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Effects of vegetation cover and season on soil nitrifiers in an African savanna: Evidence of archaeal nitrifier inhibition by grasses

Tharaniya Srikanthasamy [a,*], Sébastien Barot [b], Fulgence K. Koffi [c], Kevin Tambosco [a], Yoan Marcangeli [a], David Carmignac [a], Aya Brigitte N'Dri [c], Jonathan Gervaix [d], Julie Leloup [a], Xavier Le Roux [d], Jean-Christophe Lata [a,e,*]

[a] Sorbonne Université, Université de Paris, UPEC, CNRS, INRA, IRD, UMR 7618, Institute of Ecology and Environmental Sciences – Paris, iEES Paris, Tour 44-45, 4 place Jussieu, 75005 Paris, France
[b] IRD, Sorbonne Université, CNRS, INRA, Univ Paris Diderot Paris 07, UPEC, UMR 7618, Institute of Ecology and Environmental Sciences – Paris, Tour 44-45, 4 place Jussieu, 75005 Paris, France
[c] UFR-SN / Research Station of Lamto (CRE), Research pole Environment and Sustainable Development, Nangui Abrogoua University (ex University of Abobo-Adjamé), 02 BP 801, Abidjan 02, Côte d'Ivoire, France
[d] INRAE, CNRS, Université de Lyon, Université Lyon 1, Laboratoire d'Ecologie Microbienne, UMR INRAE 1418, UMR CNRS 5557, 43 boulevard du 11 novembre 1918, Villeurbanne 69622, France
[e] Department of Geoecology and Geochemistry, Institute of Natural Resources, Tomsk Polytechnic University, 30, Lenin Street, Tomsk 634050, Russia

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ABSTRACT

For the first time in a savanna, we analysed the joint influence of climate seasonality and plant cover type on the abundances and transcription levels of bacterial and archaeal nitrifiers, and nitrification enzyme activity (NEA). The dominant tree and grass species stimulated and inhibited nitrification, respectively: NEA was 4-fold higher and 5-fold lower under trees and grasses than in bare soil, respectively. The abundances of bacterial nitrifiers (AOB-*amoA*) followed the same trend as the abundances of total bacteria did, with higher abundances under grasses and trees than in bare soil and higher abundances during the wet season, but AOB-*amoA* transcripts were always below detection limit. In contrast, the abundances of archaeal nitrifiers (AOA-*amoA*) were 13-fold lower under grasses and 2-fold higher under trees than in bare soil, and the AOA-*amoA* transcript level decreased under grasses during the wet season. Our results show that nitrifier abundances and transcriptional activities are highly seasonal and that nitrification is likely to be dominated by AOA-*amoA* in these savanna soils. We show for the first time that a dominant grass species (*Hyparrhenia diplandra*) of humid African savannas inhibits nitrification through marked decreases in AOA-*amoA* expression levels.

1. Introduction

The first, often limiting step of nitrification (oxidation of ammonium into nitrite) is mainly driven by two microbial communities – archaeal and bacterial ammonia oxidisers that harbour the ammonia monooxygenase subunit A gene (*amoA*). The relative contribution of each community to nitrification depends on different environmental parameters such as the soil characteristics (Hatzenpichler, 2012), and their niche differentiation is strongly debated. For example, ammonia-oxidising archaea (AOA-*amoA*) play a key role in nitrification in acidic soils and soils with low ammonium concentrations or contaminated with heavy metals (Nicol et al., 2008; Cao et al., 2011; Yao et al., 2011; Prosser and Nicol, 2012). Ammonia-oxidising bacteria (AOB-*amoA*) play a key role in nitrification in soils with high total N and ammonium contents (Verhamme et al., 2011; Ma et al., 2016).

Soil microbial communities and the biogeochemical cycles under their control can be impacted by drying and rewetting stresses (Fierer et al., 2003; Kaisermann et al., 2013; Thion and Prosser, 2014). However, little is known about the AOA-*amoA* and AOB-*amoA* responses to these stresses. For example, in a grassland with no historical record of drought events, AOA-*amoA* seem to be less resistant than AOB-*amoA* to drought. They are also less resilient to rewetting in soils (Thion and

* Corresponding authors at: Sorbonne Université, Université de Paris, UPEC, CNRS, INRA, IRD, UMR 7618, Institute of Ecology and Environmental Sciences – Paris, iEES Paris, Tour 44-45, 4 place Jussieu, 75005 Paris, France (J.-C. Lata).
E-mail addresses: tharaniya.sri@gmail.com (T. Srikanthasamy), jean-christophe.lata@sorbonne-universite.fr (J.-C. Lata).

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Prosser, 2014). This could be linked to the higher concentration of ammonium during and following drought associated to lower AOA-*amoA* tolerance to these concentrations. AOA-*amoA* also appear to be sensitive to high water contents, maybe due to the subsequent decrease in available oxygen (Szukics et al. 2010). However, they have been found insensitive to oxygen availability, contrary to AOB-*amoA* (Ke et al. 2015). Finally, AOA-*amoA* have proved more sensitive than AOB-*amoA* to osmotic and matric stresses in soil microcosms (Bello et al., 2018). Changes in temperature also influence the overall archaeal and bacterial community structures (Stres et al., 2008). As far as nitrifiers are concerned, AOB-*amoA* abundances increase with increasing soil temperature, while AOA-*amoA* decrease at 25 °C in high soil water content conditions (70%) (Szukics et al. 2010). In addition, acidophilic AOA-*amoA* strains have lower optimum growing temperature than neutrophilic AOA-*amoA* (Gubry-Rangin et al., 2017). All these results suggest that the abundance and activity of archaeal and bacterial nitrifiers should vary along the year according to climate seasonality. For example, a seasonal effect was recorded on both groups of ammonia oxidisers in a Chinese upland red soil, with higher gene copy numbers in summer than in winter (He et al. 2007).

Overall, AOA-*amoA* seem more abundant in temperate soils (Prosser and Nicol 2012; Sterngren et al., 2015), but their contribution to nitrification vs. AOB-*amoA* varies among ecosystems (Le Roux et al., 2008; Nicol et al., 2008; Verhamme et al., 2011; Simonin et al., 2015). AOA-*amoA* have been found more abundant than AOB-*amoA* in savanna soils (Assémien et al., 2017; Catão et al., 2017; Rughöft et al., 2016; Srikanthasamy et al., 2018), but there is no consensus on this issue because of the very low number of studies documenting nitrification in savanna ecosystems (which yet cover 12–13% of global terrestrial surfaces; Rutten et al., 2016). AOA-*amoA* are key nitrifiers in non-fertilized cultivated soils from the West African savanna zone, but the influence of AOB-*amoA* on nitrification increases when these soils are fertilized (Assémien et al. 2017). The relative contribution of AOA-*amoA* vs. AOB-*amoA* in non-cultivated savanna soils has been determined only once, in Brazilian Cerrados (Catão et al., 2017, 2016), and has never been determined in an African savanna.

The soil nitrification rate also depends on the plant cover and its species composition (Le Roux et al., 2013). Some species of African savanna tussock grasses such as *Hyparrhenia diplandra* (Lata et al., 1999, 2000, 2004) and *Brachiaria humidicola* (Subbarao et al., 2009) inhibit nitrification. Their roots exude biological nitrification inhibitors such as brachialactone or methyl 3-(4-hydroxyphenyl) propionate (Subbarao et al., 2009; Zakir et al., 2008). These molecules can block nitrification totally or partially by inhibiting the AMO and/or HAO enzyme (Coskun et al., 2017; Subbarao et al., 2012) through a process called Biological Nitrification Inhibition (BNI). BNI molecules do inhibit nitrification by AOB-*amoA* (*e.g.*, Subbarao et al., 2009); however, whether they also inhibit nitrification by AOA-*amoA* and their impacts on AOA-*amoA* and AOB-*amoA* abundances and transcriptional activities remain to be fully deciphered. A few studies in an agricultural context suggest that nitrification by AOA-*amoA* can also be inhibited through BNI (Subbarao et al., 2009; Byrnes et al., 2017; Nuñez et al., 2018; Lu et al., 2019; Sarr et al., 2020). However, the authors only used AOA-*amoA* DNA abundance – not AOA-*amoA* transcripts. This can lead to skewed conclusions because the abundance of a microorganism is not necessarily correlated with the activity of all its potential functions (Fierer, 2017). Finally, a pure culture experiment recently showed that AOA-*amoA* seem more sensitive than AOB-*amoA* to BNI compounds, with variability among strains (Kaur-Bhambra et al., 2021).

The nitrification rate has been found 72 times higher under the dominant tree species than under the dominant grass species in the humid West African savanna of Lamto reserve (Ivory Coast) (Srikanthasamy et al., 2018). These results suggest that nitrification could be inhibited by the dominant grasses but also stimulated by the dominant tree species of this savanna. In order to verify these hypotheses, we compared nitrification enzyme activity (NEA) and the abundance and transcriptional activity of nitrifiers of soils under the dominant tree species vs. the dominant grass species with those of regularly weeded bare soil plots almost without any root influence. The Lamto savanna experiences dry and wet seasons. During the dry season, soils are much drier than during the wet season, grasses stop growing and most of their leaves dry out and are burnt by the annual fire. Thus, grasses likely produce much less root exudates during the dry season than during the wet season, which could decrease their capability to inhibit nitrification. This could change the nitrification activities, abundances and gene expression levels of archaeal and bacterial nitrifiers, in particular under grasses during the dry season.

We characterised the overall effects of the vegetation cover (dominant tree species, grass species and bare soil) and season (wet and dry) on NEA, and on the abundances and transcriptional levels of AOB-*amoA* and AOA-*amoA* in the Lamto savanna. We hypothesized that:

(1) The dominant tree and grass species stimulate and inhibit nitrification, respectively, as compared to bare soil.
(2) Soil microbial abundances and transcription levels are higher under a plant cover than in bare soil, except for the nitrifier groups inhibited under grasses. In particular, this would lead to higher AOA-*amoA*:total archaea and lower AOB-*amoA*:total bacteria ratios under trees and grasses, respectively, based on abundances and transcript abundances.
(3) Microbial abundances and transcription levels are higher during the wet season, except for the nitrifier groups targeted by BNI, as they undergo a higher inhibiting root activity under grasses during the wet season. The activity of grasses indeed strongly decreases during the dry season (Abbadie et al., 2006), which may alleviate the BNI effect.
(4) Because AOA-*amoA* have been assumed to be the most important ammonia oxidisers in this area, we also hypothesized that the dominant grass species (*H. diplandra*) in this savanna area, known to have BNI capacity, may affect the abundance and/or the *amoA* transcript level of both AOB-*amoA* and AOA-*amoA*. Overall, we expected NEA to be positively correlated to AOA-*amoA* abundance and transcriptional activity.

2. Materials and methods

2.1. Study sites

The Lamto reserve (6°13′N, 5°20′W) in Ivory Coast harbours a mosaic of savannas distinguished by tree densities, and riparian and gallery forests. The climate is sub-equatorial, and 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 2017 was 1100.5 mm, as compared to a 30-year average of 1200 mm (data from the Geophysical Station of Lamto). Temperatures are relatively constant throughout the year (27 °C on average). Soils are derived from granites and the resulting sands, and classified as tropical ferrugineous soils with a superficial gravelly horizon (acrisol according to the World Reference Base for Soil Resource – WRB). They are sandy (sands 77%; silts 14%; clays 9%), with a bulk density of *ca.* 1.65 (Lata et al., 1999).

2.2. Soil sampling

We distinguished three types of vegetation cover for soil sampling: (i) under the tussocks of the dominant grass *Hyparrhenia diplandra* (hereafter GRA), known to have BNI capacity (Lata et al., 2004; Srikanthasamy et al., 2018); (ii) under the canopy of the dominant tree *Crossopteryx febrifuga* (hereafter TRE); and (iii) in bare soil patches (2 m × 2 m plots without vegetation, weeded for one year before sampling, hereafter BS). In Lamto savanna, grasses are perennial and their

individuals form large tussocks separated by at least 20 cm of bare soil, and trees grow separately or in clumps (clumps were avoided for soil sampling). The soil under grasses was directly sampled below identified grass individuals, away from any tree individual. Therefore, the samples not only consisted of soil adhering to the roots but also of all the soil included inside the very dense grass root systems. Grass tussocks were chosen to have a similar basal diameter (ca. 15 cm), and trees were selected to have a similar diameter at breast height (ca. 22 cm). Grass (*H. diplandra*) and tree (*C. febrifuga*) individuals were randomly chosen on a surface of *ca.* 10 ha of shrub savanna, and local sources of heterogeneity (termite mounds, small depressions, rocks) were avoided.

Soil was sampled during two contrasted seasons: during the long dry season (January 2017, 107.7 mm of precipitation in the previous 2 months and 0 mm in the last 15 days, hereafter D) and the long wet season (May 2017, 344.9 mm of precipitation in the previous 2 months and 196.5 mm in the last 15 days, hereafter W). For each of the 6 combinations of season and cover type (3 covers × 2 seasons), five replicates were sampled (i.e., a total of 30 samples). One soil sample of about 1 kg was collected from the top 15 cm soil layer at each sampling point using an 8 cm-diameter auger. After a first homogenisation of the samples in the field, 10-g sub-samples were immediately placed in cryotubes and in liquid nitrogen. The rest of the soil samples was stored at 4 °C for a very short period for transport to the field station. These samples were subsequently sieved (2 mm), and 200 g of soil were stored at −20 °C for molecular biology analyses and NEA measurements. The remaining soil was air-dried in the shade and stored at ambient temperature for physico-chemical analyses. Roots were collected from all soil samples by dry sieving (2 mm mesh), and dried at 50 °C before weighing. Fine-root densities varied as follows: 2.06 to 9.08 g dry root dm^{-3} dry soil under grasses, 0.89 to 2.06 g dry root dm^{-3} dry soil under trees, and 0 to 0.30 g dry root dm^{-3} dry soil under bare soil. The aboveground biomass of each grass tussock was collected and dried at 50 °C before weighing; it varied between 1.229 and 5.884 g dry matter $tussock^{-1}$. Soil moisture was measured in the field before sampling with a previously calibrated ThetaProbe ML3 (Delta-T Devices).

2.3. Soil physico-chemical characteristics

The soil pH was measured in water (5:1 water:soil v/v) with a pH meter (Thermo Scientific Orion™ Star A211, and an Orion™ PerpHecT™ ROSS™ Combination Micro Electrode) according to the NF ISO 10390 standard. Mineral N was extracted from 2 g of frozen soil by adding 2 M KCl solution (1:4 soil:solution). Nitrate was reduced to nitrite, and nitrite and ammonium concentrations (expressed as mg N-NO_3^- and N-NH_4^+ g^{-1} dry soil) were measured with a continuous-flow N analyser (SKALAR, San Plus System, Breda, The Netherlands). Total soil C and N contents (expressed as %) were measured using a CHN Elemental Analyser (FlashEA 1112 Series, Thermo Electron Corporation, The Netherlands) after grinding at 80 μm.

2.4. Nitrifying enzyme activity assays

Potential nitrification rates were assessed with nitrifying enzyme activity (NEA) assays, i.e., short-term laboratory incubations under non-limiting conditions. This approach detects very low rates of nitrification (Lata et al., 1999; Attard et al., 2011). Frozen soil samples were placed at ambient room temperature for 2 h before the analyses. Freezing was preferred to fresh or drying conservation methods because (i) the time lapse between sampling and analysis (including transportation) was too long to use fresh soil because the analyses were performed in France, (ii) previous studies on different soil matrices showed that freezing was a preferable storage method for soil enzyme activity studies (e.g., denitrification) (Wallenius et al., 2010), and (iii) preliminary tests on fresh vs. frozen samples on our soils showed no difference. NEA was measured according to Dassonville et al. (2011) as the linear rate of nitrate production during a 72-h incubation. Soil sub-samples (3 g equivalent dry soil) were incubated with 6 ml of a solution of $(NH_4)_2SO_4$ (22 μg N-NH_4^+ g^{-1} dry soil, allowing the optimum nitrification condition for this type of soil according to preliminary analyses – Assémien et al., 2017). Distilled water was added to each sample to reach 24 ml of total liquid volume in the flasks. The 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).

2.5. Quantification of microbial abundances

Total ribonucleic acids were extracted from 4 g of fresh soil with an RNA PowerSoil® Total RNA Isolation Kit (MO BIO Laboratories), in combination with a FastPrep FP120 bead beating system (Bio-101, Inc., Ca, USA) according to the manufacturer's instructions. DNase treatment was performed using the Ambion® TURBO DNA-free TM DNase Treatment and Removal Reagents protocol, and the absence of contaminant DNA was checked on agarose gel. RNA was quantified using a Qubit™ RNA HS assay kit. All RNA samples were normalised to a similar concentration (8 ng/μl) before the reverse transcription step achieved using a SuperScript® III First-Strand Synthesis System for RT-PCR (Invitrogen). All extracted RNA samples were stored at −80 °C before analysis, and cDNA samples were stored at −20 °C.

Total deoxyribonucleic acids were extracted, purified and quantified as in Srikanthasamy et al. (2018), and archaeal and bacterial ammonia oxidiser abundances were quantified by real-time PCR following the same publication. Archaeal *amoA* genes were amplified using primers CrenamoA23f and CrenamoA616r (Tourna et al., 2008), and bacterial *amoA* genes were amplified using primers amoA 1F and amoA 2R (Rotthauwe et al., 1997). Two independent quantitative PCR assays were performed for each gene to test repeatability. Standard curves were obtained using serial dilutions of linearised plasmids containing the studied genes. Average amplification efficiency was 84.1% for AOA-*amoA* and 91.8% for AOB-*amoA* (see Table S1 for the PCR conditions). Melting curves were analysed using the Dissociation Curve Analysis software program (Applied Biosystems). Abundances were expressed as gene copy numbers per gram of dry soil.

2.6. Statistical analyses

All statistical analyses were performed using R software (R Core Team 2016). ANOVA2 was used to test the effects of vegetation cover, season and their interaction on all measured variables. When a significant effect of the cover X season interaction was observed (meaning that the effect of the cover differed with the season), all combinations of the two treatment modalities were compared using Tukey's Honestly Significant Difference test. When the interaction was non-significant, we compared the modalities of the significant simple factor(s) using the same post-hoc test. We also tested the relationship between NEA and AOB-*amoA* and AOA-*amoA* abundances or their transcript abundances using linear regressions. Normality and homoscedasticity of the residuals of all linear models were tested with Shapiro-Wilk and Bartlett tests, respectively. Data were log-transformed, square-transformed or boxcox-transformed in case of significant deviation from normality and homoscedasticity. For all tests, the null hypothesis was rejected for $p < 0.05$, and significance was represented as follows: *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$.

3. Results

3.1. Soil characteristics

The soil pH was affected by the vegetation cover and the season, but very weakly (Table 1; see also all mean values in Table S2). The pH was slightly higher under the tree cover (TRE) (6.93 ± 0.05) than under the grass cover (GRA) (6.75 ± 0.06) and in bare soil (BS) (6.70 ± 0.03; $p < 0.01$), and similar under GRA and in BS. The pH was very slightly higher

Table 1
Statistical results (ANOVA) of the effects of the vegetation cover, the season and their interaction on soil characteristics. Transformations of the variable are indicated (- for no transformation); df, degree of freedom; NS, non-significant.

Variable		Data transformation	df	p-value
NO_3^-	Cover	Boxcox transformation	2	NS
	Season	$\lambda = 2$	1	<0.01
	Cover*Season		2	NS
NH_4^+	Cover	–	2	<0.001
	Season		1	NS
	Cover*Season		2	<0.01
Water content	Cover	–	2	<0.05
	Season		1	<0.001
	Cover*Season		2	<0.05
pH	Cover	Square root	2	<0.01
	Season		1	<0.05
	Cover*Season		2	NS
N tot	Cover	–	2	<0.01
	Season		1	<0.05
	Cover*Season		2	NS
C tot	Cover	–	2	<0.01
	Season		1	NS
	Cover*Season		2	NS
C:N	Cover	–	2	<0.001
	Season		1	<0.001
	Cover*Season		2	NS

during the wet season (W) (6.86 ± 0.04) than during the dry season (D) (6.73 ± 0.04).

The soil water content (SWC) was affected by the vegetation cover, the season and their interaction (Tables 1 and S2). The SWC was higher in BS-W (20.56 ± 0.52%) than in BS-D (15.66 ± 1.10%; $p < 0.05$) and higher under GRA-W (17.94 ± 1.48%) than under GRA-D (12.18 ± 1.23%; $p < 0.01$). However, there was no difference between TRE-W (17.26 ± 0.84%) and TRE-D (16.56 ± 0.59%). The SWC was also higher in BS-W than under GRA-D ($p < 0.001$), and higher under TRE-W than under GRA-D ($p < 0.05$).

The soil N-NH_4^+ concentration was affected by the vegetation cover (Tables 1 and S2) and the cover X season interaction. N-NH_4^+ concentration was higher under TRE-W (9.69 ± 0.69 mg kg^{-1} dry soil) than under GRA-W (6.87 ± 0.26 mg kg^{-1} dry soil; $p < 0.01$) and in BS-W (5.10 ± 0.33 mg kg^{-1} soil; $p < 0.001$). N-NH_4^+ concentration was also higher under TRE-D (7.68 ± 0.36 mg kg^{-1} soil) than in BS-W ($p < 0.01$), and higher under TRE-W than under GRA-D ($p < 0.01$) and in BS-D (6.59 ± 0.29 mg kg^{-1} soil; $p < 0.001$). The soil N-NO_3^- concentration was only affected by the season and was slightly lower during the wet season (14.34 ± 1.12 mg kg^{-1} soil) than during the dry season (14.70 ± 0.70 mg kg^{-1} soil).

The total soil N concentration was affected by the vegetation cover and the season (Table 1). The total soil N concentration was higher under TRE (0.069 ± 0.003%) than in BS (0.050 ± 0.003%; $p < 0.01$). The total soil N concentration was similar under GRA (0.061 ± 0.004%) and in BS on the one hand, and under GRA and TRE on the other hand. It was higher during the dry season (0.064 ± 0.003%) than during the wet season (0.056 ± 0.004%). The total soil C concentration was affected by the cover and was higher under GRA (1.02 ± 0.07%) and TRE (1.02 ± 0.04%) than in BS (0.81 ± 0.03%; $p < 0.05$). The total soil C concentration was similar under GRA and TRE.

The C:N ratio was impacted by the vegetation cover and the season (Table 1). The C:N ratio was higher under GRA (16.84 ± 0.36) than under TRE (14.76 ± 0.35). The C:N ratio was not significantly different in BS (16.30 ± 0.60) than under GRA and TRE. The C:N ratio was higher during the wet season (16.99 ± 0.35) than during the dry season (14.94 ± 0.30).

3.2. Nitrifying enzyme activity

Nitrifying enzyme activity (NEA) was affected by the vegetation cover type (Fig. 1, ANOVA results, $p < 0.001$) and the cover X season interaction ($p < 0.01$), but not by the sole effect of the season. NEA was 18 times higher under TRE (45.17 ± 10.67 ng N-(NO_3^- + NO_2^-) h^{-1} g^{-1} dry soil) than under GRA (2.47 ± 1.60 ng N-(NO_3^- + NO_2^-) h^{-1} g^{-1} dry soil; $p < 0.001$), and 4 times higher than in BS (12.38 ± 1.73 ng N-(NO_3^- + NO_2^-) h^{-1} g^{-1} dry soil; $p < 0.01$). NEA was five times higher in BS than under GRA ($p < 0.01$). The cover X season interaction was significant: NEA was higher under TRE-D than under TRE-W, but not difference was found between GRA-D and GRA-W.

3.3. Nitrifier abundances and gene expression levels

Archaeal *amoA* (AOA-*amoA*) gene abundance was affected by the vegetation cover and the season, without any interaction effect (Table 2 and Fig. 2; see also Table S3 for mean values). Archaeal *amoA* (AOA-*amoA*) gene abundance was about 22 times higher under TRE (1.98 ± 0.44 × 10^6 copies g^{-1} dry soil) than under GRA (8.99 ± 4.04 × 10^4 copies g^{-1} dry soil; $p < 0.001$; Fig. 2A), and about 13 times higher in BS (1.18 ± 0.20 × 10^6 copies g^{-1} dry soil) than under GRA ($p < 0.001$). Archaeal *amoA* (AOA-*amoA*) gene abundance was similar under TRE and in BS. It was about 2 times higher during the wet season (1.38 ± 0.36 × 10^6 copies g^{-1} dry soil) than during the dry season (7.87 ± 2.18 × 10^5 copies g^{-1} dry soil; Fig. 2B). The abundance of AOA-*amoA* transcripts was affected by the vegetation cover and the season (Fig. 2C; see also Table S2 for mean values). There was no difference between BS, GRA and TRE during the wet season. During the dry season, the abundance of AOA-*amoA* transcripts was lower under GRA (2.10 ± 1.05 × 10^5 copies g^{-1} dry soil) than in BS (3.34 ± 1.04 × 10^4 copies g^{-1} dry soil; $p < 0.05$).

Bacterial *amoA* gene (AOB-*amoA*) abundance was affected by the vegetation cover, the season and their interaction (Table 2 and Fig. 3; see also Table S3 for mean values). Bacterial *amoA* gene was similar in BS-D (6.31 ± 2.85 × 10^5 copies g^{-1} soil) and BS-W (3.26 ± 1.42 × 10^5 copies g^{-1} dry soil). It was 7 times higher under GRA-W (1.26 ± 0.41 × 10^6 copies g^{-1} dry soil) than under GRA-D (1.62 ± 0.43 × 10^5 copies g^{-1} dry soil; $p < 0.01$), and 5 times higher under TRE-W (1.72 ± 0.17 × 10^6 copies g^{-1} dry soil) than under TRE-D (3.44 ± 0.37 × 10^5 copies g^{-1} dry soil; $p < 0.01$). Bacterial *amoA* gene was higher under GRA-W ($p < 0.05$) and TRE-W ($p < 0.01$) than in BS-W. The abundance of AOB-*amoA* transcripts was below the detection limit (*ca.* 10^3 copies g^{-1} dry soil) in all but 2 samples – a grass tussock during the wet season (8.5 × 10^3 copies g^{-1} dry soil) and a bare soil sample during the wet season (3.6 ×

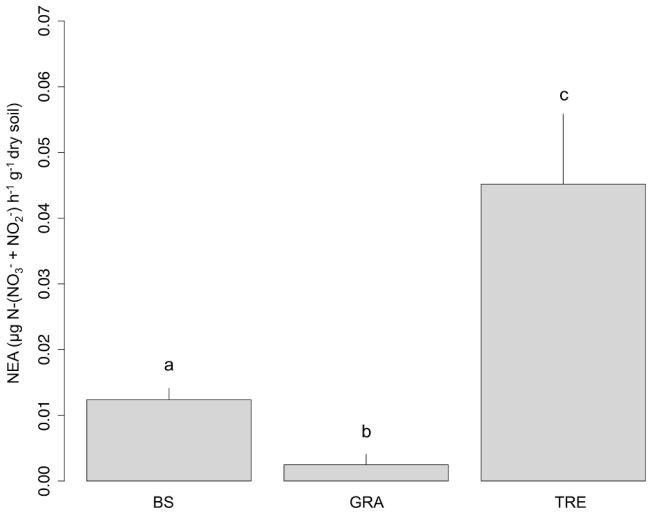

Fig. 1. Nitrifying enzyme activities (NEA) according to the vegetation cover: bare soil (BS), the dominant grass species *Hyparrhenia diplandra* (GRA) and the dominant tree species *Crossopteryx febrifuga* (TRE). Means and standard errors were calculated from ten replicates whatever the season. Different letters indicate significant differences between vegetation covers ($p < 0.05$).

Table 2
Statistical results (ANOVA) of the effects of the vegetation cover, the season and their interaction on the abundances of ammonia oxidizing archaea and bacteria (AOA and AOB, respectively), and on the corresponding transcripts (only for AOA). Transformations of the variable are indicated (- for no transformation); df, degree of freedom; NS, non-significant.

Variable		Transformation	df	p-value
AOA-*amoA*	Cover	Square root	2	<0.001
	Season		1	<0.05
	Cover*Season		2	NS
AOA-*amoA* transcripts	Cover	Square root	2	NS
	Season		1	<0.001
	Cover*Season		2	<0.05
AOB-*amoA*	Cover	Square root	2	<0.05
	Season		1	<0.001
	Cover*Season		2	<0.01
AOA:(AOA + AOB) ratio	Cover	Log	2	<0.001
	Season		1	NS
	Cover*Season		2	NS
AOA-*amoA*:total Archaea ratio	Cover	–	2	<0.001
	Season		1	<0.01
	Cover*Season		2	NS
AOA-*amoA*:Archaeal transcripts ratio	Cover	–	2	NS
	Season		1	NS
	Cover*Season		2	NS
AOB-*amoA*:total Bacteria ratio	Cover	–	2	NS
	Season		1	<0.01
	Cover*Season		2	NS

10^4 copies g^{-1} dry soil).

The AOA:(AOA + AOB) abundance ratio (Table 2; see also Table S3 for mean values) was only affected by the vegetation cover ($p < 0.001$). AOA:(AOA + AOB) abundance ratio was at least 4-fold higher under TRE (0.64 ± 0.05; $p < 0.001$) and in BS (0.73 ± 0.05; $p < 0.001$) than under GRA (0.16 ± 0.06), and no difference was observed between TRE and BS. The AOA-*amoA*:total archaeal abundance ratio (Table 2; see also Table S3 for mean values) was affected by the vegetation cover ($p < 0.001$) and the season ($p < 0.01$). AOA:(AOA + AOB) abundance ratio was higher in BS ($6.21 \pm 1.25 \times 10^{-4}$; $p < 0.01$) and under TRE ($7.12 \pm 1.34 \times 10^{-4}$; $p < 0.001$) than under GRA ($2.95 \pm 1.23 \times 10^{-5}$). AOA:(AOA + AOB) abundance ratio was higher during the wet season ($6.25 \pm 1.37 \times 10^{-4}$) than during the dry season ($2.83 \pm 0.68 \times 10^{-4}$). The AOA-*amoA*:total archaeal transcript ratio (Table S3) was unaffected by the cover and the season. Finally, the AOB-*amoA*:total bacterial abundance ratio (Table 2; see also Table S3 for mean values) was only affected by the season ($p < 0.01$) and was higher during the wet season ($6.66 \pm 1.28 \times 10^{-4}$) than during the dry season ($2.50 \pm 0.73 \times 10^{-4}$).

3.4. Links between NEA and nitrifier abundances and amoA expression levels

Regressions between NEA and nitrifier abundances and transcript abundances were performed separately for trees, grasses and bare soils. NEA increased with AOA-*amoA* abundance under trees ($NEA = 1.576 \times 10^{-8} \times$ AOA-*amoA*; $p < 0.05$, $R^2 = 0.35$; Fig. 4A), but no relationship was observed under grasses and in bare soil. A significant relationship was also observed between NEA and AOA-*amoA* abundance when considering all vegetation types ($NEA = 1.662 \times 10^{-8} \times$ AOA-*amoA*; $p < 0.001$, $R^2 = 0.42$). NEA also significantly increased with AOA-*amoA* abundance during the wet season (all vegetation covers taken together, $NEA = 0.017194 \times$ AOA-*amoA*; $p < 0.05$, $R^2 = 0.36$; Figure not shown) but did not increase during the dry season. In the same vein, NEA significantly increased with the abundance of AOA-*amoA* transcripts during the dry season (all vegetation covers taken together, $NEA = 0.004092 \times$ AOA-*amoA*; $p < 0.05$, $R^2 = 0.45$; Fig. 4C). No significant relationship was

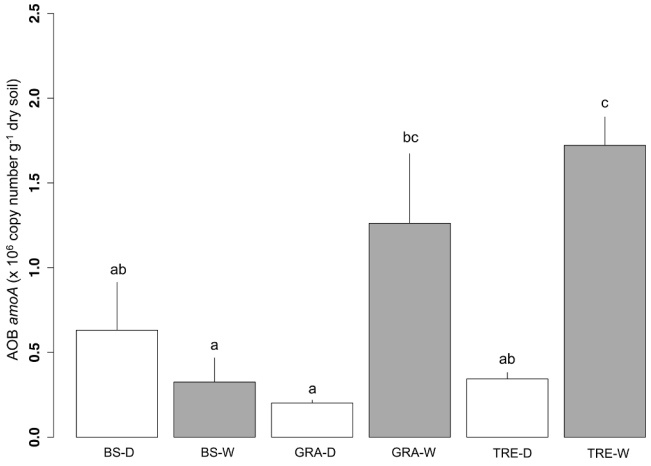

Fig. 3. Abundances of ammonia oxidizing bacteria, AOB, according to the vegetation cover (bare soil, BS; grass species *Hyparrhenia diplandra*, GRA; and tree species *Crossopteryx febrifuga*, TRE) and the season (dry season, D, white bars; wet season, W, grey bars). Means and standard errors were calculated from five replicates. Different letters indicate significant differences between treatments ($p < 0.05$).

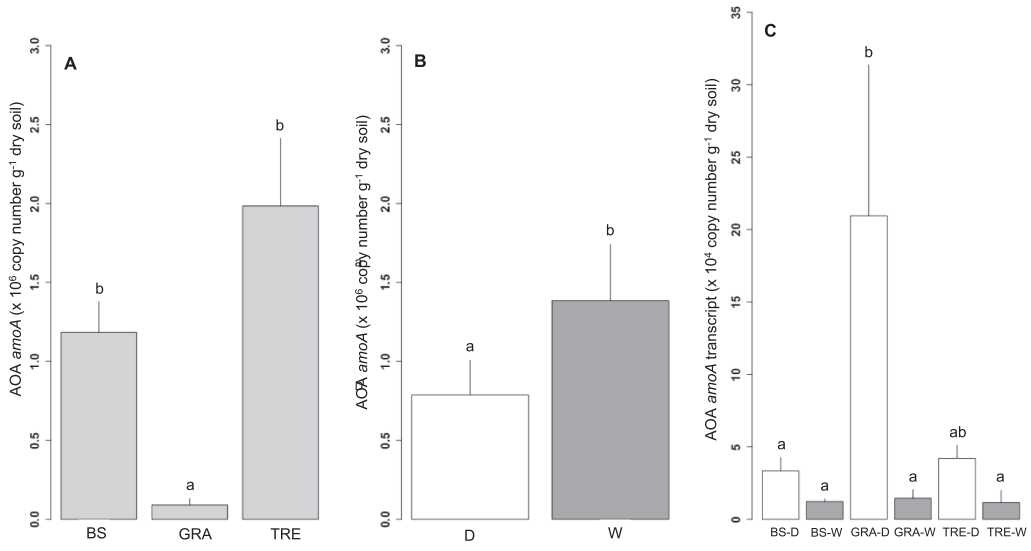

Fig. 2. Abundances of ammonia oxidizing archaea, AOA, according to (A) the vegetation cover and (B) the season, and (C) abundances of AOA-*amoA* transcripts according to vegetation cover (bare soil, BS; grass species *Hyparrhenia diplandra*, GRA; and tree species *Crossopteryx febrifuga*, TRE), and season (Dry season, D, white bars; wet season, W, grey bars). Means and standard errors were calculated from ten replicates for the cover effect (A), fifteen replicates for the season effect (B) and five replicates for the interaction (C). Different letters indicate significant differences between covers ($p < 0.05$).

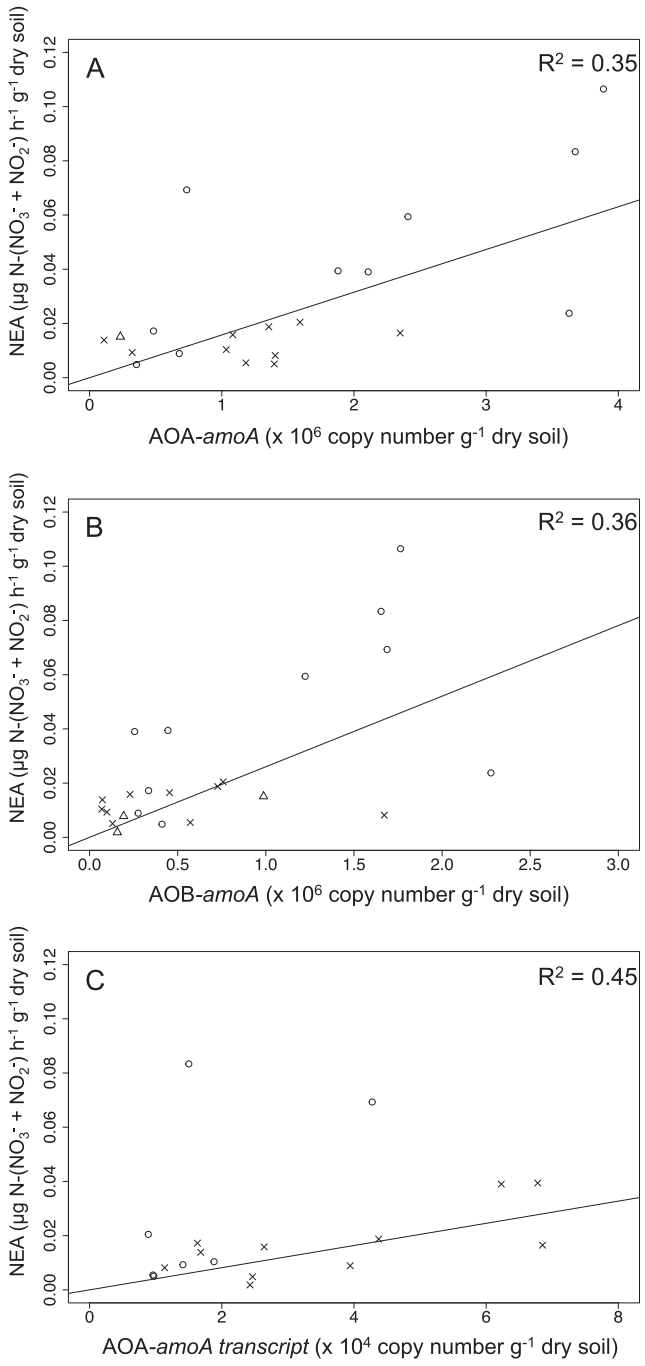

Fig. 4. (A) Regression between the nitrifying enzyme activity (NEA) and the abundance of ammonia oxidizing archaea (AOA) according to the vegetation cover whatever the season (bare soil, cross; grasses, triangle; and trees, circle). This regression was only significant for trees (as shown by the regression line) but also when considering all vegetation types together. (B) Regression between the nitrifying enzyme activity (NEA) and the abundance of ammonia oxidizing bacteria (AOB) according to the vegetation cover whatever the season (bare soil, cross; grasses, triangle; and trees, circle). This regression was only significant for all vegetation types together (as shown by the regression line).(C) Regression between the nitrifying enzyme activity (NEA) and the transcripts of AOA-*amoA* according to the season whatever the vegetation cover (dry season, cross; humid season, circle). This regression was only significant for the dry season, (as shown by the regression line).

observed between NEA and the abundance of AOA-*amoA* transcripts during the wet season (Figure not shown).

In contrast, no significant relationship was observed between NEA and AOB-*amoA* abundance for each vegetation cover type taken separately. However, NEA significantly increased with the abundance of AOB-*amoA* (NEA $= 2.604 \times 10^{-8} \times$ AOB-*amoA*, $p < 0.01$, $R^2 = 0.36$; Fig. 4B) when considering the whole dataset.

In addition to these results, the impact of the vegetation cover, the season and their interaction on the abundances and transcripts of total bacteria, archaea and fungi, and some regressions between NEA and the soil physico-chemical characteristics are described in Tables S4-1 & S4-2, Fig. S4-1 and Fig. S5-1, respectively.

4. Discussion

4.1. Influence of the vegetation cover and the season on nitrifiers

Nitrification enzyme activity (NEA) was overall 5 times lower under grasses than in bare soil. This confirms that *H. diplandra* inhibits nitrification, as already demonstrated in Lata et al. (2000). Together with the results from the other three other dominant tussock grasses of Lamto savanna (Srikanthasamy et al., 2018) – all associated to low nitrification rates –, our results confirm that the dominant grasses of this savanna have biological nitrification inhibition (BNI) capacity. However, we show for the first time that the abundance of archaeal nitrifiers was 13 times lower under grasses than in bare soil, and that the AOA-*amoA*:total archaeal abundance ratio was also significantly lower under grasses than in bare soil. This demonstrates that BNI induced by grasses decreases the competitive ability of archaeal ammonia oxidisers comparatively to other archaea, as already suggested by Subbarao et al. (2009) or Sarr et al. (2020). In addition, the abundance of AOA-*amoA* transcripts decreased strongly under grasses during the wet season, potentially due to more efficient BNI induced by more active grass roots during the wet season. Nuñez et al. (2018) reported that the effect of BNI on nitrification increases when plants are growing, and root exudate emissions increase. All these results support the view that grass BNI impact the AMO nitrifying enzyme pathway of AOA-*amoA* in the Lamto savanna. The mechanism underlying nitrification inhibition is not entirely clear in the current literature (Subbarao et al., 2015; Nuñez et al., 2018). This mechanism might be based on the inactivation of nitrification enzymes, down-regulation of the expression of nitrification genes, or a combination of both.

AOA-*amoA* abundance and transcripts, AOB-*amoA* abundance, and total fungal, bacterial and archaeal abundance (see Table S4-1 & S4-2 depend on complex interactions between the season and the vegetation cover. Seasonality is indeed often of importance when considering soil microbial abundance and transcriptional activity (Kennedy et al., 2005; Voříšková et al., 2014). On the one hand, we observed overall higher microbial (total as well as/and nitrifier) abundances during the wet season that provided better conditions for soil microorganisms (Thion & Prosser, 2014; de Castro et al., 2016). However, this seasonal difference was mainly true only for grass and tree soils, not for bare soil. This confirms that microorganisms depend as much on soil humidity as on plant activities (e.g., root exudates) that increase during the wet season (Haichar et al., 2008; Berendsen et al., 2012). On the other hand, the fact that the abundance of AOA-*amoA* transcripts decreased during the wet season under grasses in our study suggests that BNI decreased the transcription of AOA-*amoA*. In contrast, we observed higher AOB-*amoA* abundances under grasses than in bare soil during the wet season, vs. similar ones under grasses and trees. This suggests that BNI induced by grasses is very efficient in inhibiting AOA-*amoA* nitrification. New experiments would be required to test the capacity of Lamto grasses to inhibit AOB-*amoA* nitrification in other soils. This is novel for *in situ* measurements in natural ecosystems. So far, only experimental results on crop lines or pure cultures in controlled conditions have recently documented archaeal nitrifier sensitivity to BNI (Nuñez et al., 2018; Sarr

et al., 2020; Kaur-Bhambra et al., 2021). Our results show for the first time that BNI can efficiently inhibit nitrification by AOA-*amoA* in the field. The causes of this difference in sensitivity can be multiple (e.g., soil and overall abiotic characteristics, niche partitioning, biochemical, cellular or genetic differences, differences in microbial community structure (Kaur-Bhambra et al., 2021)), and they still remain to be tested.

NEA was overall 4 times higher under the tree canopy than in bare soil. This confirms that nitrification is stimulated under trees, in line with Mordelet et al. (1993) and Srikanthasamy et al. (2018). Two non-exclusive mechanisms could explain this stimulation. First, the total C and total N concentrations and ammonification are globally higher under trees in these savannas (Mordelet et al., 1997; Srikanthasamy et al., 2018; this study – see Table S2. This would stimulate the whole microbial community, increase ammonium availability for nitrifiers and in turn increase their abundance and stimulate their activity, and was confirmed by a significant positive regression between NEA and N-NH_4^+ (see supplementary data S5-1A). Moreover, since differences in total N and C contents between grasses and trees are usually low due to the overall poor soil fertility (this difference was not significant in this study (data not shown) – see Table S2), differences in the quality of organic matter might play a role in the stimulation of nitrification by trees, as suggested by the lower soil C:N ratio under trees than under grasses (Table S2) or the significant positive regression between NEA and pH (see supplementary data S5-1B). Similarly, changes in the soil moisture content could be favourable to microbial activities and nitrifiers under the tree canopy, especially in the dry season (soil humidity was higher under TRE-D than under GRA-D, and no difference was found between TRE-D and TRE-W, see Table S2).

It cannot be ruled out that Lamto savanna trees specifically stimulate nitrifiers through particular leaf leachates or root exudates. Zhang et al. (2016) documented such a process for *Picea asperata* (a coniferous tree) and Bardon et al. (2018) did so for *Pteridum aquilinum* (a fern) in the temperate zone. However, the ratios of AOA-*amoA*/total archaea and AOB-*amoA*/total bacteria were not higher under trees than in bare soil away from any tree. This supports the importance of the first mechanism, i.e., a general improvement of microbial growth conditions – in particular due to a higher organic matter content – rather than specific stimulation of nitrifiers. Finally, as previously stated (Srikanthasamy et al., 2018), the proportions of AOA-*amoA*(AOB-*amoA*) within the whole bacterial(archaeal) communities were moderately lower than what was expected for sandy soils. This probably reflects the impact of savanna climate and/or low N availability in the soils.

Based on our results, dominant trees in Lamto savanna stimulate nitrification whereas grasses inhibit it as compared to bare soil. This induces a spatially heterogeneous N cycle, which likely has important consequences for the savanna N budget and its overall functioning (Boudsocq et al., 2009; Konaré et al., 2019, 2021). BNI would decrease nitrate availability and subsequent N losses through denitrification and leaching in patches dominated by grasses, whereas the stimulation of nitrification under the tree cover should have the opposite effect. This also suggests that trees and grasses have evolved contrasted preferences for nitrate and ammonium, i.e., trees take up more nitrate that ammonium whereas grasses take up more ammonium than nitrate. This should be tested in the future, *e.g.*, under controlled conditions and using ^{15}N labelling.

4.2. Relative contribution of archaeal and bacterial nitrifiers to nitrification

As in many ecosystems (Di et al., 2010; Hirsch and Mauchline, 2012; Vasileiadis et al., 2012; Assemien et al., 2017; Xiang et al., 2017), AOA-*amoA* were on average much more abundant (2 times) than AOB-*amoA* in Lamto savanna soils. This does not necessarily mean that AOA-*amoA* are more influential than AOB-*amoA* for nitrification. However, our results strongly support the hypothesis of Srikanthasamy et al. (2018) that archaea are involved in nitrification in Lamto savanna: (i) bacterial nitrifiers were rather abundant, but the abundance of AOB-*amo*A transcripts was below the detection limit in most soil samples; (ii) the abundance of AOB-*amoA* genes was equally high under trees (with high nitrification) and grasses (with low nitrification), whereas the abundance of AOA-*amoA* genes was much higher under trees than under grasses. This means that the higher NEA under the tree cover can be explained by AOA-*amoA* abundance, not by AOB-*amoA* abundance; (iii) NEA increased with AOA-*amoA* abundance under trees when considering all vegetation types across the seasons and all vegetation types during the wet season, and with AOA-*amoA* transcripts during the dry season. Our results suggest that *C. febrifuga* can stimulate archaeal nitrifiers probably through a general improvement of microbial growth conditions (see above), whereas AOB-*amoA* remains functionally less important for nitrification in these soils. However, a role of AOB-*amoA* under trees cannot be completely excluded because we evidenced a relationship between NEA and AOB-*amoA* abundance when considering all vegetation types across the seasons, and a previous sampling campaign mixing four different tree species had shown the same result (Srikanthasamy et al., 2018).

Why there was no significant relationship between the abundance of AOA-*amoA* transcripts and nitrification (for the different vegetation covers) remains difficult to explain; so does, in the same vein, the abundance of AOA-*amoA* transcripts that was not much higher under trees than under grasses to parallel the much higher nitrification rate under trees. A first explanation is that transcription was not the last mechanistic step between the presence of AOA-*amoA* and the nitrification flux. Different mechanisms linked to the translation of transcripts into enzymes and numerous factors influencing enzyme activities (e.g., enzyme persistence and turnover, sorption/deactivation rates, substrate availability, soil organic matter/clay mineral types, soil moisture and temperature, adhesive characteristics of biofilms…) could lead to a non-uniform relationship between transcript abundance and the nitrification flux (Burns et al., 2013). In particular, a meta-analysis including the nitrification process showed how difficult it is to correlate functional genes, transcript abundance and process rates (Rocca et al. 2015). Moreover, nitrification significantly increased with the abundance of AOA-*amoA* transcripts during the dry season, all vegetation covers taken together. This confirms the role played in nitrification by AOA-*amoA* in Lamto savanna. It remains difficult to fully understand why this relationship was not significant in the wet season. A possible explanation is that the relationship between nitrification and the abundance of AOA-*amoA* transcripts depends on the season X vegetation cover interaction, so that we did not have enough replicates for each of these combinations to disentangle these complex relationships.

In the literature, significant AOA-*amoA* activities have been described in ammonium-poor or acidic temperate soils (Nicol et al., 2008; Prosser and Nicol, 2012). Lamto savanna soils are also slightly acidic and are characterised by very low ammonium availability (Abbadie and Lata, 2006). This could explain the functional importance of AOA-*amoA* for nitrification. Very few studies have documented the relative influence of archaeal and bacterial ammonia oxidisers on nitrification in savannas. In Brazilian savanna (Cerrado) soils, nitrification and AOA-*amoA* and AOB-*amoA* abundances have been found generally low (Catão et al., 2017, 2016). AOA-*amoA* predominated, and AOA-*amoA* transcripts were detected but AOB-*amoA* transcripts were not. However, nitrification was apparently not constrained by ammonium availability, a low pH or biological inhibitors in that savanna (Catão et al., 2017). In African savannas, studies have reported AOA-*amoA* as the main drivers of nitrification rather than AOB-*amoA* (Assémien et al., 2017; Rughöft et al., 2016). To confirm the preponderant influence of archaeal ammonia oxidisers on nitrification in Lamto soils and in other savannas, future studies could use a specific inhibitor of bacterial nitrifying enzymes (1-octyne, Taylor et al., 2013), and acetylene that inhibits both the AOA-*amoA* and AOB-*amoA* nitrification enzymatic pathways (Hink et al., 2017). As AOA-*amoA* tend to prefer

low NH_4^+ concentrations while AOB-*amoA* tend to prefer high NH_4^+ concentrations, it would also be useful to link AOA-*amoA* and AOB-*amoA* transcripts and abundances to a gradient of NH_4^+ concentrations in incubation or microcosm experiments. Furthermore, the persistence of AOB-*amoA* at relatively high abundances in Lamto savanna soils associated with low levels of AOB-*amoA* transcripts suggests that these AOB-*amoA* could be in a dormancy state. Another hypothesis is that the dominant AOB-*amoA* populations in these soils could grow as mixotrophs using soil organic matter rather than only oxidising ammonium into nitrite as their source of energy. Such mixotrophic growth of bacterial nitrifiers has been reported, e.g., by Kouki et al. (2011). Any experiment testing this hypothesis on savanna soils should also ensure that the mixotrophic capacity of AOA-*amoA* is tested (Alves et al., 2018).

5. Conclusions

The abundances of archaeal and bacterial ammonia oxidisers increase during the wet season, while the transcriptional activities of the AOA-*amoA* gene decrease (the abundance of AOB-*amoA* transcripts remained below the detection limit). This type of discrepancy calls for more studies in savanna soils to better link microbial abundances and activities and decipher the functional and ecological implications of microbial dormancy phenomena (Jones and Lennon, 2010) in these constrained environments.

Our results also strongly suggest that archaeal ammonia oxidisers are mostly responsible for nitrification in these West African savanna soils and that grasses can inhibit archaeal nitrification through decreased *amoA* expression, whereas trees stimulate it by improving growth conditions (higher organic matter and N contents) that benefit all microorganisms including nitrifiers. The absence of a positive correlation between NEA and the abundance of ammonia oxidisers under grasses and in bare soil suggests that nitrification is, at least at some periods, an optional process for nitrifiers (bacteria and archaea). This was suggested by Qin et al. (2014) and should be further tested in experiments in controlled conditions. Published results on nitrification in tropical savannas are currently scarce. The generality of the present results should be tested in other savannas. In particular, the frequency of BNI capacity among perennial grass species and the relative influence of archaea and bacteria on nitrification should be assessed across African savannas and in savannas of other continents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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