abundances are very likely due to BNI. If confirmed, this overall BNI capability of dominant grasses in this savanna could thus largely contribute to the rather closed N cycle and high primary production that characterize humid savannas such as Lamto (ca. 11–19 t $ha^{-1}year^{-1}$; Abbadie et al., 2006) notwithstanding very low soil fertilities and frequent fires.

Trees could benefit from high nitrifications rates *via* their deeper roots than grasses (Schenk and Jackson, 2002). Indeed, high NEA under trees should increase NO_3^- availability and leaching towards deeper soil layers and tree root systems could still reach an important fraction of this NO_3^- no longer available for grasses. A complementary view is that trees have evolved an acquisitive strategy for N while grasses have developed a conservative strategy (Grime, 2001; Barot et al., 2016). This calls for complementary studies on N cycling and microbial activities in the soil below 15 cm depth. Besides, our results suggest that grasses should uptake more ammonium while trees should uptake more NO_3^-. Such partitioning of the mineral N resource could favour the coexistence between trees and grasses (Boudsocq et al., 2012).

Finally, the contrasting impacts of trees and grasses on N cycling should be taken into account to assess the N budget of savannas and their N_2O emissions. Indeed, tropical savannas and grasslands face worldwide threats (increase in atmospheric CO_2, fire suppression and afforestation for the carbon market) that are often inducing an increase in woody cover (Bond, 2016). This could lead to a functional shift of savannas towards a more open, less conservative N cycling under increased woody cover or land conversion to agriculture (*e.g.* Brazilian Cerrados). This could in turn increase N losses through nitrate leaching and/or the production of N-compound gases through denitrification (in particular N_2O). On the contrary, the introduction and wide use of African grasses *e.g.* in South or Central American pastures, could decrease the emissions of N_2O if these grasses inhibit nitrification.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.11.016.

References

Abbadie, L., Gignoux, J., Le Roux, X., Lepage, M., 2006. Lamto Structure, Functioning and Dynamics of a Savanna Ecosystem. Ecological Studies. vol. 179 Springer, New York 419p.

Abbadie, L., Lata, J.C., 2006. Nitrogen dynamics in the soil-plant system. In: Lamto Structure Functioning and Dynamics of a Savanna Ecosystem. Ecological Studies, vol. 179. Springer, New York, pp. 278–297. http://dx.doi.org/10.1016/j.eja.2007.05.003.

Abbadie, L., Mariotti, A., Menaut, J.C., 1992. Independence of savanna grasses from soil organic matter for their nitrogen supply. Ecology 73, 608–613.

Andrianarisoa, K.S., Zeller, B., Poly, F., Siegenfuhr, H., Bienaimé, S., Ranger, J., Dambrine, E., 2010. Control of nitrification by tree species in a common-garden experiment. Ecosystems 13, 1171–1187. http://dx.doi.org/10.1007/s10021-010-9390-x.

Assémien, F.L., Pommier, T., Gonnety, J.T., Gervaix, J., Le Roux, X., 2017. Adaptation of soil nitrifiers to very low nitrogen level jeopardizes the efficiency of chemical fertilization in west african moist savannas. Scientific Reports 7, 10275. http://dx.doi.org/10.1038/s41598-017-10185-5.

Attard, E., Recous, S., Chabbi, A., De Berranger, C., Guillaumaud, N., Labreuche, J., Philippot, L., Schmid, B., Le Roux, X., 2011. Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. Global Change Biology 17, 1975–1989. http://dx.doi.org/10.1111/j.1365-2486.2010.02340.x.

Banning, N.C., Maccarone, L.D., Fisk, L.M., Murphy, D.V., 2015. Ammonia-oxidising bacteria not archaea dominate nitrification activity in semi-arid agricultural soil. Scientific Reports 5, 11146. http://dx.doi.org/10.1038/srep11146.

Bardon, C., Piola, F., Bellvert, F., el Zahar Haichar, F., Comte, G., Meiffren, G., Pommier, T., Puijalon, S., Tsafack, N., Poly, F., 2014. Evidence for biological denitrification inhibition (BDI) by plant secondary metabolites. New Phytologist 204, 620–630. http://dx.doi.org/10.1111/nph.12944.

Bardon, C., Piola, F., el Haichar Zahar, F., Meiffren, G., Comte, G., Missery, B., Balby, M., Poly, F., 2015. Identification of B-type procyanidins in *Fallopia* spp. involved in biological denitrification inhibition (BDI). Environmental Microbiology 18, 644–655. http://dx.doi.org/10.1111/1462-2920.13062.

Barot, S., Bornhofen, S., Boudsocq, S., Raynaud, X., Loeuille, N., 2016. Evolution of nutrient acquisition: when space matters. Functional Ecology 30, 283–294. http://dx.doi.org/10.1111/1365-2435.12494.

Barot, S., Gignoux, J., 2004. Mechanisms promoting plant coexistence: can all the proposed processes be reconciled? Oikos 106, 185–192. http://dx.doi.org/10.1111/j.0030-1299.2004.13038.x.

Bárta, J., Melichová, T., Vaněk, D., Picek, T., Šantrůčková, H., 2010. Effect of pH and dissolved organic matter on the abundance of nirK and nirS denitrifiers in spruce forest soil. Biogeochemistry 101, 123–132. http://dx.doi.org/10.1007/s10533-010-9430-9.

Bartholomeus, R.P., Witte, J.P.M., van Bodegom, P.M., van Dam, J.C., Aerts, R., 2008. Critical soil conditions for oxygen stress to plant roots: substituting the Feddes-function by a process-based model. Journal of Hydrology 360, 147–165. http://dx.

doi.org/10.1016/j.jhydrol.2008.07.029.
Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2010. Examining the global distribution of dominant archaeal populations in soil. The ISME Journal 5, 908–917. http://dx.doi.org/10.1038/ismej.2010.171.
Bonanomi, G., Rietkerk, M., Dekker, S.C., Mazzoleni, S., 2008. Islands of fertility induce co-occurring negative and positive plant-soil feedbacks promoting coexistence. Plant Ecology 197, 207–218. http://dx.doi.org/10.1007/s11258-007-9371-0.
Bond, W.J., 2016. Ancient grasslands at risk. Science 351, 120–122. http://dx.doi.org/10.1126/science.aad5132.
Boudsocq, S., Lata, J.C., Mathieu, J., Abbadie, L., Barot, S., 2009. Modelling approach to analyse the effects of nitrification inhibition on primary production. Functional Ecology 23, 220–230. http://dx.doi.org/10.1111/j.1365-2435.2008.01476.x.
Boudsocq, S., Niboyet, A., Lata, J.C., Raynaud, X., Loeuille, N., Mathieu, J., Blouin, M., Abbadie, L., Barot, S., 2012. Plant preference for ammonium versus nitrate: a neglected determinant of ecosystem functioning? The American Naturalist 180, 60–69. http://dx.doi.org/10.1086/665997.
Brandt, A.J., De Kroon, H., Reynolds, H.L., Burns, J.H., 2013. Soil heterogeneity generated by plant-soil feedbacks has implications for species recruitment and coexistence. Journal of Ecology 101, 277–286. http://dx.doi.org/10.1111/1365-2745.12042.
Catão, E.C.P., Lopes, F.A.C., Rubini, M.R., Nardoto, G.B., Prosser, J.I., Krüger, R.H., 2016. Short-term impact of soybean management on ammonia oxidizers in a Brazilian savanna under restoration as revealed by coupling different techniques. Biology and Fertility of Soils 52, 401–412. http://dx.doi.org/10.1007/s00374-015-1086-0.
Chen, Z., Luo, X., Hu, R., Wu, M., Wu, J., Wei, W., 2010. Impact of long-term fertilization on the composition of denitrifier communities based on nitrite reductase analyses in a paddy soil. Microbial Ecology 60, 850–861. http://dx.doi.org/10.1007/s00248-010-9700-z.
Dambreville, C., Hallet, S., Nguyen, C., Morvan, T., Germon, J.C., Philippot, L., 2006. Structure and activity of the denitrifying community in a maize-cropped field fertilized with composted pig manure or ammonium nitrate. FEMS Microbiology Ecology 56, 119–131. http://dx.doi.org/10.1111/j.1574-6941.2006.00064.x.
Dandie, C.E., Wertz, S., Leclair, C.L., Goyer, C., Burton, D.L., Patten, C.L., Zebarth, B.J., Trevors, J.T., 2011. Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. FEMS Microbiology Ecology 77, 69–82. http://dx.doi.org/10.1111/j.1574-6941.2011.01084.x.
Dassonville, N., Guillaumaud, N., Piola, F., Meerts, P., Poly, F., 2011. Niche construction by the invasive Asian knotweeds (species complex *Fallopia*): impact on activity, abundance and community structure of denitrifiers and nitrifiers. Biological Invasions 13, 1115–1133. http://dx.doi.org/10.1007/s10530-011-9954-5.
Del' Alamo Guarda, V., Del' Alamo Guarda, R., 2014. Brazilian tropical grassland ecosystems : distribution and research advances. Scientific Research 5, 924–932.
Desnues, C., Michotey, V.D., Wieland, A., Zhizang, C., Fourçans, A., Duran, R., Bonin, P.C., 2007. Seasonal and diel distributions of denitrifying and bacterial communities in a hypersaline microbial mat (Camargue, France). Water Research 41, 3407–3419. http://dx.doi.org/10.1016/j.watres.2007.04.018.
Enwall, K., Throbäck, I.N., Stenberg, M., Söderström, M., Hallin, S., 2010. Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management. Applied and Environmental Microbiology 76, 2243–2250. http://dx.doi.org/10.1128/AEM.02197-09.
Grime, J.P., 2001. Plant Strategies, Vegetation Processes, and Ecosystem Properties, second ed. John Wiley & Sons, Chichester, UK (ed).
Hatzenpichler, R., 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. Applied and Environmental Microbiology 78, 7501–7510. http://dx.doi.org/10.1128/AEM.01960-12.
Jones, C.M., Spor, A., Brennan, F.P., Breuil, M.C., Bru, D., Lemanceau, P., Griffiths, B., Hallin, S., Philippot, L., 2014. Recently identified microbial guild mediates soil N_2O sink capacity. Nature Climate Change 4, 801–805. http://dx.doi.org/10.1038/Nclimate2301.
Lammel, D.R., Feigl, B.J., Cerri, C.C., Nüsslein, K., 2015. Specific microbial gene abundances and soil parameters contribute to C, N, and greenhouse gas process rates after land use change in southern amazonian soils. Frontiers in Microbiology 6, 1–14. http://dx.doi.org/10.3389/fmicb.2015.01057.
Lata, J.C., 1999. Interactions between Microbial Processes, Nutrient Cycle and Grass Cover Functioning: Study of Soil Nitrification Under the Gramineae *Hyparrhenia diplandra* in a Wet Tropical Savanna of Ivory Coast. University of Paris VI, Paris (PhD Thesis).
Lata, J.C., Durand, J., Lensi, R., Abbadie, L., 1999. Stable coexistence of contrasted nitrification statuses in a wet tropical savanna ecosystem. Funct. Ecol. 13, 762–768. http://dx.doi.org/10.1046/j.1365-2435.1999.00380.x.
Lata, J.C., Guillaume, K., Degrange, V., Abbadie, L., Lensi, R., 2000. Relationships between root density of the African grass *Hyparrhenia diplandra* and nitrification at the decimetric scale: an inhibition-stimulation balance hypothesis. Proc. Biol. Sci./Roy. Soc. 267, 595–600. http://dx.doi.org/10.1098/rspb.2000.1043.
Lata, J.C., Degrange, V., Raynaud, X., Maron, P.A., Lensi, R., Abbadie, L., 2004. Grass populations control nitrification in savanna soils. Funct. Ecol. 18, 605–611. http://dx.doi.org/10.1111/j.0269-8463.2004.00880.x.
Le Roux, X., Schmid, B., Poly, F., Barnard, R.L., Niklaus, P.A., Guillaumaud, N., Habekost, M., Oelmann, Y., Philippot, L., Salles, J.F., Schloter, M., Steinbeiss, S., Weigelt, A., 2013. Soil environmental conditions and microbial build-up mediate the effect of plant diversity on soil nitrifying and denitrifying enzyme activities in temperate grasslands. PLoS One 8, e61069. http://dx.doi.org/10.1371/journal.pone.0061069.
Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809. http://dx.doi.org/10.1038/nature04983.
Ma, W., Jiang, S., Assemien, F., Qin, M., Ma, B., Xie, Z., Liu, Y., Feng, H., Du, G., Ma, X., Le Roux, X., 2016. Response of microbial functional groups involved in soil N cycle to N, P and NP fertilization in Tibetan alpine meadows. Soil Biology and Biochemistry 101, 195–206. http://dx.doi.org/10.1016/j.soilbio.2016.07.023.
Mordelet, P., Abbadie, L., Menaut, J.C., 1993. Effects of tree clumps on soil characteristics in a humid savanna of West Africa (Lamto, Côte d'Ivoire). Plant and Soil 153, 103–111. http://dx.doi.org/10.1007/BF00010549.
Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology 10, 2966–2978. http://dx.doi.org/10.1111/j.1462-2920.2008.01701.x.
Patra, A.K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S.J., Guillaumaud, N., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J.I., Le Roux, X., 2006. Effects of management regime and plant species on the enzyme activity and genetic structure of N-fixing, denitrifying and nitrifying bacterial communities in grassland soils. Environmental Microbiology 8, 1005–1016. http://dx.doi.org/10.1111/j.1462-2920.2006.00992.x.
Philippot, L., Čuhel, J., Saby, N.P.A., Chèneby, D., Chroňáková, A., Bru, D., Arrouays, D., Martin-Laurent, F., Šimek, M., 2009. Mapping field-scale spatial patterns of size and activity of the denitrifier community. Environmental Microbiology 11, 1518–1526. http://dx.doi.org/10.1111/j.1462-2920.2009.01879.x.
Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends in Microbiology 20, 523–531. http://dx.doi.org/10.1016/j.tim.2012.08.001.
Qiu, X.Y., Hurt, R.A., Wu, L.Y., Chen, C.H., Tiedje, J.M., Zhou, J.Z., 2004. Detection and quantification of copper-denitrifying bacteria by quantitative competitive PCR. Journal of Microbiological Methods 59, 199–210. http://dx.doi.org/10.1016/j.mimet.2004.07.008.
Ribbons, R.R., Levy-Booth, D.J., Masse, J., Grayston, S.J., McDonald, M.A., Vesterdal, L., Prescott, C.E., 2016. Linking microbial communities, functional genes and nitrogen-cycling processes in forest floors under four tree species. Soil Biology and Biochemistry 103, 181–191. http://dx.doi.org/10.1016/j.soilbio.2016.07.024.
Rughöft, S., Herrmann, M., Lazar, C.S., Cesarz, S., Levick, S.R., Trumbore, S.E., Küsel, K., 2016. Community composition and abundance of bacterial, archaeal and nitrifying populations in savanna soils on contrasting bedrock material in Kruger National Park, South Africa. Frontiers in Microbiology 7, 1–16. http://dx.doi.org/10.3389/fmicb.2016.01638.
Rutten, G., Prati, D., Hemp, A., Fischer, M., 2016. Plant–soil feedback in East-African savanna trees. Ecology 97, 294–301. http://dx.doi.org/10.1007/s13398-014-0173-7.2.
Sankaran, M., Ratnam, J., Hanan, N.P., 2004. Tree-grass coexistence in savannas revisited - insights from an examination of assumptions and mechanisms invoked in existing models. Ecology Letters 7, 480–490. http://dx.doi.org/10.1111/j.1461-0248.2004.00596.x.
Schenk, H.J., Jackson, R.B., 2002. Rooting depths, lateral root spreads and belowground aboveground allometries of plants in water limited ecosystems. Journal of Ecology 90, 480–494. http://dx.doi.org/10.1046/j.1365-2745.2002.00682.x.
Simonin, M., Le Roux, X., Poly, F., Lerondelle, C., Hungate, B.A., Nunan, N., Niboyet, A., 2015. Coupling between and among ammonia oxidizers and nitrite oxidizers in grassland mesocosms submitted to elevated CO_2 and nitrogen supply. Microbial Ecology 70, 809–818. http://dx.doi.org/10.1007/s00248-015-0604-9.
Stempfhuber, B., Engel, M., Fischer, D., Neskovic-Prit, G., Wubet, T., Schöning, I., Gubry-Rangin, C., Kublik, S., Schloter-Hai, B., Rattei, T., Welzl, G., Nicol, G.W., Schrumpf, M., Buscot, F., Prosser, J.I., Schloter, M., 2015. pH as a driver for ammonia-oxidizing archaea in forest soils. Microbial Ecology 69, 879–883. http://dx.doi.org/10.1007/s00248-014-0548-5.
Sterngren, A.E., Hallin, S., Bengtson, P., 2015. Archaeal ammonia oxidizers dominate in numbers, but bacteria drive gross nitrification in N-amended grassland soil. Frontiers in Microbiology 6, 1–8. http://dx.doi.org/10.3389/fmicb.2015.01350.
Subbarao, G.V., Nakahara, K., Hurtado, M.P., Ono, H., Moreta, D.E., Salcedo, A.F., Yoshihashi, A.T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I.M., Lascano, C.E., Berry, W.L., Ito, O., 2009. Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proceedings of the National Academy of Sciences 106, 17302–17307. http://dx.doi.org/10.1073/pnas.0903694106.
Subbarao, G.V., Sahrawat, K.L., Nakahara, K., Ishikawa, T., Kishii, M., Rao, I.M., Hash, C.T., George, T.S., Srinivasa Rao, P., Nardi, P., Bonnett, D., Berry, W., Suenaga, K., Lata, J.C., 2012. Biological nitrification inhibition. A novel strategy to regulate nitrification in agricultural systems. Advances in Agronomy 114, 249–302. http://dx.doi.org/10.1016/B978-0-12-394275-3.00001-8.
Taylor, A.E., Zeglin, L.H., Wanzek, T.A., Myrold, D.D., Bottomley, P.J., 2012. Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. The ISME Journal 6, 2024–2032. http://dx.doi.org/10.1038/ismej.2012.51.
Verhamme, D.T., Prosser, J.I., Nicol, G.W., 2011. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. The ISME Journal 5, 1067–1071. http://dx.doi.org/10.1038/ismej.2010.191.
Wallenius, K., Rita, H., Simpanen, S., Mikkonen, A., Niemi, R.M., 2010. Sample storage for soil enzyme activity and bacterial community profiles. Journal of Microbiological Methods 81, 48–55. http://dx.doi.org/10.1016/j.mimet.2010.01.021.
Wild, S., 2016. Quest to map Africa's soil microbiome begins. Nature 539, 152. http://dx.doi.org/10.1038/539152a.
Xie, Z., Le Roux, X., Wang, C., Gu, Z., An, M., Nan, H., Chen, B., Li, F., Liu, Y., Du, G., Feng, H., Ma, X., 2014. Identifying response groups of soil nitrifiers and denitrifiers to grazing and associated soil environmental drivers in Tibetan alpine meadows. Soil Biology and Biochemistry 77, 89–99. http://dx.doi.org/10.1016/j.soilbio.2014.06.024.
Zakir, H.A.K.M., Subbarao, G.V., Pearse, S.J., Gopalakrishnan, S., Ito, O., Ishikawa, T., Kawano, N., Nakahara, K., Yoshihashi, T., Ono, H., Yoshida, M., 2008. Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum bicolor*). New Phytologist 180, 442–451.