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1 **Effects of vegetation cover and season on soil nitrifiers in an African savanna: evidence**
2 **of archaeal nitrifier inhibition by grasses**

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24 Inhibition), Poaceae, Savanna, Tree

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28

29 **ABSTRACT**

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

43 1. Introduction

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

53 Soil microbial communities and the biogeochemical cycles under their control can be impacted by
54 drying and rewetting stresses (Fierer et al., 2003; Kaisermann et al., 2013; Thion and Prosser, 2014).
55 However, little is known about the AOA-*amoA* and AOB-*amoA* responses to these stresses. For
56 example, in a grassland with no historical record of drought events, AOA-*amoA* seem to be less
57 resistant than AOB-*amoA* to drought. They are also less resilient to rewetting in soils (Thion and
58 Prosser, 2014). This could be linked to the higher concentration of ammonium during and following
59 drought associated to lower AOA-*amoA* tolerance to these concentrations. AOA-*amoA* also appear to
60 be sensitive to high water contents, maybe due to the subsequent decrease in available oxygen
61 (Szukics et al. 2010). However, they have been found insensitive to oxygen availability, contrary to
62 AOB-*amoA* (Ke et al. 2015). Finally, AOA-*amoA* have proved more sensitive than AOB-*amoA* to
63 osmotic and matric stresses in soil microcosms (Bello et al. 2019). Changes in temperature also
64 influence the overall archaeal and bacterial community structures (Stres et al., 2008). As far as
65 nitrifiers are concerned, AOB-*amoA* abundances increase with increasing soil temperature, while
66 AOA-*amoA* decrease at 25°C in high soil water content conditions (70%) (Szukics et al. 2010). In
67 addition, acidophilic AOA-*amoA* strains have lower optimum growing temperature than neutrophilic
68 AOA-*amoA* (Gubry-Rangin et al., 2017). All these results suggest that the abundance and activity of
69 archaeal and bacterial nitrifiers should vary along the year according to climate seasonality. For

70 example, a seasonal effect was recorded on both groups of ammonia oxidisers in a Chinese upland red
71 soil, with higher gene copy numbers in summer than in winter (He et al. 2007) .

72 Overall, AOA-*amoA* seem more abundant in temperate soils (Prosser and Nicol 2012; Sterngren et
73 al., 2015), but their contribution to nitrification vs. AOB-*amoA* varies among ecosystems (Le Roux et
74 al., 2008; Nicol et al., 2008; Verhamme et al., 2011; Simonin et al., 2015). AOA-*amoA* have been
75 found more abundant than AOB-*amoA* in savanna soils (Assémien et al., 2017; Catão et al., 2017;
76 Rughöft et al., 2016; Srikanthasamy et al., 2018), but there is no consensus on this issue because of the
77 very low number of studies documenting nitrification in savanna ecosystems (which yet cover 12–
78 13% of global terrestrial surfaces; Rutten et al., 2016). AOA-*amoA* are key nitrifiers in non-fertilized
79 cultivated soils from the West African savanna zone, but the influence of AOB-*amoA* on nitrification
80 increases when these soils are fertilized (Assémien et al. 2017). The relative contribution of AOA-
81 *amoA* vs. AOB-*amoA* in non-cultivated savanna soils has been determined only once, in Brazilian
82 Cerrados (Catão et al., 2017, 2016), and has never been determined in an African savanna.

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

97 functions (Fierer, 2017). Finally, a pure culture experiment recently showed that AOA-*amoA* seem
98 more sensitive than AOB-*amoA* to BNI compounds, with variability among strains (Kaur-Bhambra et
99 al. 2021).

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

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

- 116 (1) The dominant tree and grass species stimulate and inhibit nitrification, respectively, as
117 compared to bare soil.
- 118 (2) Soil microbial abundances and transcription levels are higher under a plant cover than in
119 bare soil, except for the nitrifier groups inhibited under grasses. In particular, this would
120 lead to higher AOA-*amoA*:total archaea and lower AOB-*amoA*:total bacteria ratios under
121 trees and grasses, respectively, based on abundances and transcript abundances.

122 (3) Microbial abundances and transcription levels are higher during the wet season, except for
123 the nitrifier groups targeted by BNI, as they undergo a higher inhibiting root activity under
124 grasses during the wet season. The activity of grasses indeed strongly decreases during the
125 dry season (Abbadie et al., 2006), which may alleviate the BNI effect.

126 (4) Because AOA-*amoA* have been assumed to be the most important ammonia oxidisers in this
127 area, we also hypothesized that the dominant grass species (*H. diplandra*) in this savanna
128 area, known to have BNI capacity, may affect the abundance and/or the *amoA* transcript
129 level of both AOB-*amoA* and AOA-*amoA*. Overall, we expected NEA to be positively
130 correlated to AOA-*amoA* abundance and transcriptional activity.

131 2. Materials and methods

132 2.1. Study sites

133 The Lamto reserve (6°13'N, 5°20'W) in Ivory Coast harbours a mosaic of savannas distinguished
134 by tree densities, and riparian and gallery forests. The climate is sub-equatorial, and four seasons can
135 be distinguished: (i) a long dry season from December to February; (ii) a long wet season from March
136 to July; (iii) a short dry season in August; (iv) a short wet season from September to November.
137 Annual precipitation in 2017 was 1100.5 mm, as compared to a 30-year average of 1200 mm (data
138 from the Geophysical Station of Lamto). Temperatures are relatively constant throughout the year (27
139 °C on average). Soils are derived from granites and the resulting sands, and classified as tropical
140 ferruginous soils with a superficial gravelly horizon (acrisol according to the World Reference Base
141 for Soil Resource - WRB). They are sandy (sands 77%; silts 14%; clays 9%), with a bulk density of
142 *ca.* 1.65 (Lata et al., 1999).

143 2.2. Soil sampling

144 We distinguished three types of vegetation cover for soil sampling: (i) under the tussocks of the
145 dominant grass *Hyparrhenia diplandra* (hereafter GRA), known to have BNI capacity (Lata et al.,
146 2004; Srikanthasamy et al., 2018); (ii) under the canopy of the dominant tree *Crossopteryx febrifuga*
147 (hereafter TRE); and (iii) in bare soil patches (2 m x 2 m plots without vegetation, weeded for one year
148 before sampling, hereafter BS). In Lamto savanna, grasses are perennial and their individuals form

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

157 Soil was sampled during two contrasted seasons: during the long dry season (January 2017, 107.7
158 mm of precipitation in the previous 2 months and 0 mm in the last 15 days, hereafter D) and the long
159 wet season (May 2017, 344.9 mm of precipitation in the previous 2 months and 196.5 mm in the last
160 15 days, hereafter W). For each of the 6 combinations of season and cover type (3 covers X 2
161 seasons), five replicates were sampled (i.e., a total of 30 samples). One soil sample of about 1 kg was
162 collected from the top 15 cm soil layer at each sampling point using an 8 cm-diameter auger. After a
163 first homogenisation of the samples in the field, 10-g sub-samples were immediately placed in
164 cryotubes and in liquid N. The rest of the soil samples was stored at 4 °C for a very short period for
165 transport to the field station. These samples were subsequently sieved (2 mm), and 200 g of soil were
166 stored at -20 °C for molecular biology analyses and NEA measurements. The remaining soil was air-
167 dried in the shade and stored at ambient temperature for physico-chemical analyses. Roots were
168 collected from all soil samples by dry sieving (2 mm mesh), and dried at 50 °C before weighing. Fine-
169 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
170 root dm⁻³ dry soil under trees, and 0 to 0.30 g dry root dm⁻³ dry soil under bare soil. The aboveground
171 biomass of each grass tussock was collected and dried at 50 °C before weighing; it varied between
172 1.229 and 5.884 g dry matter tussock⁻¹. Soil moisture was measured in the field before sampling with
173 a previously calibrated ThetaProbe ML3 (Delta-T Devices).

174 2.3 Soil physico-chemical characteristics

175 The soil pH was measured in water (5:1 water:soil v/v) with a pH meter (Thermo Scientific
176 Orion™ Star A211, and an Orion™ PerpHecT™ ROSS™ Combination Micro Electrode) according to
177 the NF ISO 10390 standard. Mineral N was extracted from 2 g of frozen soil by adding 2 M KCl
178 solution (1:4 soil:solution). Nitrate was reduced to nitrite, and nitrite and ammonium concentrations
179 (expressed as mg N-NO₃⁻ and N-NH₄⁺ g⁻¹ dry soil) were measured with a continuous-flow N analyser
180 (SKALAR, San Plus System, Breda, The Netherlands). Total soil C and N contents (expressed as %) were
181 measured using a CHN Elemental Analyser (FlashEA 1112 Series, Thermo Electron
182 Corporation, The Netherlands) after grinding at 80 μm.

183 *2.4. Nitrifying enzyme activity assays*

184 Potential nitrification rates were assessed with nitrifying enzyme activity (NEA) assays, i.e., short-
185 term laboratory incubations under non-limiting conditions. This approach detects very low rates of
186 nitrification (Lata et al., 1999; Attard et al., 2011). Frozen soil samples were placed at ambient room
187 temperature for 2 h before the analyses. Freezing was preferred to fresh or drying conservation
188 methods because (i) the time lapse between sampling and analysis (including transportation) was too
189 long to use fresh soil because the analyses were performed in France, (ii) previous studies on different
190 soil matrices showed that freezing was a preferable storage method for soil enzyme activity studies
191 (e.g., denitrification) (Wallenius et al., 2010), and (iii) preliminary tests on fresh vs. frozen samples on
192 our soils showed no difference. NEA was measured according to Dassonville et al. (2011) as the linear
193 rate of nitrate production during a 72-h incubation. Soil sub-samples (3 g equivalent dry soil) were
194 incubated with 6 ml of a solution of (NH₄)₂SO₄ (22 μg N-NH₄⁺ g⁻¹ dry soil, allowing the optimum
195 nitrification condition for this type of soil according to preliminary analyses – Assemien et al., 2017).
196 Distilled water was added to each sample to reach 24 ml of total liquid volume in the flasks. The soil
197 nitrate content was measured after 5, 24, 48 and 72 h during the aerobic incubation under constant
198 agitation (180 rpm) by ion chromatography (DX120, Dionex, Salt Lake City, USA).

199 *2.5. Quantification of microbial abundances*

200 Total ribonucleic acids were extracted from 4 g of fresh soil with an RNA PowerSoil® Total RNA
201 Isolation Kit (MO BIO Laboratories), in combination with a FastPrep FP120 bead beating system

202 (Bio-101, Inc., Ca, USA) according to the manufacturer's instructions. DNase treatment was
203 performed using the Ambion® TURBO DNA-free™ DNase Treatment and Removal Reagents
204 protocol, and the absence of contaminant DNA was checked on agarose gel. RNA was quantified
205 using a Qubit™ RNA HS assay kit. All RNA samples were normalised to a similar concentration (8
206 ng/μl) before the reverse transcription step achieved using a SuperScript® III First-Strand Synthesis
207 System for RT-PCR (Invitrogen). All extracted RNA samples were stored at -80 °C before analysis,
208 and cDNA samples were stored at -20 °C.

209 Total deoxyribonucleic acids were extracted, purified and quantified as in Srikanthasamy et al.
210 (2018), and archaeal and bacterial ammonia oxidiser abundances were quantified by real-time PCR
211 following the same publication. Archaeal *amoA* genes were amplified using primers CrenamoA23f
212 and CrenamoA616r (Tourna et al., 2008), and bacterial *amoA* genes were amplified using primers
213 amoA 1F and amoA 2R (Rotthauwe et al., 1997). Two independent quantitative PCR assays were
214 performed for each gene to test repeatability. Standard curves were obtained using serial dilutions of
215 linearised plasmids containing the studied genes. Average amplification efficiency was 84.1% for
216 AOA-*amoA* and 91.8% for AOB-*amoA* (see Table S1 for the PCR conditions). Melting curves were
217 analysed using the Dissociation Curve Analysis software program (Applied Biosystems). Abundances
218 were expressed as gene copy numbers per gram of dry soil.

219 2.6. Statistical analyses

220 All statistical analyses were performed using R software (R Core Team 2016). ANOVA2 was used
221 to test the effects of vegetation cover, season and their interaction on all measured variables. When a
222 significant effect of the cover X season interaction was observed (meaning that the effect of the cover
223 differed with the season), all combinations of the two treatment modalities were compared using
224 Tukey's Honestly Significant Difference test. When the interaction was non-significant, we compared
225 the modalities of the significant simple factor(s) using the same post-hoc test. We also tested the
226 relationship between NEA and AOB-*amoA* and AOA-*amoA* abundances or their transcript abundances
227 using linear regressions. Normality and homoscedasticity of the residuals of all linear models were
228 tested with Shapiro-Wilk and Bartlett tests, respectively. Data were log-transformed, square-

229 transformed or boxcox-transformed in case of significant deviation from normality and
230 homoscedasticity. For all tests, the null hypothesis was rejected for $p < 0.05$, and significance was
231 represented as follows: *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$.

232 **3. Results**

233

234 *3.1. Soil characteristics*

235 The soil pH was affected by the vegetation cover and the season, but very weakly so (Table 1; see
236 also all mean values in Table S2). The pH was slightly higher under the tree cover (TRE) (6.93 ± 0.05)
237 than under the grass cover (GRA) (6.75 ± 0.06) and in bare soil (BS) (6.70 ± 0.03 ; $p < 0.01$), and similar
238 under GRA and in BS. The pH was very slightly higher during the wet season (W) (6.86 ± 0.04) than
239 during the dry season (D) (6.73 ± 0.04).

240 The soil water content (SWC) was affected by the vegetation cover, the season and their interaction
241 (Tables 1 and S2). The SWC was higher in BS-W ($20.56 \pm 0.52\%$) than in BS-D ($15.66 \pm 1.10\%$;
242 $p = 0.025$) and higher under GRA-W ($17.94 \pm 1.48\%$) than under GRA-D ($12.18 \pm 1.23\%$; $p < 0.01$).
243 However, there was no difference between TRE-W ($17.26 \pm 0.84\%$) and TRE-D ($16.56 \pm 0.59\%$). The
244 SWC was also higher in BS-W than under GRA-D ($p < 0.001$), and higher under TRE-W than under
245 GRA-D ($p < 0.05$).

246 The soil N-NH_4^+ concentration was affected by the vegetation cover (Tables 1 and S2) and the
247 cover X season interaction. N-NH_4^+ concentration was higher under TRE-W ($9.69 \pm 0.69 \text{ mg kg}^{-1}$ dry
248 soil) than under GRA-W ($6.87 \pm 0.26 \text{ mg kg}^{-1}$ dry soil; $p < 0.01$) and in BS-W ($5.10 \pm 0.33 \text{ mg kg}^{-1}$ soil;
249 $p < 0.001$). N-NH_4^+ concentration was also higher under TRE-D ($7.68 \pm 0.36 \text{ mg kg}^{-1}$ soil) than in BS-W
250 ($p < 0.01$), and higher under TRE-W than under GRA-D ($p < 0.01$) and in BS-D ($6.59 \pm 0.29 \text{ mg kg}^{-1}$
251 soil; $p < 0.001$). The soil N-NO_3^- concentration was only affected by the season and was slightly lower
252 during the wet season ($14.34 \pm 1.12 \text{ mg kg}^{-1}$ soil) than during the dry season ($14.70 \pm 0.70 \text{ mg kg}^{-1}$ soil).

253 The total soil N concentration was affected by the vegetation cover and the season (Table 1). The
254 total soil N concentration was higher under TRE ($0.069 \pm 0.003\%$) than in BS ($0.050 \pm 0.003\%$;
255 $p = 0.0023$). The total soil N concentration was similar under GRA ($0.061 \pm 0.004\%$) and in BS on the

256 one hand, and under GRA and TRE on the other hand. It was higher during the dry season
257 (0.064±0.003%) than during the wet season (0.056±0.004%). The total soil C concentration was
258 affected by the cover and was higher under GRA (1.02±0.07%) and TRE (1.02±0.04%) than in BS
259 (0.81±0.03%; p= 0.013). The total soil C concentration was similar under GRA and TRE.

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

264 3.2. Nitrifying enzyme activity

265 Nitrifying enzyme activity (NEA) was affected by the vegetation cover type (Fig. 1, ANOVA
266 results, p<0.001) and the cover X season interaction (p<0.01), but not by the sole effect of the
267 season. NEA was 18 times higher under TRE (45.17±10.67 ng N-(NO₃⁻ + NO₂⁻) h⁻¹ g⁻¹ dry soil) than
268 under GRA (2.47±1.60 ng N-(NO₃⁻ + NO₂⁻) h⁻¹ g⁻¹ dry soil; p< 0.001), and 4 times higher than in BS
269 (12.38±1.73 ng N-(NO₃⁻ + NO₂⁻) h⁻¹ g⁻¹ dry soil; p< 0.01). NEA was five times higher in BS than under
270 GRA (p< 0.01). The cover X season interaction was significant: NEA was higher under TRE-D than
271 under TRE-W, but not difference was found between GRA-D and GRA-W.

272 3.3. Nitrifier abundances and gene expression levels

273 Archaeal *amoA* (AOA-*amoA*) gene abundance was affected by the vegetation cover and the season,
274 without any interaction effect (Table 2 and Fig. 2; see also Table S3 for mean values). Archaeal *amoA*
275 (AOA-*amoA*) gene abundance was about 22 times higher under TRE (1.98±0.44×10⁶ copies g⁻¹ dry
276 soil) than under GRA (8.99±4.04×10⁴ copies g⁻¹ dry soil; p< 0.001; Fig. 2A), and about 13 times
277 higher in BS (1.18±0.20×10⁶ copies g⁻¹ dry soil) than under GRA (p< 0.001). Archaeal *amoA* (AOA-
278 *amoA*) gene abundance was similar under TRE and in BS. It was about 2 times higher during the wet
279 season (1.38±0.36×10⁶ copies g⁻¹ dry soil) than during the dry season (7.87±2.18×10⁵ copies g⁻¹ dry
280 soil; Fig. 2B). The abundance of AOA-*amoA* transcripts was affected by the vegetation cover and the
281 season (Fig. 2C; see also Table S2 for mean values). There was no difference between BS, GRA and
282 TRE during the wet season. During the dry season, the abundance of AOA-*amoA* transcripts was

283 lower under GRA ($2.10 \pm 1.05 \times 10^5$ copies g^{-1} dry soil) than in BS ($3.34 \pm 1.04 \times 10^4$ copies g^{-1} dry soil;
284 $p < 0.05$).

285 Bacterial *amoA* gene (AOB-*amoA*) abundance was affected by the vegetation cover, the season and
286 their interaction (Table 2 and Fig. 3; see also Table S3 for mean values). Bacterial *amoA* gene was
287 similar in BS-D ($6.31 \pm 2.85 \times 10^5$ copies g^{-1} soil) and BS-W ($3.26 \pm 1.42 \times 10^5$ copies g^{-1} dry soil). It was
288 7 times higher under GRA-W ($1.26 \pm 0.41 \times 10^6$ copies g^{-1} dry soil) than under GRA-D ($1.62 \pm 0.43 \times 10^5$
289 copies g^{-1} dry soil; $p < 0.01$), and 5 times higher under TRE-W ($1.72 \pm 0.17 \times 10^6$ copies g^{-1} dry soil) than
290 under TRE-D ($3.44 \pm 0.37 \times 10^5$ copies g^{-1} dry soil; $p < 0.01$). Bacterial *amoA* gene was higher under
291 GRA-W ($p < 0.05$) and TRE-W ($p < 0.01$) than in BS-W. The abundance of AOB-*amoA* transcripts was
292 below the detection limit (*ca.* 10^3 copies g^{-1} dry soil) in all but 2 samples – a grass tussock during the
293 wet season (8.5×10^3 copies g^{-1} dry soil) and a bare soil sample during the wet season (3.6×10^4 copies
294 g^{-1} dry soil).

295 The AOA:(AOA+AOB) abundance ratio (Table 2; see also Table S3 for mean values) was only
296 affected by the vegetation cover ($p < 0.001$). AOA:(AOA+AOB) abundance ratio was at least 4-fold
297 higher under TRE (0.64 ± 0.05 ; $p < 0.001$) and in BS (0.73 ± 0.05 ; $p < 0.001$) than under GRA
298 (0.16 ± 0.06), and no difference was observed between TRE and BS. The AOA-*amoA*:total archaeal
299 abundance ratio (Table 2; see also Table S3 for mean values) was affected by the vegetation cover ($p <$
300 0.001) and the season ($p < 0.01$). AOA:(AOA+AOB) abundance ratio was higher in BS ($6.21 \pm 1.25 \times 10^{-4}$;
301 $p < 0.01$) and under TRE ($7.12 \pm 1.34 \times 10^{-4}$; $p < 0.001$) than under GRA ($2.95 \pm 1.23 \times 10^{-5}$).
302 AOA:(AOA+AOB) abundance ratio was higher during the wet season ($6.25 \pm 1.37 \times 10^{-4}$) than during
303 the dry season ($2.83 \pm 0.68 \times 10^{-4}$). The AOA-*amoA*:total archaeal transcript ratio (Table S3) was
304 unaffected by the cover and the season. Finally, the AOB-*amoA*:total bacterial abundance ratio (Table
305 2; see also Table S3 for mean values) was only affected by the season ($p < 0.01$) and was higher during
306 the wet season ($6.66 \pm 1.28 \times 10^{-4}$) than during the dry season ($2.50 \pm 0.73 \times 10^{-4}$).

307 3.4. Links between NEA and nitrifier abundances and *amoA* expression levels

308 Regressions between NEA and nitrifier abundances and transcript abundances were performed
309 separately for trees, grasses and bare soils. NEA increased with AOA-*amoA* abundance under trees

310 (NEA= $1.576 \times 10^{-8} \times \text{AOA-amoA}$; $p < 0.05$, $R^2 = 0.35$; Fig. 4A), but no relationship was observed under
311 grasses and in bare soil. A significant relationship was also observed between NEA and AOA-*amoA*
312 abundance when considering all vegetation types (NEA= $1.662 \times 10^{-8} \times \text{AOA-amoA}$; $p < 0.001$, $R^2 =$
313 0.42). NEA also significantly increased with AOA-*amoA* abundance during the wet season (all
314 vegetation covers taken together, NEA= $0.017194 \times \text{AOA-amoA}$; $p < 0.05$, $R^2 = 0.36$; Figure not
315 shown) but did not increase during the dry season. In the same vein, NEA significantly increased with
316 the abundance of AOA-*amoA* transcripts during the dry season (all vegetation covers taken together,
317 NEA= $0.004092 \times \text{AOA-amoA}$; $p < 0.05$, $R^2 = 0.45$; Fig. 4C). No significant relationship was observed
318 between NEA and the abundance of AOA-*amoA* transcripts during the wet season (Figure not shown).

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

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

327 **4. Discussion**

328 *4.1. Influence of the vegetation cover and the season on nitrifiers*

329 Nitrification enzyme activity (NEA) was overall 5 times lower under grasses than in bare soil. This
330 confirms that *H. diplandra* inhibits nitrification, as already demonstrated in Lata et al. (2000).
331 Together with the results from the other three other dominant tussock grasses of Lamto savanna
332 (Srikanthasamy et al., 2018) – all associated to low nitrification rates –, our results confirm that the
333 dominant grasses of this savanna have biological nitrification inhibition (BNI) capacity. However, we
334 show for the first time that the abundance of archaeal nitrifiers was 13 times lower under grasses than
335 in bare soil, and that the AOA-*amoA*:total archaeal abundance ratio was also significantly lower under
336 grasses than in bare soil. This demonstrates that BNI induced by grasses decreases the competitive

337 ability of archaeal ammonia oxidisers comparatively to other archaea, as already suggested by
338 Subbarao et al. (2009) or Sarr et al. (2020). In addition, the abundance of AOA-*amoA* transcripts
339 decreased strongly under grasses during the wet season, potentially due to more efficient BNI induced
340 by more active grass roots during the wet season. Nuñez et al. (2018) reported that the effect of BNI
341 on nitrification increases when plants are growing, and root exudate emissions increase. All these
342 results support the view that grass BNI impact the AMO nitrifying enzyme pathway of AOA-*amoA* in
343 the Lamto savanna. The mechanism underlying nitrification inhibition is not entirely clear in the
344 current literature (Subbarao et al., 2015; Nuñez et al., 2018). This mechanism might be based on the
345 inactivation of nitrification enzymes, down-regulation of the expression of nitrification genes, or a
346 combination of both.

347 AOA-*amoA* abundance and transcripts, AOB-*amoA* abundance, and total fungal, bacterial and
348 archaeal abundance (see Table S4-1 & S4-2) depend on complex interactions between the season and
349 the vegetation cover. Seasonality is indeed often of importance when considering soil microbial
350 abundance and transcriptional activity (Kennedy et al., 2005; Voříšková et al., 2014). On the one hand,
351 we observed overall higher microbial (total as well as/and nitrifier) abundances during the wet season
352 that provided better conditions for soil microorganisms (Thion & Prosser, 2014; de Castro et al.,
353 2016). However, this seasonal difference was mainly true only for grass and tree soils, not for bare
354 soil. This confirms that microorganisms depend as much on soil humidity as on plant activities (e.g.,
355 root exudates) that increase during the wet season (Haichar et al., 2008; Berendsen et al., 2012). On
356 the other hand, the fact that the abundance of AOA-*amoA* transcripts decreased during the wet season
357 under grasses in our study suggests that BNI decreased the transcription of AOA-*amoA*. In contrast,
358 we observed higher AOB-*amoA* abundances under grasses than in bare soil during the wet season, vs.
359 similar ones under grasses and trees. This suggests that BNI induced by grasses is very efficient in
360 inhibiting AOA-*amoA* nitrification. New experiments would be required to test the capacity of Lamto
361 grasses to inhibit AOB-*amoA* nitrification in other soils. This is novel for *in situ* measurements in
362 natural ecosystems. So far, only experimental results on crop lines or pure cultures in controlled
363 conditions have recently documented archaeal nitrifier sensitivity to BNI (Nuñez et al., 2018; Sarr et
364 al., 2020; Kaur-Bhambra et al., 2021). Our results show for the first time that BNI can efficiently

365 inhibit nitrification by AOA-*amoA* in the field. The causes of this difference in sensitivity can be
366 multiple (e.g., soil and overall abiotic characteristics, niche partitioning, biochemical, cellular or
367 genetic differences, differences in microbial community structure (Kaur-Bhambra et al., 2021)), and
368 they still remain to be tested.

369 NEA was overall 4 times higher under the tree canopy than in bare soil. This confirms that
370 nitrification is stimulated under trees, in line with Mordelet et al. (1993) and Srikanthasamy et al.
371 (2018). Two non-exclusive mechanisms could explain this stimulation. First, the total C and total N
372 concentrations and ammonification are globally higher under trees in these savannas (Mordelet et al.,
373 1997; Srikanthasamy et al., 2018; this study – see Table S2). This would stimulate the whole microbial
374 community, increase ammonium availability for nitrifiers and in turn increase their abundance and
375 stimulate their activity, and was confirmed by a significant positive regression between NEA and N-
376 NH_4^+ (see supplementary data S5-1A). Moreover, since differences in total N and C contents between
377 grasses and trees are usually low due to the overall poor soil fertility (this difference was not
378 significant in this study (data not shown) – see Table S2), differences in the quality of organic matter
379 might play a role in the stimulation of nitrification by trees, as suggested by the lower soil C:N ratio
380 under trees than under grasses (Table S2 or the significant positive regression between NEA and pH
381 (see supplementary data S5-1B). Similarly, changes in the soil moisture content could be favourable to
382 microbial activities and nitrifiers under the tree canopy, especially in the dry season (soil humidity was
383 higher under TRE-D than under GRA-D, and no difference was found between TRE-D and TRE-W,
384 see Table S2).

385 It cannot be ruled out that Lamto savanna trees specifically stimulate nitrifiers through particular
386 leaf leachates or root exudates. Zhang et al. (2016) documented such a process for *Picea asperata* (a
387 coniferous tree) and Bardon et al. (2018) did so for *Pteridium aquilinum* (a fern) in the temperate zone.
388 However, the ratios of AOA-*amoA*/total archaea and AOB-*amoA*/total bacteria were not higher under
389 trees than in bare soil away from any tree. This supports the importance of the first mechanism, i.e., a
390 general improvement of microbial growth conditions – in particular due to a higher organic matter
391 content – rather than specific stimulation of nitrifiers. Finally, as previously stated (Srikanthasamy et

392 al., 2018), the proportions of AOA-*amoA*(AOB-*amoA*) within the whole bacterial(archaeal)
393 communities were moderately lower than what was expected for sandy soils. This probably reflects
394 the impact of savanna climate and/or low N availability in the soils.

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

404

405 *4.2. Relative contribution of archaeal and bacterial nitrifiers to nitrification*

406 As in many ecosystems (Di et al., 2010; Hirsch and Mauchline, 2012; Vasileiadis et al., 2012;
407 Assemien et al., 2017; Xiang et al., 2017), AOA-*amoA* were on average much more abundant (2
408 times) than AOB-*amoA* in Lamto savanna soils. This does not necessarily mean that AOA-*amoA* are
409 more influential than AOB-*amoA* for nitrification. However, our results strongly support the
410 hypothesis of Srikanthasamy et al. (2018) that archaea are involved in nitrification in Lamto savanna:
411 (i) bacterial nitrifiers were rather abundant, but the abundance of AOB-*amoA* transcripts was below
412 the detection limit in most soil samples; (ii) the abundance of AOB-*amoA* genes was equally high
413 under trees (with high nitrification) and grasses (with low nitrification), whereas the abundance of
414 AOA-*amoA* genes was much higher under trees than under grasses. This means that the higher NEA
415 under the tree cover can be explained by AOA-*amoA* abundance, not by AOB-*amoA* abundance; (iii)
416 NEA increased with AOA-*amoA* abundance under trees when considering all vegetation types across
417 the seasons and all vegetation types during the wet season, and with AOA-*amoA* transcripts during the
418 dry season. Our results suggest that *C. febrifuga* can stimulate archaeal nitrifiers probably through a

419 general improvement of microbial growth conditions (see above), whereas AOB-*amoA* remains
420 functionally less important for nitrification in these soils. However, a role of AOB-*amoA* under trees
421 cannot be completely excluded because we evidenced a relationship between NEA and AOB-*amoA*
422 abundance when considering all vegetation types across the seasons, and a previous sampling
423 campaign mixing four different tree species had shown the same result (Srikanthasamy et al., 2018).

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

442 In the literature, significant AOA-*amoA* activities have been described in ammonium-poor or acidic
443 temperate soils (Nicol et al., 2008; Prosser and Nicol, 2012). Lamto savanna soils are also slightly
444 acidic and are characterised by very low ammonium availability (Abbadie and Lata, 2006). This could
445 explain the functional importance of AOA-*amoA* for nitrification. Very few studies have documented
446 the relative influence of archaeal and bacterial ammonia oxidisers on nitrification in savannas. In

447 Brazilian savanna (Cerrado) soils, nitrification and AOA-*amoA* and AOB-*amoA* abundances have
448 been found generally low (Catão et al., 2017, 2016). AOA-*amoA* predominated, and AOA-*amoA*
449 transcripts were detected but AOB-*amoA* transcripts were not. However, nitrification was apparently
450 not constrained by ammonium availability, a low pH or biological inhibitors in that savanna (Catão et
451 al., 2017). In African savannas, studies have reported AOA-*amoA* as the main drivers of nitrification
452 rather than AOB-*amoA* (Assémien et al., 2017; Rughöft et al., 2016). To confirm the preponderant
453 influence of archaeal ammonia oxidisers on nitrification in Lamto soils and in other savannas, future
454 studies could use a specific inhibitor of bacterial nitrifying enzymes (1-octyne, Taylor et al., 2013),
455 and acetylene that inhibits both the AOA-*amoA* and AOB-*amoA* nitrification enzymatic pathways
456 (Hink et al., 2017). As AOA-*amoA* tend to prefer low NH_4^+ concentrations while AOB-*amoA* tend to
457 prefer high NH_4^+ concentrations, it would also be useful to link AOA-*amoA* and AOB-*amoA*
458 transcripts and abundances to a gradient of NH_4^+ concentrations in incubation or microcosm
459 experiments. Furthermore, the persistence of AOB-*amoA* at relatively high abundances in Lamto
460 savanna soils associated with low levels of AOB-*amoA* transcripts suggests that these AOB-*amoA*
461 could be in a dormancy state. Another hypothesis is that the dominant AOB-*amoA* populations in
462 these soils could grow as mixotrophs using soil organic matter rather than only oxidising ammonium
463 into nitrite as their source of energy. Such mixotrophic growth of bacterial nitrifiers has been reported,
464 e.g., by Kouki et al. (2011). Any experiment testing this hypothesis on savanna soils should also
465 ensure that the mixotrophic capacity of AOA-*amoA* is tested (Alves et al., 2018).

466 **5. Conclusions**

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

473 Our results also strongly suggest that archaeal ammonia oxidisers are mostly responsible for
474 nitrification in these West African savanna soils and that grasses can inhibit archaeal nitrification
475 through decreased *amoA* expression, whereas trees stimulate it by improving growth conditions
476 (higher organic matter and N contents) that benefit all microorganisms including nitrifiers. The
477 absence of a positive correlation between NEA and the abundance of ammonia oxidisers under grasses
478 and in bare soil suggests that nitrification is, at least at some periods, an optional process for nitrifiers
479 (bacteria and archaea). This was suggested by Qin et al. (2014) and should be further tested in
480 experiments in controlled conditions. Published results on nitrification in tropical savannas are
481 currently scarce. The generality of the present results should be tested in other savannas. In particular,
482 the frequency of BNI capacity among perennial grass species and the relative influence of archaea and
483 bacteria on nitrification should be assessed across African savannas and in savannas of other
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485

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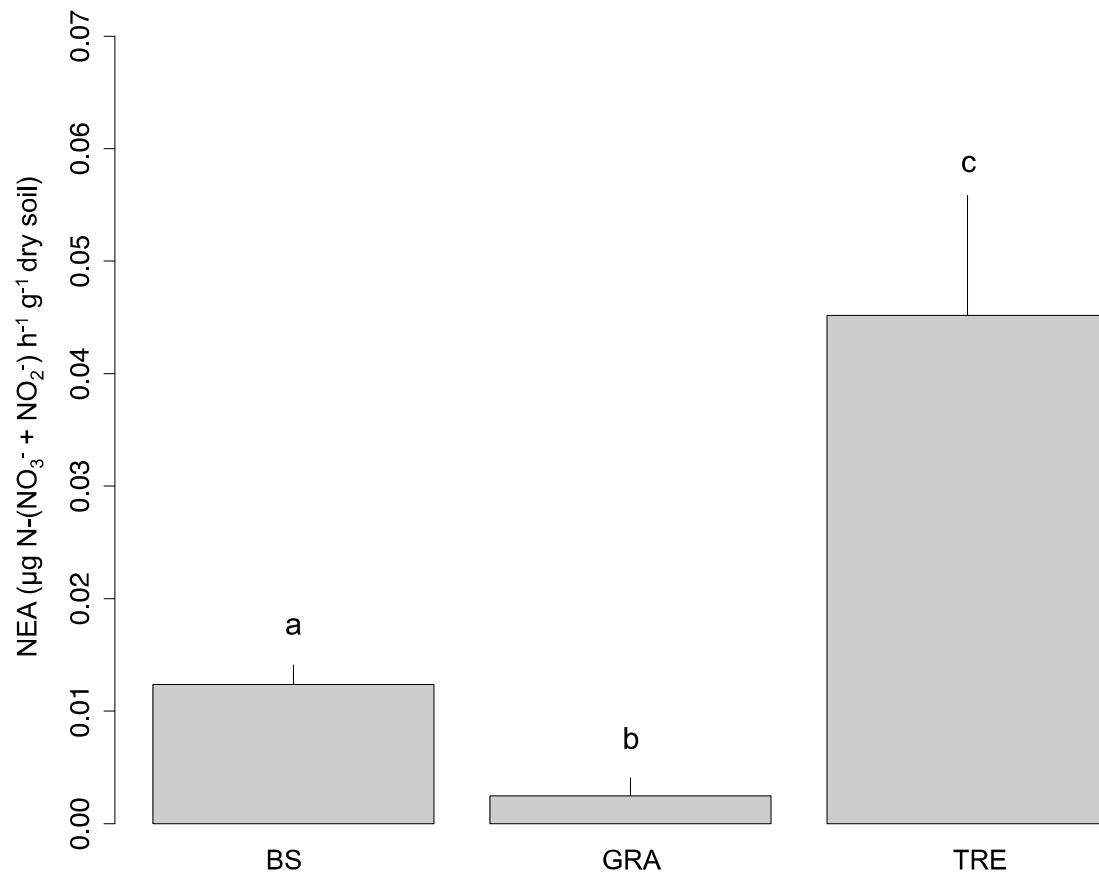


Fig. 1. Nitrifying enzyme activities (NEA) according to the vegetation cover: bare soil (BS), the dominant grass species *Hyparrhenia diplandra* (GRA) and the dominant tree species *Crossopteryx febrifuga* (TRE). Means and standard errors were calculated from ten replicates whatever the season. Different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

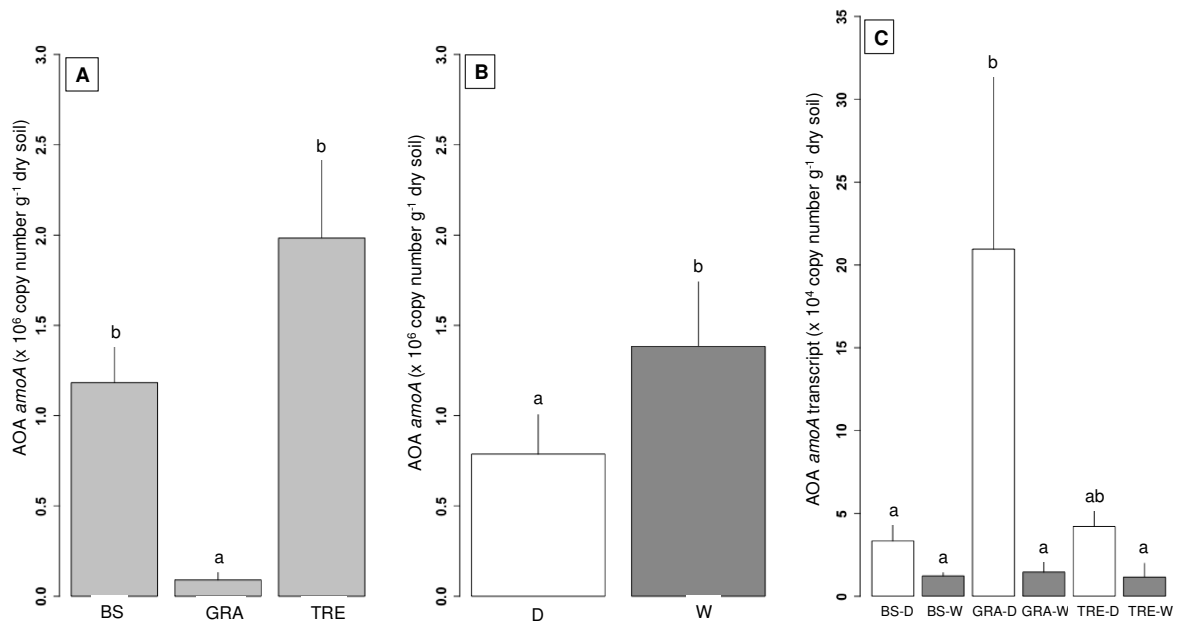


Fig. 2. Abundances of ammonia oxidizing archaea, AOA, according to (A) the vegetation cover and (B) the season, and (C) abundances of AOA-*amoA* transcripts according to vegetation cover (bare soil, BS; grass species *Hyparrhenia diplandra*, GRA; and tree species *Crossopteryx febrifuga*, TRE), and season (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 ($p < 0.05$).

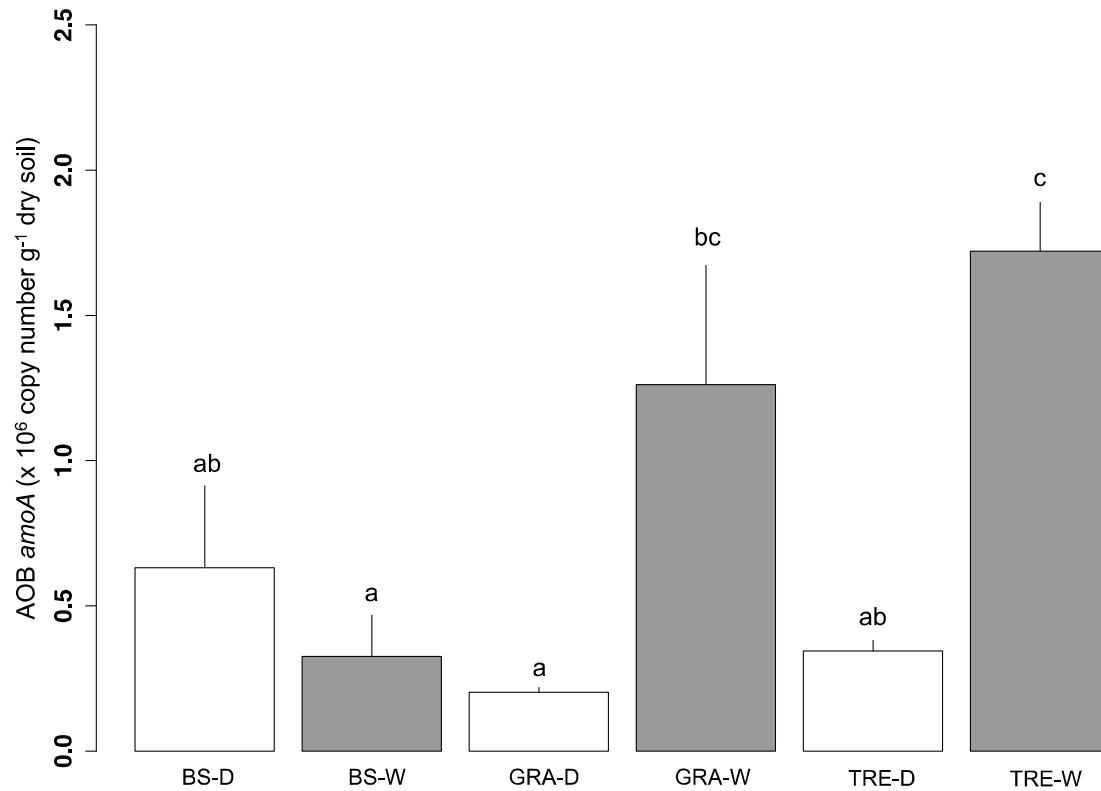


Fig. 3. Abundances of ammonia oxidizing bacteria, AOB, according to the vegetation cover (bare soil, BS; grass species *Hyparrhenia diplandra*, GRA; and tree species *Crossopteryx febrifuga*, TRE) and the season (dry season, D, white bars; wet season, W, grey bars). Means and standard errors were calculated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

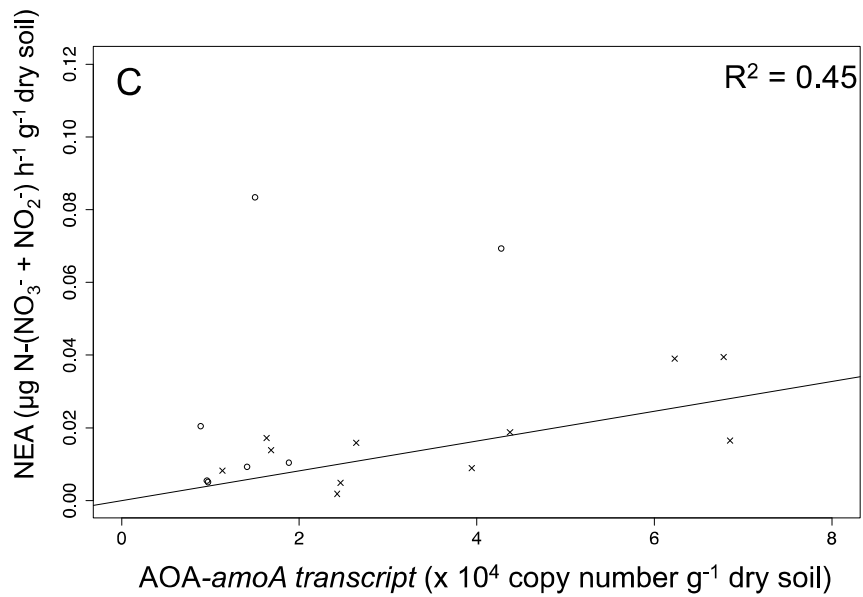
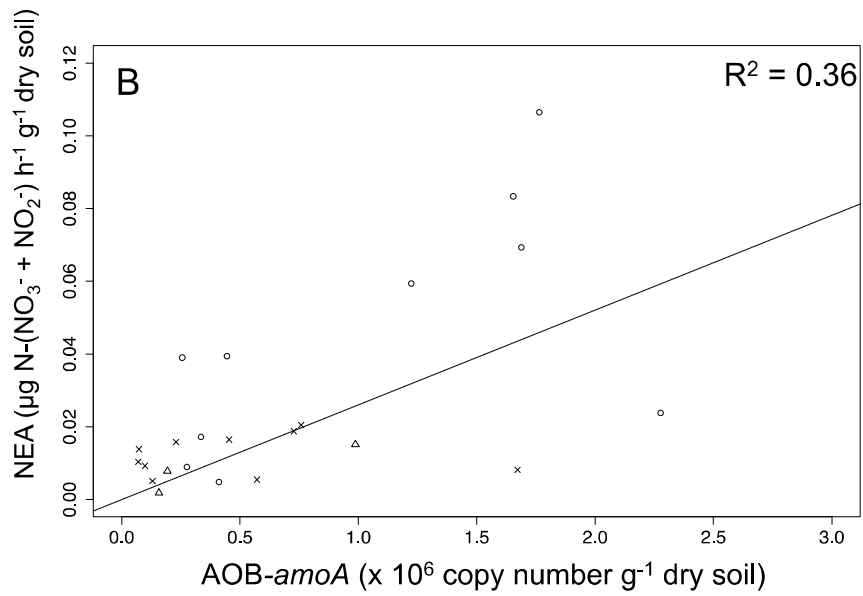
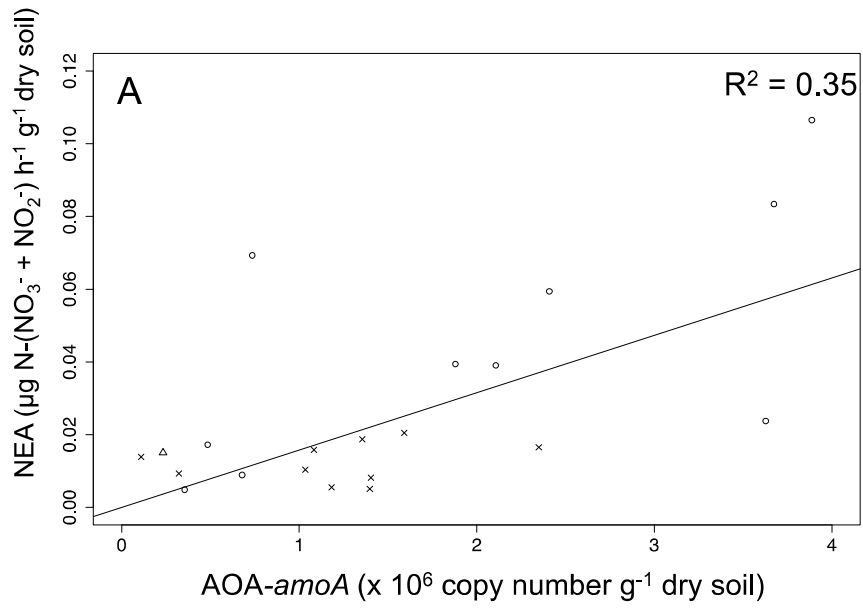


Fig. 4: (A) Regression between the nitrifying enzyme activity (NEA) and the abundance of ammonia oxidizing archaea (AOA) according to the vegetation cover whatever the season (bare soil, cross; grasses, triangle; and trees, circle). This regression was only significant for trees (as shown by the regression 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).

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 λ= 2	2	NS
	Season		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

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- <i>amoA</i>	Cover	2	<0.001	
	Season	Square root	1	<0.05
	Cover*Season		2	NS
AOA- <i>amoA</i> transcripts	Cover	2	NS	
	Season	Square root	1	<0.001
	Cover*Season		2	<0.05
AOB- <i>amoA</i>	Cover	2	<0.05	
	Season	Square root	1	<0.001
	Cover*Season		2	<0.01
AOA:(AOA+AOB) ratio	Cover	2	<0.001	
	Season	Log	1	NS
	Cover*Season		2	NS
AOA- <i>amoA</i> :total Archaea ratio	Cover	2	<0.001	
	Season	-	1	<0.01
	Cover*Season		2	NS
AOA- <i>amoA</i> :Archaeal transcripts ratio	Cover	2	NS	
	Season	-	1	NS
	Cover*Season		2	NS
AOB- <i>amoA</i> :total Bacteria ratio	Cover	2	NS	
	Season	-	1	<0.01
	Cover*Season		2	NS