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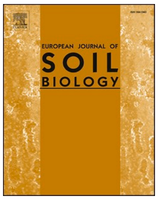

Response of ammonia-oxidizing bacteria and archaea abundance and activity to land use changes in agricultural systems of the Central Andes

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ABSTRACT

Ammonia-oxidation is the first rate-limiting step of the nitrification process performed by ammonia-oxidizing bacteria (AOB) and archaea (AOA). The response of ammonia oxidizers to agricultural management determines the forms of nitrogen available for plant nutrition and the potential for nitrate leaching, nitrous oxide emissions, and soil acidification. We investigated the potential nitrification rates (PNR) of AOA and AOB through the use of a specific inhibitor of bacterial nitrification, and the *amoA* gene abundance of AOB and AOA under potato, fallow and eucalyptus land uses in an agricultural system in the Central Andes of Bolivia. AOA dominated PNR and *amoA* gene abundance under all land uses. The ratio of AOA to AOB abundance decreased with soil pH, due to higher AOB abundances under the less acid soils of potato crops. Eucalyptus led to reduced AOB *amoA* abundances and PNR of both AOA and AOB, while PNR were highest under potato soils, and the contribution of AOB to total PNR increased. Specific PNR, as expressed per *amoA* gene copy numbers, was 12, 14 and 62 times higher for AOB than for AOA in potato, fallow and eucalyptus soils, respectively. AOB and AOA PNR were positively related to their respective *amoA* gene copy numbers, but for AOA the relationship depended on land use. This study demonstrates the interest for measuring separately nitrification rates of AOA and AOB for a mechanistic understanding of nitrification in different environments, as well as the importance of measuring process rates for assessing the environmental consequences of land use management.

1. Introduction

Nitrification is the biological oxidation of ammonia to nitrate followed by the oxidation of nitrite to nitrate. Ammonia oxidation is carried out by ammonia-oxidizing archaea (AOA) and bacteria (AOB), while the recently discovered comammox bacteria are capable of performing both steps [1,2]. When AOB and AOA are involved, ammonia oxidation is the rate limiting process, and their relative contribution to nitrate production rates in soils is still under debate. Niche differentiation of ammonia oxidizers was initially attributed to differences in ammonia affinities, with AOB showing lower affinity and requiring higher substrate availability [3–5]. For example, Martens-Habbena et al. [3] demonstrated that AOA are capable of oxidizing ammonia and to replicate at ammonium concentration below 10 nM, which is 100 times lower than the minimum concentration required for AOB activity and replication [6]. AOA were thus considered to be more relevant in oligotrophic environments, but recent research on their metabolic characteristics revealed similar affinities between AOA and AOB in soils [2,7]. Further, pH strongly influences ammonia-oxidizing prokaryotes through controls on free ammonia, and lower ammonia availability under acid conditions [8,9] would favor archaea over bacteria. Also specific metabolic adaptations, such as the vacuole-type ATPases, may explain growth of *Thaumarchaeota* in low pH environments [10].

Concomitantly, increased relative abundances of AOB and AOA in environmental samples through the measurement of the *amoA* gene (which encodes for the alpha-subunit of the ammonia-monooxygenase enzyme) indicated AOA dominance over AOB in numerous environments [11–13], and specifically under acidic conditions [14-18]. However, while in some acidic environments potential nitrification rates (PNR, assessing the activity of autotroph nitrifiers), show strong positive

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associations with AOA *amoA* gene copies [16], other studies report weak or no correlation [19–21]. These discrepancies may be attributed to higher specific oxidation rates of AOB, as shown in cell cultures [4,5,22], and evidence that at some sites acid-tolerant AOB may significantly contribute to nitrification [23,24]. Given that studies linking ammonia-oxidizer activity and abundance in environmental soil samples rarely differentiate between the contribution of AOA and AOB to total PNR, it has been difficult to interpret the ecological relevance of AOA or AOB *amoA* gene abundances, and moreover, it is not known whether the higher specific oxidation rates of AOB compared to AOA determined under laboratory conditions are also relevant in the environment. Evaluation of the relative contribution of archaea and bacteria to PNR provides evidence of their ecological relevance and the potential consequences of the changes in the AOA and AOB abundances.

The contribution of AOA and AOB to nitrification can be evaluated by the use of specific inhibitors, such as 1-octyne and allylthiourea. Allylthiourea (ATU) is a metabolic inhibitor that selectively chelates the copper in the active site of the ammonia mono-oxygenase enzyme and inhibits AOB activity [5,25,26]. ATU concentrations above 80 μM have been demonstrated to inhibit bacterial ammonia oxidation, while some AOA are not affected, even at concentrations of 500 μM [5,26,27]. ATU has been successfully used in kinetics studies of nitrification [5,28] and studies of waste water treatment [29–31]. Its incorporation in PNR measurements can thus be used to differentiate the nitrification performed by AOB and AOA, and contribute to the understanding of their respective role in the environment.

Changes in soil chemistry, especially pH, caused by land use can also differentially affect the nitrification process and the abundance and activity of AOB and AOA. For example, a study across different land uses under the same soil type reported changes in nitrification and *amoA* abundance following a pH change through acidification or liming [32]. Similarly, several studies of agricultural land uses reported that soil pH is the main variable explaining changes in the ammonia oxidizers community and potential nitrification rates under different fertilization regimes but with a different effect on AOA and AOB [33–35]. However, while the central role of soil pH on determining niche differentiation has been recognized, the relative contribution of AOB and AOA to nitrification under different land uses remains poorly understood. Evaluation of AOB and AOA potential nitrification and abundances is essential to understand their role in the environment and the potential consequences of land use change on the N cycle, particularly in agricultural systems where their activity determines the type of mineral nitrogen available for plants, but also contributes to nitrate leaching and ground water contamination [36], nitric and nitrous oxide gas emissions [37], and soil acidification [32,38].

We studied AOB and AOA PNR in relation to *amoA* gene abundance in agricultural areas of the mountainous Andean range of Bolivia characterized by acidic soil pH [39]. These Andean landscapes are dominated by three types of land use: (1) intensively cultivated fields in which potato constitutes the main crop planted during 2–4 consecutive growing seasons, followed by secondary crops (*Vicia faba* and *Ordeum vulgare*) after which the cycle is repeated again or fields are left fallow; (2) agricultural fields that are left fallow for a maximum of 6 years and are colonized by self-regenerated herbaceous vegetation, aiming at fertility restoration; (3) introduced *Eucalyptus globulus* L. plantations that replaced agricultural areas as an alternative economic income to farmers.

The objective of this study was to determine the relationship between AOA and AOB abundances and total, AOA and AOB nitrification rates in response to the three land uses. Given the acidity of the soils we hypothesized a dominance of AOA activity and abundance under fallow fields and eucalyptus plantations, but due to organic and mineral fertilization of cultivated fields, we expect higher activity and abundance of AOB in the potato cultivated fields.

2. Materials and methods

2.1. Study site

The study was conducted in the fields of the Chullchunqani Community (17°32′30'' - 17°33′30″ S, 065°20′08'' - 065°21′36″ W, altitude range 3100–3400 m), an agricultural community located in the Eastern branch of the Andes range, characterized by a rainy summer season (November–March) and a dry winter season (April-October) [40,41], with a mean annual rainfall of 500.7 mm, and a mean annual temperature of 17.9 °C [42]. The topography is characterized by secondary mountain ranges of the Andean region, with soils classified as Cambisols [43].

The potato crops, fallow fields and eucalyptus plantations are interspersed within the landscape in areas of ca. 0.5–1 ha, defined here as "plots". Potato fields are tilled (ca. 20 cm depth) for soil preparation, and chemical N, P, K inputs as well as chicken manure are added when sowing. Additional fertilization and irrigation is applied in varying amounts according to farmers' personal judgement and harvesting is conducted manually. Before conversion to fallow, remaining aboveground biomass is incorporated by tillage and the fields are then left unmanaged. Fields converted to eucalyptus plantations are not managed after seedling plantation. Eight plots of each land use type were selected within an area of ca. 4 km^2 based on the following criteria: agricultural plots in which potato had been grown during the last rainy season, fallow plots (2–6 years old) in which the spontaneously grown vegetation (grass-shrubland of semiarid high Andes [40]) fully covered the soil, and Eucalyptus (*E. globulus)* plantations (minimum 5 years since plantation).

Soils were sampled in February 2017, at the end of the rainy season. Within each plot, three randomly selected sampling points were established at a minimum distance of 10 m. At each sampling point, one composite soil sample was taken with a shovel (one central sample and four individual samples taken 2 m from this central sample; 20 cm depth). The thin organic layer (<0.5 cm) of the eucalyptus plots was discarded. Samples were homogenized, sieved (2 mm mesh) under sterile conditions and stored at 4 °C for potential nitrification measurements. From one randomly selected soil sample per plot, a subsample was freeze-dried and stored for molecular analyses.

2.2. Soil pH and potential nitrification

Soil pH_{KCl} and potential nitrification rates (PNR) were determined on all three soil samples taken in each plot. Soil pH was determined in KCl 1 M (1:2 w/v) (HI2550, HANNA instruments) [44]. PNR was determined using the shaken soil slurry method [45], with and without allylthiourea (80 μM), a selective inhibitor of bacterial nitrification [25]. Briefly, 15 g of soil were shaken at 180 rpm in 100 ml nutrient solution (pH 7.2, 1 mM PO_4^{3-}, 1.5 mM NH_4^+) at 20 °C in the dark. After 2, 5, 23 and 26 h of incubation, 15 ml of homogenized soil slurry was sampled, centrifuged, filtered (Whatmann 595 ½) and stored at −20 °C until analyses. Nitrate content of the extracts was analyzed colorimetrically with a continuous flow autoanalyzer (BranLuebbe, SPX Process Equipment, Germany). PNR were calculated by linear regression of NO_3^--N concentrations over time. We were thus able to determine total PNR and AOA-PNR (allylthiourea treated soil-slurry). The AOB-PNR were calculated as the difference between total PNR and AOA-PNR. PNR assesses the maximum nitrification rate of the nitrifier population present in a soil sample at the time of sampling. The short incubation time, high NH_4^+ concentrations and oxygenation assure the absence of nitrifier's population growth, denitrification and N immobilization during the incubation, so that net rates are equivalent to gross rates [45]. Further, the buffer solution is dilute enough so that the final pH of the soil slurry is close to the soil-water solution [45]. The high ammonium supply might influence the activity of some species (both AOA and AOB), but not lead to a selective advantage of one group, as recent studies revealed a

Table 1
Soil pH_{KCl}, potential nitrification rates (PNR), *amoA* gene copy numbers and *amoA*-AOA:*amoA*-AOB ratio under three land uses (mean ± standard deviation; n = 8). Different letters indicate significant differences between land uses (*P*-value < 0.05, nested ANOVA and Tukey tests). Values were calculated using the average values of the three samples taken in each plot.

	Soil pH_{KCl}	Total PNR (mg NO_3^--N kg^{-1} d^{-1})	AOB-PNR (mg NO_3^--N kg^{-1} d^{-1})	AOA-PNR (mg NO_3^--N kg^{-1} d^{-1})	AOB *amoA* (gene kg^{-1})	AOA *amoA* (gene kg^{-1})	*amoA*-AOA:*amoA*-AOB ratio
Potato	4.3 ± 0.3 a	0.29 ± 0.11 a	0.11 ± 0.07 a	0.18 ± 0.07 a	$3.7 \times 10^9 \pm 2.3 \times 10^9$ a	$5.9 \times 10^{10} \pm 3.0 \times 10^{10}$ a	19.94 ± 13.61 b
Fallow	4.3 ± 0.3 a	0.16 ± 0.07 b	0.03 ± 0.03 b	0.13 ± 0.05 a	$1.3 \times 10^9 \pm 7.0 \times 10^8$ a	$9.4 \times 10^{10} \pm 5.2 \times 10^{10}$ a	124.46 ± 148.88 ab
Eucalyptus	4.0 ± 0.3 a	0.03 ± 0.03 c	0.00 ± 0.01 b	0.04 ± 0.03 b	$4.9 \times 10^8 \pm 7.5 \times 10^8$ b	$4.3 \times 10^{10} \pm 4.9 \times 10^9$ a	140.71 ± 118.21 a

similar range of ammonia affinities between AOA and AOB from soils [2, 7].

2.3. *amoA* gene abundance

DNA was extracted from 0.25 g freeze-dried soil samples using the DNeasy PowerSoil Pro kit (QIAGEN) according the manufacturer's protocol on one randomly selected sample per plot. The DNA concentration and quality were measured using a Qubit™ fluorometer (Invitrogen). Archaeal and bacterial nitrifier abundances were determined by quantitative PCR targeting the *amoA* gene sequences of each domain. The gene primers amoA_1F and amoA_2R were used for bacteria [46], and the primers CrenamoA23f and CrenamoA616r for archaea [47]. A reaction mix containing 0.5 μM of each bacterial *amoA* primer or 0.75 μM of CrenamoA616r and 1 μM of CrenamoA23f primers, 2% bovine serum albumin (BSA), 1X of QuantiTect SybrGreen PCR Master Mix (Qiagen, Courtaboeuf, France) and 10 ng of soil DNA where run in duplicate on Lightcycler 480 (Roche Diagnostics, Meylan, France). The qPCR efficiency for the bacteria *amoA* was 88.8%, and for the archaea *amoA* was 79.2%. For Archaea, the highest qPCR efficiency and the best melting curve quality with the LightCycler (Roche Molecular Biochemicals) were obtained using different concentrations for forward and reverse primers. Such asymmetric amplification had been shown to improve the qPCR efficiency and melting curve quality using the LightCycler [48]. Run details are given in Simonin et al. [49]. We calculated the *amoA* gene abundance for AOA (AOA *amoA*) and AOB (AOB *amoA*), and the *amoA*-AOA:*amoA*-AOB ratio. PNR per unit *amoA* gene copies for AOA and AOB were calculated by dividing their PNR by their respective number of *amoA* copies.

2.4. Statistical analyses

Differences in soil pH_{KCl} and PNR between land uses were assessed by linear mixed effects models (data for three samples per plot) for AOA and AOB, followed by post hoc Tukey test for linear mixed models [50, 51]. The models included "plot" as random factor to account for the non-independence of the three soil samples taken in each plot [50].

Differences in *amoA* gene copy numbers, *amoA*-AOA: *amoA*-AOB, and PNR per unit *amoA* for AOB and AOA between land uses were tested with one-way ANOVA and post hoc Tukey tests (data from one sample per plot). The data of *amoA* gene copy numbers and *amoA*-AOA: *amoA*-AOB were log-transformed to meet normality and homoscedasticity assumptions.

We assessed the relationships of PNR with soil pH_{KCl}, AOA *amoA* and AOB *amoA* by fitting linear mixed effects models that allowed for random intercepts and slopes [52]. The marginal r-square $R^2_{LMM(m)}$ (variance explained by fixed effects of the model) were calculated according to Jaeger et al. [53], and separate models for each land use were used to calculate individual regression coefficients (R^2). To determine if slopes differed between land uses, we compared the models fitted with and without random slopes. A model with Akaike information criterion (AIC) lower by 2 units is considered to represent a better fit. The relationship of *amoA*-AOA:*amoA*-AOB with soil pH_{KCl} was assessed by fitting a simple non-linear model.

All statistical analyses were conducted with R software [54] using the packages car [55], nlme [56], nltoosl [57], r2glmm [58], and TukeyC [51].

3. Results

3.1. Effects of land use on soil pH and potential nitrification rates

Average soil pH_{KCl} was 4.3 for soils in potato and fallow plots, and 4.0 for soils under eucalyptus without significant differences between land uses (Table 1). Total PNR ranged from 0.00 to 0.51 mg NO_3^--N kg^{-1} d^{-1} with significantly higher rates under potato, and lower rates under

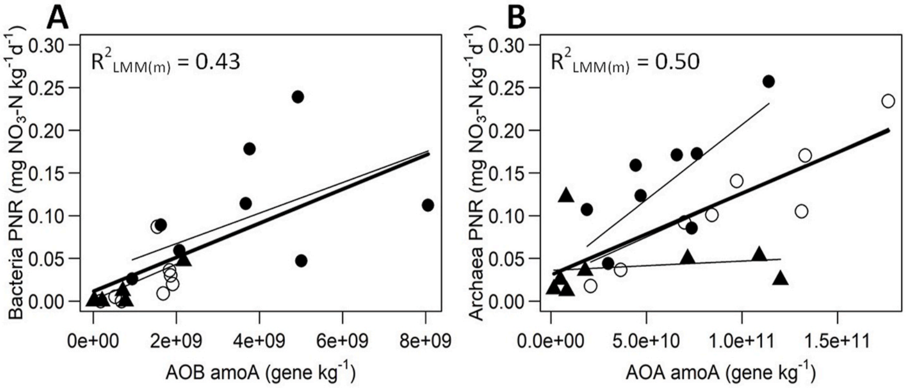

Fig. 1. Relationships of AOB and AOA *amoA* gene copy numbers with their PNR (A and B, respectively). The thick lines were fitted for all data points (n = 24) and thin lines were fitted for each land use separately (n = 8), points represent soils under potato (●), fallow (○) and eucalyptus (▲) land use. In A the fitted lines of potato and fallow overlap and in B the fitted line for fallow overlaps with the general regression line.

eucalyptus plantations. The AOB-PNR ranged from 0.00 to 0.24 mg NO_3^--N kg^{-1} d^{-1} with significantly higher values in potato crops compared to eucalyptus and fallow, whereas AOA-PNR ranged from 0.01 to 0.35 mg NO_3^--N kg^{-1} d^{-1} with significantly higher rates in potato crops and in fallow fields. Archaeal contribution to total nitrification rates ranged from 39 to 100% and, on average, AOA accounted for 64, 82 and 96% of total nitrification in potato, fallow and eucalyptus soils, respectively (Fig. A1 of Appendix A).

3.2. *Abundance of AOA and AOB amoA gene copies under the three land uses*

The AOB *amoA* abundance ranged from 1.1×10^7 to 8.1×10^9 gene copies per kg of dry soil with significantly lower values under eucalyptus plantations compared to potato and fallow soils (Table 1). AOA *amoA* abundance ranged from 1.5×10^9 to 1.8×10^{11} gene copies per kg of dry soil without significant differences between land uses. The proportion of AOA *amoA* to the total *amoA* gene copies ranged between 86 and 100%, and on average accounted for 93% of total *amoA* copies under potato, and for 98% under fallow and eucalyptus land uses (Fig. A1 of Appendix). Except for one extreme value under eucalyptus (6777), *amoA*-AOA: *amoA*-AOB ratios ranged from 6 to 468. The mean *amoA*-AOA:*amoA*-AOB ratios were significantly higher under eucalyptus plantations, compared to potato land use (Table 1).

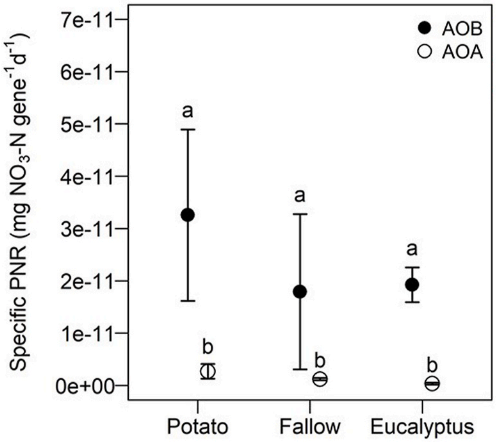

Fig. 2. Specific potential nitrification rates per land use. AOA and AOB specific potential nitrification rates (potential nitrification per unit of *amoA* gene copies) were no significantly different between land uses (P-value > 0.05, ANOVA and Tukey tests). Letters indicate significant differences between AOA and AOB for each land use (P-value < 0.05, Tukey tests).

3.3. *Relationships between nitrification potential, amoA gene abundance and pH*

Total PNR showed a positive relationship with both AOB *amoA* ($R^2_{LMM(m)} = 0.39$) and AOA *amoA* ($R^2_{LMM(m)} = 0.61$) gene abundances at landscape level (Fig. A2 of Appendix). At land use level total PNR was positively correlated with AOA *amoA* under potato and fallow (R^2 = 0.64 and 0.69, respectively), and with AOB *amoA* under eucalyptus (R^2 = 0.94, Table A1 of Appendix). AOB-PNR and AOA-PNR had positive relationships with their respective *amoA* gene copy numbers, with marginal r-squares $R^2_{LMM(m)}$ of 0.50 for AOA, and 0.43 for AOB (Fig. 1). For AOA, the random slope model performed better than the fixed slope model ($\Delta_{AIC} = 3.7$, P-value = 0.02), but not for AOB ($\Delta_{AIC} = 4$, P-value = 0.98). The individual relationships between AOA-PNR and AOA *amoA* within each land use were positive, with regression coefficients of 0.50, 0.86, 0.23 for potato, fallow and eucalyptus soils, respectively (Table A2 of Appendix A). The relationships of AOB-PNR and AOB *amoA* in potato soils was not significant ($R^2 = 0.01$), it was weak in fallow soils (R^2 = 0.14) and strong under eucalyptus plantations ($R^2 = 0.86$, Table A2 of Appendix).

Specific PNR (AOA and AOB PNR per unit of their respective *amoA* gene copies) indicated no significant differences between land uses (Fig. 2), and significantly higher specific PNR for AOB (*t*-test, data not shown). On average, the specific PNR of AOB was 12, 14 and 62 times higher than the specific PNR of AOA under potato, fallow and eucalyptus soils, respectively.

Total PNR and AOB-PNR showed weak positive relationships with soil pH_{KCl} ($R^2_{LMM(m)} = 0.11$ and 0.21, respectively) with statistical significant slopes (P-value < 0.01), while there was no significant relationship (P-value of slope = 0.54) between AOA-PNR and PH_{KCl} ($R^2_{LMM(m)} = 0.01$). Regression coefficients of total PNR and soil pH_{KCl} were 0.14, 0.10 and 0.00 for potato, fallow and eucalyptus soils, and the regression coefficients of AOB-PNR with soil pH_{KCl} were 0.37, 0.16, 0.00 under potato, fallow and eucalyptus soils, respectively (Fig. 3, Table A3 of Appendix). The *amoA*-AOA:*amoA*-AOB ratio and soil pH_{KCl} showed a negative power-convex relationship (Fig. 4).

4. Discussion

4.1. *AOB and AOA activity and abundance in response to land use*

This study presents the first report addressing the consequences of land use on the activity and abundance of ammonia-oxidizer bacteria and archaea in agricultural ecosystems in the Central Andean region. Total PNR under potato and fallow were relatively low (0.00–0.51 mg NO_3^--N kg^{-1} d^{-1}, Table 1) compared to potential nitrification measured in fertilized agricultural soils (2.2–105 mg NO_3^--N kg^{-1} d^{-1}, [59,60]),

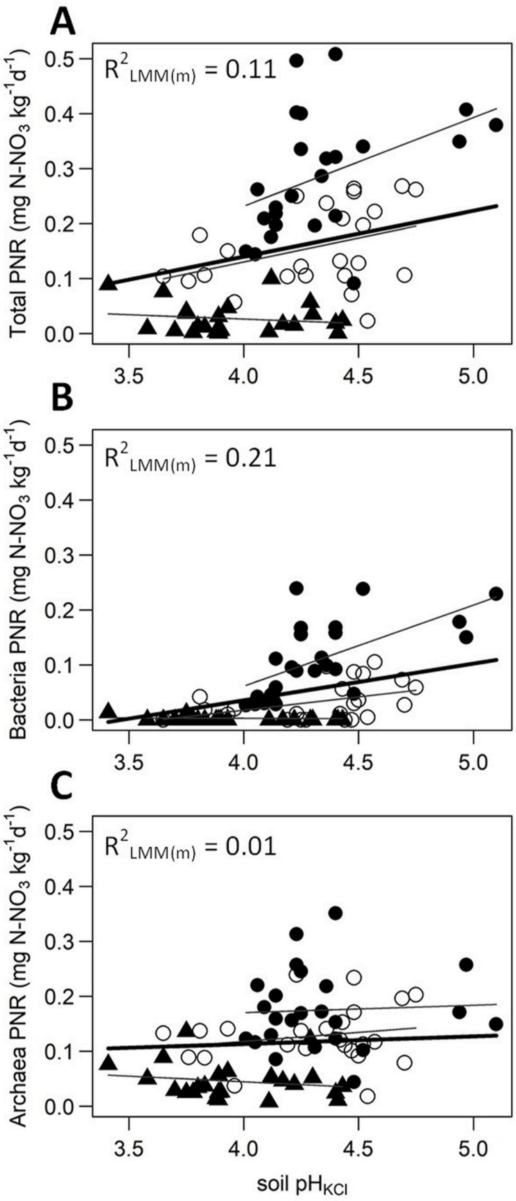

Fig. 3. Relationship of potential nitrification rates (PNR) and soil pH$_{KCl}$. Relationship of total (A), bacteria (B) and archaea (C) PNR with soil pH$_{KCl}$. The thick lines were fitted for all data points (n = 72, three samples per plot) and thin lines were fitted for each land use (n = 24), points represent soils under potato (●), fallow (○) and eucalyptus (▲) land uses.

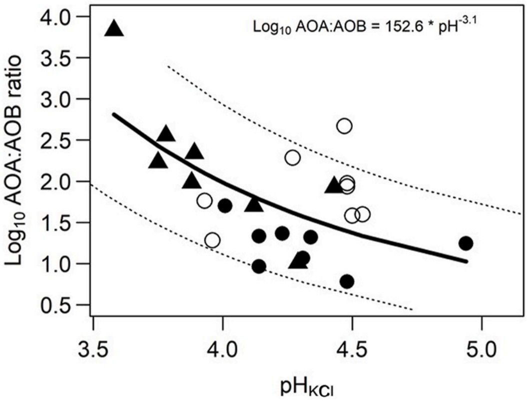

Fig. 4. Archaea-to-bacteria *amoA* ratio and soil pH$_{KCl}$. Relationship between the *amoA*-AOA: *amoA*-AOB ratio and soil pH$_{KCl}$ for potato soils (●), fallow soils (○) and eucalyptus plantations (▲). The thick line represents the fitted curve and the dashed lines are confidence intervals (n = 24).

grassland soils (1.1–3.4 mg NO$_3^-$-N kg^{-1} d^{-1}, [61]), but total PNR under eucalyptus (Table 1) were similar to previous reports for eucalyptus plantations (0.01–0.03 mg NO$_3^-$-N kg^{-1} d^{-1}, [20]). Nitrification was largely attributed to AOA, responsible for 64%–96% of total potential nitrification (Fig. A1 of Appendix). Under eucalyptus AOA accounted for 96% of total PNR but both rates for AOB and AOA were low compared to the other land uses (Table 1), which can be explained by the inhibitory effect of organic compounds (i.e. terpenes and caffeic acid) produced by this tree on bacterial and archaeal nitrification [62,63]. Potential nitrification under potato was dominated by AOA but the relative contribution of AOB was 36% of total PNR despite they accounted for only 7% of total *amoA* copies (Fig. A1 of Appendix). The higher relative contribution of AOB to potential nitrification could be explained by the competitive advantage of AOB under higher ammonium concentrations due to inorganic N fertilization [64,65]. Also, chicken manure additions may have contributed to increased AOB activity by promoting the formation of soil aggregates with favorable micro environmental conditions (neutral pH and high ammonia concentration [66,67]). Moreover, the significant increase in AOB activity could be related to the higher ammonia oxidation rate per AOB cell (4–23 fmol NO$_2^-$ cell^{-1} h^{-1}, [68]) compared to AOA (0.07–2.5 fmol NO$_2^-$ cell^{-1} h^{-1} [69]), which is reflected in the higher specific PNR (PNR per *amoA* gene copies) of AOB. Although *amoA* gene copy numbers do not strictly reflect cell numbers, as AOB and AOA vary in the number of *amoA* copies in their genetic material [70–73], the specific PNR indicate that AOB contribution to nitrification per unit of *amoA* copy is 12, 14 and 62 times higher than AOA under potato, fallow and eucalyptus, respectively (Fig. 2). Upon conversion to fallow, AOB-PNR decreased and AOA contribution to total nitrification increased to 82% (Fig. A1 of Appendix). This could be due to the absence of N fertilization and the dependency on N supplied by mineralization of organic matter entering the soil from colonizing vegetation.

Results revealed that AOA were also dominant in terms of gene abundance with the AOA *amoA* copy numbers one to two orders of magnitude higher than AOB *amoA* (Table 1). The *amoA*-AOA:*amoA*-AOB ratios were thus situated between 20 and 141 (Table 1), and the differences between land uses resulted from changes of AOB *amoA* abundance, as the AOA *amoA* remained unchanged (Table 1). Despite soil pH did not differ between land uses, a wider pH range was observed under potato, and changes in the dominance ratio are likely driven by soil pH because minor variations have strong effects on AOB abundance [74]. Increasing acidic conditions would thus lead to stronger AOA dominance as observed under eucalyptus, which had the highest *amoA*-AOA: *amoA*-AOB ratios and reported one extreme value (6777) that corresponded to the soil sample with the lowest pH (3.5). Yao et al. [21] reported a similar relationship between *amoA*-AOA:*amoA*-AOB and soil pH, in accordance with studies indicating that pH is the major factor determining AOB and AOA niche differentiation [34,35]. Soil pH showed a significant positive relationship with total PNR (R^2 = 0.14, Appendix Table A3) and with AOB-PNR under potato and fallow (R^2 = 0.37 and R^2 = 0.16 respectively, Appendix Table A3). These results suggest that under higher pH, nitrification rates would be mostly controlled by AOB, likely due to increased ammonia availability. This is in accordance with previous studies indicating reduced AOB activity with increasing soil acidity [65,75]. In contrast, under low pH, AOA would be most important, but the control of nitrification rates would also depend on other factors than pH or AOA gene copy number, as illustrated by the absence of correlation between pH$_{KCl}$ and AOA-PNR (Fig. 3C, Table A3 of Appendix), and varying slopes between AOA *amoA* and AOA-PNR under the three land uses (Fig. 1B). Further, ammonia-oxidizer community composition and diversity [18] could be important in controlling AOA activity within the acidic range of the studied soils.

4.2. *Relationship of potential nitrification rates with amoA abundance*

Since the discovery of AOA, numerous studies have documented their dominance in different environments through the measurement of their *amoA* gene copy numbers [11–13,16–18], and particularly, in agricultural systems where AOA and AOB activities determine the N fertilization efficiency and potential N loss through nitrate leaching [76, 77]. These studies have raised the question whether this dominance reflects their functional importance in nitrification, but few have included nitrification rate measurements. Studies assessing *amoA* gene abundance and total PNR usually have associated the presence of correlations as indicative of the functional importance of AOB or AOA in the environment [18–21]. However, our results show that these relationships do not reflect the real contribution of AOA and AOB ammonia oxidizers to nitrification, as well as previous studies reporting negative correlations [19]. Indeed, in our study, total PNR was strongly positively correlated with AOB *amoA*, despite that rate measurements showed that nitrification was mainly due to AOA (Table 1, Fig. A1 of Appendix). AOB and AOA relevance to nitrification process rates should thus be determined by separately measuring their nitrification rates and not be inferred from relationships of *amoA* with total PNR.

Increases in the AOB *amoA* and AOA *amoA* abundances were associated with their respective PNR (Fig. 1), and consequently with ammonia-oxidizers community size [78] but the relationships differed for AOB and AOA. The AOB *amoA* and AOB-PNR relationship was strong at landscape level, but relationships were weak within land uses and had similar low slopes (Table A2 of Appendix), likely due to the low nitrification rates and narrow *amoA* abundance range. These results suggest that soil management practices known to increase the AOB *amoA* abundance (e.g. fertilization, liming) could be expected to increase AOB nitrification [74,79]. The AOA *amoA* and AOA-PNR relationships differed between land uses, with strong positive relationships and greater slopes under potato and fallow soils, but no significant relation under eucalyptus plantations (Table A2 of Appendix). Changes in the environmental factors under eucalyptus may contribute to explain lower AOA activity than expected by the AOA *amoA* abundance alone. For example, the presence of AOA nitrification inhibitors produced by this tree [62] may have contributed to the low AOA-PNR despite the presence of AOA. Also, changes in the organic carbon content, which have been reported to affect AOA activity by increasing competition for available ammonia and oxygen with heterotrophs [33,34,80] could explain low PNR under eucalyptus. Consequently, the relationships between AOA abundance and activity at low pH is land use-dependent, and changes in AOA activity cannot be inferred from changes in the AOA *amoA* abundance alone.

5. Conclusions

We demonstrated that AOA dominated in terms of abundance and activity under the three land uses studied. Eucalyptus led to decreased AOB abundance, and AOA and AOB nitrification rates, while under potato nitrification rates were highest, and the relative contribution of AOB increased. The AOA-to-AOB *amoA* ratio decreased with pH, and was driven by the increase of AOB *amoA* with decreasing soil acidity. The relation of *amoA* gene copy numbers and nitrification rates depended on land use, indicating that gene abundance measurements alone are not sufficient for inferring AOB and AOA activity. Therefore, rate measurements of AOB and AOA are essential to determine the environmental consequences of land use management. Our results indicate that in these agricultural soils, the role of AOB might become predominant in controlling nitrification rates with increasing pH and N inputs, while under low pH AOA would be most important with process rates controlled by other factors than pH and gene abundances.

Author contributions

Alejandro Coca-Salazar and Monique Carnol designed the study and wrote the manuscript. Material preparation, data collection and analysis were performed by Alejandro Coca-Salazar. Agnes Richaume and Alessandro Florio performed qPCR analyses. All authors commented, read and approved the final manuscript.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2020.103263.

References

[1] L.Y. Stein, Insights into the physiology of ammonia-oxidizing microorganisms, Curr. Opin. Chem. Biol. 49 (2019) 9–15, https://doi.org/10.1016/j.cbpa.2018.09.003.

[2] D.K. Kits, C.J. Sedlacek, E.V. Lebedeva, P. Han, A. Bulaev, P. Pjevac, A. Daebeler, S. Romano, M. Albertsen, L.Y. Stein, H. Daims, M. Wagner, Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle, Nature 549 (2017) 269–272, https://doi.org/10.1038/nature23679.

[3] W. Martens-Habbena, P.M. Berube, H. Urakawa, J.R. De la Torre, D.A. Stahl, Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria, Nat. Lett. 461 (2009) 976–979, https://doi.org/10.1038/nature08465.

[4] B. Park, S. Park, D. Yoon, S. Schouten, J.S. Sinninghe, S.K. Rhee, Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria, Appl. Environ. Microbiol. 76 (2010) 7575–7587, https://doi.org/10.1128/AEM.01478-10.

[5] M. Jung, S. Park, D. Min, J. Kim, W.I.C. Rijpstra, G. Kim, E.L. Madsen, S.K. Rhee, Enrichment and characterization of an autotrophic ammonia-oxidizing archaeon of mesophilic crenarchaeal group I.1a from an agricultural soil, Appl. Environ. Microbiol. 77 (2011) 8635–8647, https://doi.org/10.1128/AEM.05787-11.

[6] A. Bollmann, M.-J. Bär-Gilissen, H.J. Laanbroek, Growth at low ammonium concentrations and starvation response as potential factors involved in niche differentiation among ammonia-oxidizing bacteria, Appl. Environ. Microbiol. 68 (2002) 4751–4757, https://doi.org/10.1128/AEM.68.10.4751.

[7] Y. Zhang, W. Qin, L. Hou, E.J. Zakem, X. Wan, Z. Zhao, L. Liu, K.A. Hunt, N. Jiao, S. J. Kao, K. Tang, X. Xie, J. Shen, Y. Li, M. Chen, X. Dai, C. Liu, W. Deng, M. Dai, A. E. Ingalls, D.A. Stahl, G.J. Herndl, Nitrifier adaptation to low energy flux controls inventory of reduced nitrogen in the dark ocean, Proc. Natl. Acad. Sci. U. S. A 117 (2020) 4823–4830, https://doi.org/10.1073/pnas.1912367117.

[8] I. Suzuki, U. Dular, S.C. Kwok, Ammonia or ammonium ion as substrate for oxidation by *Nitrosomonas europaea* cells and extracts, J. Bacteriol. 120 (1974) 556–558.

[9] R. Gonzales-Cabaleiro, T.P. Curtis, D.I. Ofiteru, Bioenergetics analysis of ammonia-oxidizing bacteria and the estimation of their maximum growth yield, Water Res. 154 (2019) 238–245, https://doi.org/10.1016/j.watres.2019.01.054.

[10] B. Wang, W. Qin, Y. Ren, X. Zhou, M.Y. Jung, P. Han, E.A. Eloe-Fadrosh, M. Li, Y. Zheng, L. Lu, X. Yan, J. Ji, Y. Liu, L. Liu, C. Heiner, R. Hall, W. Martens-Habbena, C.W. Herbold, S. keun Rhee, D.H. Bartlett, L. Huang, A.E. Ingalls, M. Wagner, D. A. Stahl, Z. Jia, Expansion of *Thaumarchaeota* habitat range is correlated with horizontal transfer of ATPase operons, ISME J. 13 (2019) 3067–3079, https://doi.org/10.1038/s41396-019-0493-x.

[11] D.R. Clark, B.A. Mckew, L.F. Dong, G. Leung, A.J. Dumbrell, A. Stott, H. Grant, D. B. Nedwell, M. Trimmer, C. Whitby, Mineralization and nitrification: archaea dominate ammonia-oxidising communities in grassland soils, Soil Biol. Biochem. 143 (2020) 107725, https://doi.org/10.1016/j.soilbio.2020.107725.

[12] A. Sterngren, S. Hallin, P. Bengtson, Archaeal ammonia oxidizers dominate in numbers, but bacteria drive gross nitrification in N-amended grassland soil, Front. Microbiol. 6 (2015) 1–8, https://doi.org/10.3389/fmicb.2015.01350.

[13] X. Li, Y. Xiao, W. Ren, Z. Liu, J. Shi, Z. Quan, Abundance and composition of ammonia-oxidizing bacteria and archaea in different types of soil in the Yangtze River estuary, Biomed. Biotechnol. 13 (2012) 769–782, https://doi.org/10.1631/jzus.B1200013.

[14] Ji-zheng He, Ju-pei Shen, Li-mei Zhang, Yong-guan Zhu, Yuan-ming Zheng, Ming-gang Xu, Hongjie Di, Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices, Environ. Microbiol. 9 (2007) 2364–2364–2374, https://doi.org/10.1111/j.1462-2920.2007.01358.x.

[15] J.Z. He, H.W. Hu, L.M. Zhang, Current insights into the autotrophic thaumarchaeal ammonia oxidation in acidic soils, Soil Biol. Biochem. 55 (2012) 146–154, https://doi.org/10.1016/j.soilbio.2012.06.006.

[16] X. Lu, P.J. Bottomley, D.D. Myrold, Contributions of ammonia-oxidizing archaea and bacteria to nitrification in Oregon forest soils, Soil Biol. Biochem. 85 (2015) 54–62, https://doi.org/10.1016/j.soilbio.2015.02.034.

[17] S. Leininger, T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I. Prosser, S. C. Schuster, C. Schleper, Archaea predominate among ammonia-oxidizing prokaryotes in soils, Nature 442 (2006) 806–809, https://doi.org/10.1038/nature04983.

[18] H. Qin, H. Yuan, H. Zhang, Ammonia-oxidizing archaea are more important than ammonia-oxidizing bacteria in nitrification and NO3-N loss in acidic soil of sloped land, Biol. Fertil. Soils 49 (2013) 767–776, https://doi.org/10.1007/s00374-012-0767-1.

[19] S. Gao, D. Chang, C. Zou, W. Cao, J. Gao, J. Huang, J. Bai, N. Zeng, R.M. Rees, K. Thorup-Kristensen, Archaea are the predominant and responsive ammonia oxidizing prokaryotes in a red paddy soil receiving green manures, Eur. J. Soil Biol. 88 (2018) 27–35, https://doi.org/10.1016/j.ejsobi.2018.05.008.

[20] F. Zhang, W. Pan, J. Gu, B. Xu, W. Zhang, B.-Z. Zhu, Y.-X. Wang, Y.-F. Wang, Dominance of ammonia-oxidizing archaea community induced by land use change from Masson pine to eucalyptus plantation in subtropical China, Appl. Microbiol. Biotechnol. (2016), https://doi.org/10.1007/s00253-016-7506-8.

[21] H. Yao, Y. Gao, G.W. Nicol, C.D. Campbell, J.I. Prosser, L. Zhang, W. Han, B. K. Singh, Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils, Appl. Environ. Microbiol. 77 (2011) 4618–4625, https://doi.org/10.1128/AEM.00136-11.

[22] Q.Q. Jiang, L.R. Bakken, Comparison of *Nitrosospira* strains isolated from terrestrial environments, FEMS Microbiol. Ecol. 30 (1999) 171–186.

[23] M. Carnol, G.A. Kowalchuk, W. De Boer, Nitrosomonas europaea-like bacteria detected as the dominant β-subclass Proteobacteria ammonia oxidisers in reference and limed acid forest soils, Soil Biol. Biochem. 34 (2002) 1047–1050.

[24] M. Hayatsu, K. Tago, I. Uchiyama, A. Toyoda, Y. Wang, Y. Shimomura, T. Okubo, F. Kurisu, Y. Hirono, K. Nonaka, H. Akiyama, T. Itoh, H. Takami, An acid-tolerant ammonia-oxidizing γ-proteobacterium from soil, ISME J. 11 (2017) 1130–1141, https://doi.org/10.1038/ismej.2016.191.

[25] A.E. Taylor, L.H. Zeglin, S. Dooley, D.D. Myrold, P.J. Bottomley, Evidence for different contributions of archaea and bacteria to the ammonia-oxidizing potential of diverse Oregon soils, Appl. Environ. Microbiol. 76 (2010) 7691–7698, https://doi.org/10.1128/AEM.01324-10.

[26] P. Ginestet, J. Audic, V. Urbain, J. Block, Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors Allylthiourea and Azide, Appl. Environ. Microbiol. 64 (1998) 2266–2268.

[27] W. Martens-Habbena, W. Qin, R.E.A. Horak, H. Urakawa, A.J. Schauer, J. W. Moffett, E.V. Armbrust, A.E. Ingalls, A.H. Devol, D.A. Stahl, The production of nitric oxide by marine ammonia-oxidizing archaea and inhibition of archaeal ammonia oxidation by a nitric oxide scavenger, Environ. Microbiol. 17 (2015) 2261–2274, https://doi.org/10.1111/1462-2920.12677.

[28] G. Munz, G. Mori, C. Vannini, C. Lubello, Kinetic parameters and inhibition response of ammonia- and nitrite-oxidizing bacteria in membrane bioreactors and conventional activated sludge processes, Environ. Technol. 31 (2010) 1557–1564, https://doi.org/10.1080/09593331003793828.

[29] J. Adamczyk, M. Hesselsoe, N. Iversen, M. Horn, A. Lehner, P.H. Nielsen, M. Schloter, P. Roslev, M. Wagner, The isotope array, a new tool that employs substrate-mediated labeling of rRNA for determination of microbial community structure and function, Appl. Environ. Microbiol. 69 (2003) 6875–6887, https://doi.org/10.1128/AEM.69.11.6875.

[30] X. Zhou, J.A. Oleszkiewicz, Biodegradation of oestrogens in nitrifying activated sludge, Environ. Toxicol. 31 (2010) 1263–1269, https://doi.org/10.1080/09593331003674549.

[31] M. Rattier, J. Reungoat, J. Keller, W. Gernjak, Removal of micropollutants during tertiary wastewater treatment by biofiltration: role of nitrifiers and removal mechanisms, Water Res. 54 (2014) 89–99, https://doi.org/10.1016/j.watres.2014.01.030.

[32] J. Zhao, Y. Meng, J. Drewer, U.M. Skiba, J.I. Prosser, C. Gubry-Rangin, Differential ecosystem function stability of ammonia-oxidizing archaea and bacteria following short-term environmental perturbation, Appl. Environ. Sci. 5 (2020) 1–14.

[33] E. Wessén, K. Nyberg, J.K. Jansson, S. Hallin, Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management, Appl. Soil Ecol. 45 (2010) 193–200, https://doi.org/10.1016/j.apsoil.2010.04.003.

[34] R. Sun, D.D. Myrold, D. Wang, X. Guo, H. Chu, AOA and AOB communities respond differently to changes of soil pH under long-term fertilization, Soil Ecol. Lett. 1 (2019) 126–135.

[35] G.W. Nicol, S. Leininger, C. Schleper, J.I. Prosser, The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria, Environ. Microbiol. 10 (2008) 2966–2978, https://doi.org/10.1111/j.1462-2920.2008.01701.x.

[36] G.V. Subbarao, K. Sahrawat, K. Nakahara, T. Ishikawa, M. Kishii, I. Rao, C. Hash, T. George, P. Rao, P. Nardi, D. Bonnett, W. Berry, K. Suenaga, J. Lata, Biological nitrification inhibition - a novel strategy to regulate nitrification in agricultural systems. Advances in Agronomy 114, Elsevier, 2012, pp. 249–302.

[37] M.J. Kampschreur, R.L. van der Star, Wouter, H.A. Wielders, J.W. Mulder, M.S. M. Jetten, M.C.M. van Loosdrecht, Dynamics of nitric oxide and nitrous oxide emission during full-scale reject water treatment, Appl. Environ. Microbiol. 42 (2010) 812–826, https://doi.org/10.1016/j.watres.2007.08.022.

[38] N.S. Bolan, M.J. Hedley, R.E. White, Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures, Plant Soil 134 (1991) 53–63, https://doi.org/10.1007/BF00010717.

[39] B. Condori, A. Devaux, P. Mamani, Efecto residual de la fertilización del cultivo de papa sobre el cultivo de haba (*Vicia faba* L.) en el sistema de rotación, Rev. Latinoam. La Papa. 9 (1997) 171–187.

[40] G. Navarro, M. Maldonado, Geografía Ecológica de Bolivia - Vegetación y Ambientes Acuáticos, Patiño, Simón I., Cochabamba, Bolivia, 2002.

[41] H. Pestalozzi, Sectoral fallow systems and management of soil fertility: the rationality of indigenous knowledge in the High Andes of Bolivia, Mt. Res. Dev. 20 (2000) 64–71, https://doi.org/10.1659/0276-4741(2000)020[0064:SFSATM]2.0.CO;2.

[42] SENAMHI, Servicio Nacional de Meteorología e Hidrología - Estado Plurinacional de Bolivia. http://www.senamhi.gob.bo/web/public/, 2016. (Accessed 20 July 2016).

[43] Ministerio de Medio Ambiente y Agua, Atlas Cuenca del Rio Grande, La Paz, Bolivia, 2014.

[44] S.E. Allen, Chemical Analysis of Ecological Materials, second ed., Blackwell Scientific Publications, Oxford, UK, 1989.

[45] S.C. Hart, J.M. Stark, E.A. Davidson, K.M. Firestone, Nitrogen mineralization, immobilization, and nitrification, in: A. Page, R. Miller, D. Keeney (Eds.), Methods Soil Anal. Part 2. Microbiol. Biochem. Prop., Soil Science Society of America, Madison, Wisconsin, 1994, pp. 985–1081.

[46] J. Rotthauwe, K. Witzel, W. Liesack, The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations, Appl. Environ. Microbiol. 63 (1997) 4704–4712.

[47] M. Tourna, T.E. Freitag, G.W. Nicol, J.I. Prosser, Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms, Environ. Microbiol. 10 (2008) 1357–1364, https://doi.org/10.1111/j.1462-2920.2007.01563.x.

[48] K. Barratt, J.F. Mackay, Improving real-time PCR genotyping assays by asymmetric amplification, J. Clin. Microbiol. 40 (2002) 1571–1572, https://doi.org/10.1128/JCM.40.4.1571-1572.2002.

[49] M. Simonin, A. Richaume, J.P. Guyonnet, A. Dubost, J.M.F. Martins, T. Pommier, Titanium dioxide nanoparticles strongly impact soil microbial function by affecting archaeal nitrifiers, Sci. Rep. (2016) 1–10, https://doi.org/10.1038/srep33643.

[50] S.S. Mangiafico, An R Companion for the Handbook of Biological Statistics, Rutgers Cooperative Extension, New Brunswick, NJ, 2015, 1.3.2.

[51] J.C. Faria, E.G. Jelihovschi, I.B. Allaman, Conventional Tukey test. https://github.com/jcfaria/TukeyC, 2018, 28.

[52] A.F. Zuur, E.N. Ieno, N.J. Walker, A.A. Saveliev, G.M. Smith, Mixed Effects Models and Extensions in Ecology with R, Springer Science+Business Media, New York, 2009.

[53] B.C. Jaeger, L.J. Edwards, K. Das, P.K. Sen, An R statistic for fixed effects in the generalized linear mixed model, J. Appl. Stat. 44 (2016) 1086–1105, https://doi.org/10.1080/02664763.2016.1193725.

[54] R. R Core Team, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2018.

[55] J. Fox, S. Weisger, An {R} Companion to Applied Regression, second ed., SAGE Publications, Inc., Thousand Oaks California, 2011. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

[56] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, S. Heisterkamp, B. Van Willigen, Nlme: Linear and Nonlinear Mixed Effects Models, 2018, p. 321.

[57] F. Baty, C. Ritz, S. Charles, M. Brutsche, A toolbox for nonlinear regression in R : the package nlstools, J. Stat. Software 66 (2015).

[58] B.C. Jaeger, Computes R squared for mixed (multilevel) model, Packag. 'R2glmm.' 12 (2017), https://doi.org/10.1002/sim.3429>is.

[59] H. Chu, T. Fujii, S. Morimoto, X. Lin, K. Yagi, J. Hu, J. Zhang, Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil, Appl. Environ. Microbiol. 73 (2007) 485–491, https://doi.org/10.1128/AEM.01536-06.

[60] Y. Zhang, G. Ji, Quantitative responses of potential nitrification and denitrification rates to the size of microbial communities in rice paddy soils, Chemosphere 211 (2018) 970–977, https://doi.org/10.1016/j.chemosphere.2018.08.047.

[61] S.D. Veresoglou, R. Sen, A.P. Mamolos, D.S. Veresoglou, Plant species identity and arbuscular mycorrhizal status modulate potential nitrification rates in nitrogen-limited grassland soils, J. Ecol. 99 (2011) 1339–1349, https://doi.org/10.1111/j.1365-2745.2011.01863.x.

[62] L.A. Sauder, A.A. Ross, J.D. Neufeld, Nitric oxide scavengers differentially inhibit ammonia oxidation in ammonia-oxidizing archaea and bacteria, FEMS Microbiol. Lett. (2016) 1–8, https://doi.org/10.1093/femsle/fnw052.

[63] C.S. White, Volatile and water-soluble inhibitors of nitrogen mineralization and nitrification in a ponderosa pine ecosystem, Biol. Fertil. Soils 2 (1986) 97–104.

[64] H. Chu, T. Fujii, S. Morimoto, X. Lin, K. Yagi, Population size and specific nitrification potential of soil ammonia-oxidizing bacteria under long-term fertilizer management, Soil Biol. Biochem. 40 (2008) 1960–1963, https://doi.org/10.1016/j.soilbio.2008.01.006.

[65] E. French, J.A. Kozlowski, M. Mukherjee, G. Bullerjahn, A. Bollmann, Ecophysiological characterization of ammonia-oxidizing archaea and bacteria from freshwater, Appl. Environ. Microbiol. 78 (2012) 5773–5780, https://doi.org/10.1128/AEM.00432-12.

[66] S.A.Q. Burton, J. im I. Prosser, Autotrophic ammonia oxidation at low pH through urea hydrolysis, Appl. Environ. Microbiol. 67 (2001) 2952–2957, https://doi.org/10.1128/AEM.67.7.2952.

[67] W. de Boer, P.J.A.K. Gunnewiek, M. Veenhuis, E. Bock, H.J. Laanbroek, Nitrification at low pH by aggregated chemolithotrophic bacteria, Appl, Environ. Microbiol. 57 (1991) 3600–3604.

[68] J.I. Prosser, G.W. Nicol, Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation, Trends Microbiol. 20 (2012) 523–531, https://doi.org/10.1016/j.tim.2012.08.001.

[69] L.E. Lehtovirta-Morley, J. Ross, L. Hink, E.B. Weber, C. Gubry-Rangin, C. Thion, J. I. Prosser, G.W. Nicol, Isolation of "*Candidatus* Nitrosocosmicus franklandus", a novel ureolytic soil archaeal ammonia oxidiser with tolerance to high ammonia concentration, FEMS Microbiol. Ecol. 92 (2016) 1–10, https://doi.org/10.1093/femsec/fiw057.

[70] M.G. Klotz, J.M. Norton, Sequence of an ammonia monooxygenase subunit A-encoding gene from *Nitrosospira* sp. NpAV, Gene 163 (1995) 159–160.

[71] J.M. Norton, J.M. Low, M.G. Klotz, The gene encoding ammonia monooxygenase subunit A exists in three nearly identical copies in *Nitrosospira* sp. NpAV, FEMS Microbiol. Lett. 139 (1996) 181–188.

[72] S. Wang, Y. Chen, Phylogenomic analysis demonstrates a pattern of rare and long-lasting concerted evolution in prokaryotes, Commun. Biol. (2018) 1–12, https://doi.org/10.1038/s42003-018-0014-x.

[73] E. Pedneault, P.E. Galand, M. Polvin, J.-E. Tremblay, C. Lovejoy, Archaeal amoA and ureC genes and their transcriptional activity in the Arctic Ocean, Sci. Rep. 4 (2014) 1–10, https://doi.org/10.1038/srep04661.

[74] J. Shen, L.M. Zhang, Y.G. Zhu, J.B. Zhang, J.Z. He, Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam, Environ. Microbiol. 10 (2008) 1601–1611, https://doi.org/10.1111/j.1462-2920.2008.01578.x.

[75] L. Zhang, H. Hu, J. Shen, J. He, Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils, ISME J. 6 (2012) 1032–1045, https://doi.org/10.1038/ismej.2011.168.

[76] G.V. Subbarao, M. Kishii, K. Nakahara, T. Ishikawa, T. Ban, H. Tsujimoto, T. S. George, W.L. Berry, C.T. Hash, O. Ito, Biological nitrification inhibition (BNI)—is there potential for genetic interventions in the Triticeae? Breed Sci. 59 (2009) 529–545, https://doi.org/10.1270/jsbbs.59.529.

[77] J. Norton, Y. Ouyang, Controls and adaptive management of nitrification in agricultural soils, Front. Microbiol. 10 (2019) 1–18, https://doi.org/10.3389/fmicb.2019.01931.

[78] M. Rahalkar, J. Deutzmann, B. Schink, I. Bussmann, Abundance and activity of methanotrophic bacteria in littoral and profundal sediments of lake Constance (Germany), Appl. Environ. Microbiol. 75 (2009) 119–126, https://doi.org/10.1128/AEM.01350-08.

[79] W.J. Bossolani, C.A. Costa, Cruciol, L.F. Merloti, L.G. Moretti, N.R. Costa, S.M. Tsai, E.E. Kuramae, Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system, Geoderma 375 (2020) 114476, https://doi.org/10.1016/j.geoderma.2020.114476.

[80] H. Liu, J. Li, Y. Zhao, K. Xie, X. Tang, S. Wang, Z. Li, Y. Liao, J. Xu, H. Di, Y. Li, Ammonia oxidizers and nitrite-oxidizing bacteria respond differently to long-term manure application in four paddy soils of south of China, Sci. Total Environ. 633 (2018) 641–648, https://doi.org/10.1016/j.scitotenv.2018.03.108.