RESEARCH ARTICLE

Vascular plants mediate the effects of aridity and soil properties on ammonia-oxidizing bacteria and archaea

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Abstract

An integrated perspective of the most important factors driving the abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in natural ecosystems is lacking, especially in drylands. We evaluated how different climatic, abiotic, and nutrient-related factors determine AOA and AOB abundance in bare and vegetated microsites from grasslands throughout the Mediterranean Basin. We found a strong negative relationship between the abundance of AOA genes and soil fertility (availability of C, N, and P). Aridity and other abiotic factors (pH, sand content, and electrical conductivity) were more important than soil fertility in modulating the AOA/AOB ratio. AOB were more abundant under vegetated microsites, while AOA, highly resistant to stressful conditions, were more abundant in bare ground areas. These results suggest that AOA may carry out nitrification in less fertile microsites, while AOB predominate under more fertile conditions. Our results indicate that the influence of aridity and pH on the relative dominance of AOA and AOB genes is ultimately determined by local-scale environmental changes promoted by perennial vegetation. Thus, in spatially heterogeneous ecosystems such as drylands, there is a mutual exclusion and niche division between these microorganisms, suggesting that they may be functionally complementary.

Introduction

Prior to the recent discovery of archaeal ammonia oxidizers, it was assumed that the critical process of autotrophic nitrification was performed exclusively by bacteria. Many recent studies have detected variants of the ammonia monooxygenase (*amoA*) gene associated with ammonia-oxidizing archaea (AOA) in a variety of environments, often in greater abundance than those associated with ammonia-oxidizing bacteria (AOB; Leininger *et al.*, 2006). The physiology of AOA differs substantially from AOB. For example, AOA are thought to be better adapted to water-stress environments such as drylands (Adair & Schwartz, 2008) and may be able to function under more stressful conditions than AOB (Valentine, 2007). In addition, AOA microorganisms have been showed to preserve a high level of ammonia-oxidizing activity under low ammonium conditions (Martens-Habbena *et al.*, 2009; Verhamme *et al.*, 2011).

The potential for AOA to perform nitrification in stressful aquatic and terrestrial environments, such as thermal hot springs (You *et al.*, 2009) and drylands (Adair & Schwartz, 2008), challenges traditional assumptions about the types of environments where this process occurs and is important. Since Leininger *et al.* (2006) suggested that AOA were the dominant ammonia-oxidizing prokaryotes in soils, other studies have showed similar or lower abundance of AOA regarding AOB in these environments (Di *et al.*, 2009, 2010; Jia & Conrad, 2009; Xia *et al.*, 2011). The abundance of AOA and AOB seems to be modulated by climate (i.e. temperature and water availability; Szukics *et al.*, 2010), nutrient conditions (i.e. Wessén *et al.*, 2010, 2011; Verhamme *et al.*, 2011), and by other abiotic factors such as soil texture, pH, and salinity (Nicol *et al.*, 2008; Moin *et al.*, 2009; Gubry-Rangin *et al.*, 2011; Wessén *et al.*, 2011). However, field and laboratory studies carried out to date have shown inconsistent responses of both AOA and AOB to variations in environmental

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conditions. For example, increases in both AOB and AOA gene abundances have been observed with increasing temperature in some studies (Szukics *et al.*, 2010), but others have found the opposite or have reported inconsistent responses (Jung *et al.*, 2011; Rasche *et al.*, 2011). Similarly, contradictory responses of both AOA and AOB genes have been observed with changes in pH (He *et al.*, 2007; Nicol *et al.*, 2008), soil nitrogen (Wessén *et al.*, 2010; Rasche *et al.*, 2011), and soil carbon (C; He *et al.*, 2007; Shen *et al.*, 2008). The inconsistent results observed to date might be caused by differences in plant communities, soil types, and climates studied, which mask the effects of these environmental variables on the distribution of AOA and AOB. Most studies conducted so far have also focused on single environmental variables that have been manipulated in the laboratory or under field conditions (i.e. Nicol *et al.*, 2008; Verhamme *et al.*, 2011). Although some studies have studied the abundance of AOA and AOB in soils along natural gradients in factors such as pH (Gubry-Rangin *et al.*, 2011), C to N ratios (Bates *et al.*, 2011), salinity (Moin *et al.*, 2009), or temperature (Fierer *et al.*, 2009), we lack an integrated perspective on the most important environmental drivers modulating their abundance under natural conditions, particularly in drylands (López *et al.*, 2003; Adair & Schwartz, 2008; Bastida *et al.*, 2009; Gleeson *et al.*, 2010).

Drylands are of paramount importance for the Earth system, as they cover about 41% of Earth's land surface and support over 38% of the total global population (Reynolds *et al.*, 2007). In addition, these ecosystems are highly heterogeneous, typically having sparse plant coverage with open spaces located between plant canopies (Schlesinger *et al.*, 1996; Maestre & Cortina, 2002), which may provide different potential niches for AOA and AOB microorganisms. However, the effects of vegetated microsites on the abundance of AOA and AOB microorganisms have not, to our knowledge, been studied before.

This study had two major objectives. First, we aimed to evaluate the relationships between the abundance of AOA and AOB and climate (aridity), abiotic factors (pH, sand content, and electrical conductivity), and variables related to soil carbon (organic C, hexoses, and activity of β-glucosidase), nitrogen (ammonium, nitrate, dissolved organic N, and total available N), and phosphorus (phosphate and phosphatase activity) in semi-arid Mediterranean grasslands along a broad aridity gradient (from Spain to Tunisia). Factors such as climate (temperature and water availability; Szukics *et al.*, 2010), pH (Nicol *et al.*, 2008; Gubry-Rangin *et al.*, 2011), soil texture (Wessén *et al.*, 2011), electrical conductivity (i.e. salinity; Moin *et al.*, 2009), and nutrients (indicators of C, N, and P availability; He *et al.*, 2007; Wessén *et al.*, 2010, 2011) were included in our study because they have been shown be correlated with the abundance of AOA and AOB in terrestrial ecosystems. Second, we aimed to evaluate the relative importance of climate, abiotic factors, and soil nutrient variables as potential modulators of AOA and AOB abundance in contrasting microsites (vegetated and bare ground areas). We hypothesized that because AOB have been observed to be stimulated under high ammonium conditions (Verhamme *et al.*, 2011) and AOA have been suggested to be adapted to low nutrient availability and energy-limiting conditions (Valentine, 2007; Verhamme *et al.*, 2011), AOA would be more abundant than AOB under less fertile conditions (lower nutrient content and higher aridity). Moreover, AOA should dominate in bare ground areas because of their apparent adaptation to high temperatures, low water availability, and energetically stressful conditions (Valentine, 2007; Adair & Schwartz, 2008; You *et al.*, 2009), while AOB may prefer vegetated microsites, typically characterized by higher availability of water and nutrients (Maestre *et al.*, 2001, 2003; Cortina & Maestre, 2005). Improving our knowledge of the factors driving the abundance of AOA and AOB in soils may help us to clarify the contribution of these organisms to the accumulation of NO_3^- often reported in drylands (Cookson *et al.*, 2006) and thus to achieve a better understanding of the role of these microorganisms in the N cycle.

Materials and methods

Study site

This study was conducted in 16 *Stipa tenacissima* L. grasslands from Spain, Morocco, and Tunisia (Supporting Information, Fig. S1; Table S1). The area sampled covers the core of the geographic distribution of this vegetation type in the Mediterranean Basin, which spans from Spain to Lybia (Le Houérou, 2001). Mean annual precipitation (MAP) and temperature (MAT) of the study sites varied from 141 to 465 mm and from 13 to 20 °C, respectively (Table S2). The content of sand and electrical conductivity of the sites studied range from 33.5% to 80.5% and from 63.5 to 238.9 μS cm^{-1}, respectively. As typically found in drylands (FAO, 1998), the range of pH found at our sites is narrow (from 7.56 to 8.57), which may restrict the prokaryotic communities at these sites to specific lineages (Gubry-Rangin *et al.*, 2011). Perennial vegetation cover ranged between 8% and 64% and was in all cases an open steppe dominated by *S. tenacissima* (Fig. S1), with shrub species such as *Quercus coccifera L.*, *Rosmarinus officinalis L.*, and *Thymus vulgaris L.* in Spain, *Cistus clusii* and *Helianthemum apenninum L.* (Mill.) in Morocco, and *Artemisia herba-alba Asso* and *Hammada scoparia* (Pomel) Iljin in Tunisia. Detailed information on the characteristics of each site can be found in Tables S1 and S2.

Sampling design and measurements

We established a 30 × 30 m plot representative of the vegetation found at each site. We obtained mean annual temperature and precipitation data from the WorldClim global database (Hijmans *et al.*, 2005). The UNEP (1992) aridity index (AI = P/PET, where P is annual precipitation and PET is annual potential evapotranspiration) of each site was gathered using data from WorldClim as described in the study by Maestre *et al.* (2012). The AI decreases as aridity increases, and for clarity, we used the inverse of AI in this work. Thus, aridity = 1 − AI. This index is strongly related to both annual average rainfall ($R^2 = -0.98$) and temperature ($R^2 = 0.74$) in our study sites.

Soil sampling was carried out during the summer season of 2006 (Spain) and in 2010 (Morocco and Tunisia). Five composite soil samples from the top 7.5 cm of the mineral soil profile were collected at each plot from two microsites: bare ground areas devoid of vascular vegetation (hereafter Bare) and under the canopy of *S. tenacissima* (hereafter Stipa), totaling 160 soil samples. Soils were transported to the laboratory and sieved (2-mm mesh). A portion of this soil was frozen at −80 °C for molecular analysis, while the rest was air-dried at room temperature for 4 weeks for physiochemical analyses (pH, texture, electrical conductivity, organic C, activity of β-glucosidase, hexose content, total available N, and phosphate and phosphatase activity). Previous studies have found that these properties are hardly affected by air-drying in semi-arid Mediterranean soils (Zornoza *et al.*, 2006, 2009). Organic C was determined following Anderson & Ingramm (1993). The activity of β-glucosidase was assayed following Tabatabai (1982). Total available N (sum of ammonium, nitrate, and DON) and hexoses were colorimetrically analyzed from K_2SO_4 0.5 M soil extracts using a 1 : 5 soil/extract ratio as described in the study by Delgado-Baquerizo *et al.* (2013) and Chantigny *et al.* (2006), respectively. Phosphate was determined by colorimetry from a 0.5 M $NaHCO_3$ extraction (Bray & Kurtz, 1945). Phosphatase activity was measured following Tabatabai & Bremner (1969). Soil pH was measured for all of the soil samples with a pH meter in a 1 : 2.5 mass/volume soil and water suspension. One composite sample per microsite and site was analyzed for sand, clay, and silt content according to Kettler *et al.* (2001). Electrical conductivity was determined using a conductivity meter in the laboratory.

qPCR analysis

Soil DNA was extracted from 0.5 g of defrosted soil sample using the MoBio Powersoil DNA Isolation kit (Carlsbad) according to the instructions provided by the manufacturer and stored at −20 °C. We performed quantitative PCRs in triplicate using 96-well plates on an iCycler iQ thermal cycler (Bio-Rad). The *amoA* genes of AOB and AOA were amplified using the primers *amoA1F* (GGGGTTTCTACTGGTGGT)/*amoA2R* (CCCCTCKGSAA AGCCTTCTTC) and *Arch-amoAF* (STAATGGTCTGGCT TAGACG)/*Arch amoAR* (GCGGCCATCCATCTGTATG T), respectively, as described previously by Rotthauwe *et al.* (1997) and Francis *et al.* (2005). The 25 μL reaction mixture contained 12.5 μL FastStart Universal SYBR Green Master (Rox), 0.75 μL (10 mM) each primer, 1 μL BSA, 1–10 ng template DNA, and ultraclean water to volume. The cycling conditions were 95 °C for 10 min, followed by 35 cycles of 95 °C for 60 s, 55 °C for 45 s, and 72 °C for 60 s for both primer sets. Standards were run in triplicate in each assay, and our standard calibration curve was developed using a serial 10^{-3} and 10^{-9} dilution from 30 ng μL^{-1}. We generated melting curves for each run to verify product specificity by increasing the temperature from 55 to 95 °C. Efficiencies for all quantification reactions were higher than 90%, with R^2 values ranging from 0.90 to 0.99. Results were expressed as number of copies of genes g $soil^{-1}$. The total amount of the AMO gene (*amoA*) was calculated as the sum of AOA and AOB genes.

qPCR standard curve preparation

The *amoA* bacterial and archaeal primers described above were used to amplify *amoA* genes from DNA extracted from composite soil samples. In parallel, both PCR products were cloned into *Escherichia coli* using a TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. One specific clone was selected for AOA and AOB cultures to generate the standard curves. Plasmid DNA was extracted with a Plasmid Mini kit (Invitrogen), and the insert was sequenced using M13F and M13R primers to check that AOA and AOB were correctly inserted into their respective plasmids (sequences from selected AOA and AOB clones are available in Table S3). These sequences were compared with known *amoA* genes in the GenBank database (http://www.ncbi.nlm.nih.gov) using BLAST. BLAST analysis showed that the sequences were > 99% similar to known AOA (i.e. uncultured archaeon clone E1-76 ammonia monooxygenase gene) and AOB (i.e. *Nitrospira* sp.) genes. We also checked the length of the insert AOA and AOB amplicon in their respective plasmids with PCR using the AOA, AOB, and M13 primers and conducting electrophoresis in agarose gels to check the integrity of the fragment.

Statistical analyses

Due to the low number of study sites as compared to the number of climatic (aridity), abiotic (pH, sand content,

and electrical conductivity), and nutrient (organic C, hexoses, activity of β-glucosidase, total available N, ammonium, nitrate, DON, and phosphate and phosphatase activity) variables studied and to the significant relationships between some of the variables from each group (Table S4), we reduced the dimensionality of our data set. To accomplish this, we conducted a principal component analysis (PCA) with abiotic and nutrient variables separately to reduce them to an abiotic and a nutrient component, respectively. These analyses were carried out separately for the Stipa and Bare microsites. We kept the first component from the PCAs for further analyses, which had an eigenvalue higher than 1 and explained 75% and 64% of the variance from the PCA for the nutrient (nutrient-ax1) and abiotic (abiotic-ax1) variables in the Stipa microsite and 62% and 58% of this variance in the Bare microsite, respectively. The nutrient-ax1 was positively related to all of the nutrient variables evaluated in this study (Table S4). The abiotic-ax1 component was negatively related to pH and sand content, but positively related to electrical conductivity.

We used univariate linear regression analyses to examine the relationship between AOA and AOB with the climatic (aridity), abiotic (abiotic-ax1), and nutrient (nutrient-ax1) variables. AOA, AOB, and AOA/AOB were log-transformed to normalize them prior to regression analyses. Next, we used a multimodel inference approach based on information theory and ordinary least squares (OLS) regression to evaluate the relative importance of climatic (aridity), abiotic (abiotic-ax1), and nutrient (nutrient-ax1) variables on the AOA, AOB, and AOA/AOB ratio (Burnham & Anderson, 2002). This approach does not rely on hypothesis testing for fitting models, but instead uses information theory to assess the probability that a given model is the most appropriate description of the observed data. Multimodel inference approaches are recommended when dealing with observational data collected along environmental gradients, as in this study (Chatterjee & Price, 2001; de Albuquerque *et al.*, 2011; Maestre *et al.*, 2012). We calculated the relative importance of aridity, abiotic factors (abiotic-ax1), and nutrient variables (nutrient-ax1) as predictors of the abundance of AOA, AOB, and AOA/AOB ratio as the sum of the Akaike weights of all models that included the predictor of interest, taking into account the number of models in which each predictor appears (Burnham & Anderson, 2002). We conducted separate analyses for Bare and Stipa microsites.

We tested for differences between *amoA* organisms (AOA and AOB), microsites (Bare and Stipa), and sites on the abundance of *amoA* genes using a three-way ANOVA approach. We evaluated differences between microsites and sites on the AOA/AOB ratio using a two-way ANOVA. In these analyses, we considered the *amoA* organisms (AOA and AOB) and the microsites (Bare and Stipa) as fixed factors, and site as a random factor. When significant interactions between factors were found, data were divided into subsets based on one of the factors of the interaction and then were subjected to two-way or one-way ANOVA as appropriate.

Multimodel analyses were conducted using SAM 4.0 (Rangel *et al.*, 2010); other statistical analyses were carried out with SPSS 15.0 Statistics Software (SPSS Inc., Chicago, IL).

Results

Both AOA gene abundance and the AOA/AOB ratio were positively related to aridity and to the abiotic-ax1 (lower pH and sand content, but higher electrical conductivity) in the Bare microsites along the gradient studied (Fig. 1; Table S5). The nutrient-ax1 was negatively related to AOA gene abundance and the AOA/AOB ratio in the Bare microsite and to AOA in both Bare and Stipa microsites, along this gradient (Fig. 1). AOB gene abundance was significant negatively related to nutrient-ax1 in the Stipa microsites (Fig. 1; Table S5); a positive trend between both variables was observed in the Bare microsites (Fig. 1; Table S5).

Soil nutrients (nutrient-ax1 component) were the most important factor modulating AOA gene abundance in both Stipa and Bare microsites (Fig. 2). However, contradictory results were found when analyzing the importance of abiotic, nutrient, and climatic variables (Fig. 2). Aridity was positively correlated with the AOA/AOB ratio in the Bare microsite, but this ratio did not show significant relationships with any of the variables measured in Stipa microsites (Fig. 2; Tables S5 and 1). The abiotic component always explained more variance of the abundance of AOA, AOB, and AOA/AOB ratio, in Bare than in Stipa microsites (Fig. 2).

Significant differences between sites were found for both AOA and AOB gene abundances in Bare and Stipa microsites ($P < 0.01$; Fig. 3; Table S6). However, significant *amoA* organisms (AO) × microsite (MI) × site (SI), AO × MI, and AO × SI interactions were found when analyzing these data ($P < 0.01$; Table S6; Fig. 3). In Bare microsites, differences between *amoA* organisms and sites were observed ($P < 0.01$), although the interaction AO × SI suggested that differences between AOA and AOB varied depending on the site considered ($P < 0.01$; Table S6; Fig. 3). In Stipa microsites, AOB were more abundant than AOA in all the sites ($P < 0.01$; Table S6; Fig. 3). A higher concentration of AOA was also observed in Bare microsites across the different sites ($P < 0.01$; Table S6; Fig. 3). Differences between microsites and sites

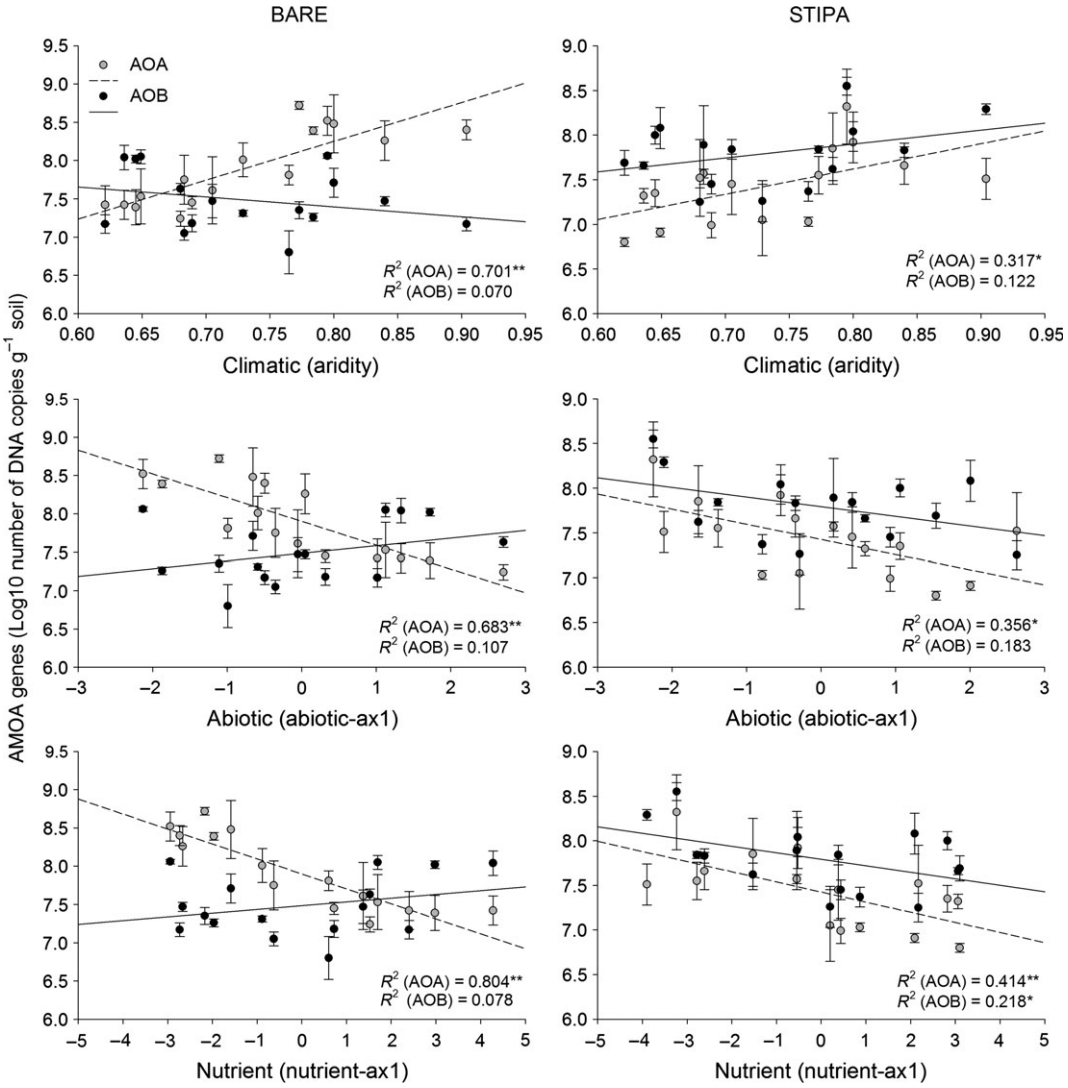

Fig. 1. *amoA* gene abundance (AOA and AOB) in relation to aridity, abiotic factors (abiotic-ax1), and nutrient variables (nutrient-ax1) in both *Stipa tenacissima* (STIPA) and bare ground (BARE) areas. Results of linear regression analyses (R^2 and fitted lines, when significant) are shown; * $P < 005$, ** $P < 001$, *** $P < 0001$.

in the abundance of AOB were also observed ($P < 0.01$). The MI × SI interaction found for this variable, however, indicated that differences between Bare and Stipa microsites were site-dependent ($P < 0.01$; Table S6; Fig. 3).

Discussion

The relative abundance of AOA and AOB should be related to the rate at which nitrite is produced through nitrification (Verhamme *et al.*, 2011), because their growth is primarily coupled to autotrophic ammonia oxidation [although some AOA may be nonautotrophic (Mussmann *et al.*, 2011)]. Thus, it is important to understand the factors that drive their abundance in soils. The present study complements previous work that focused on single nutrient variables and strongly suggests that AOA are generally inhibited by increasing soil fertility conditions in drylands. We found that the abundance of AOA was inversely related to the nutrient-ax1, an aggregate index of organic C, hexoses, β-glucosidase activity, ammonium, DON, total available N, and phosphatase activity (Table S4), in both Bare and Stipa microsites across the studied aridity gradient, where low nutrient concentrations are typical (Zak *et al.*, 2003; Delgado-Baquerizo *et al.*, 2011). In addition, a decrease in the AOA/AOB ratio was observed with increasing carbon (i.e. organic C), nitrogen (i.e. ammonium), and phosphorus (i.e. activity of phosphatase) variables in the Bare microsites

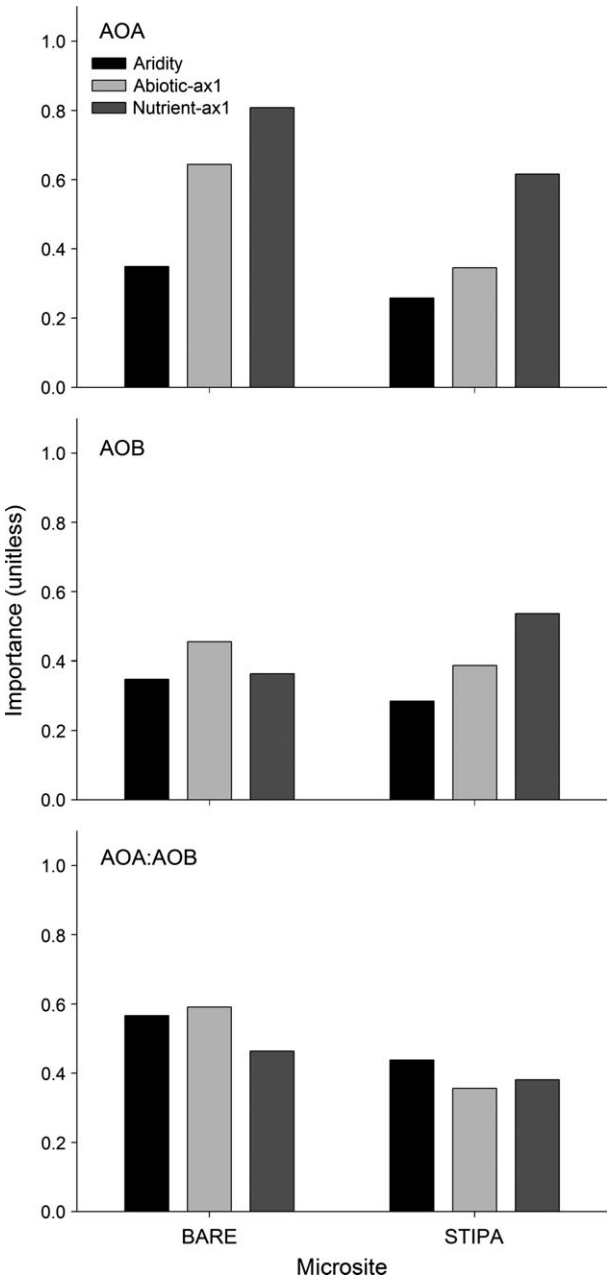

Fig. 2. Relative importance of aridity, abiotic factors (abiotic-ax1), and nutrient variables (nutrient-ax1) as predictors of the abundance of AOA, AOB, and AOA/AOB ratio in *Stipa tenacissima* (STIPA) and bare ground (BARE) areas. The height of each bar is the sum of the Akaike weights of all models that included the predictor of interest, taking into account the number of models in which each predictor appears (see Table 1).

Table 1. Top five best-fitting regression models for the AOA, AOB, and AOA/AOB ratio, ranked according to their AICc value, are presented. Each column represents a different predictor variable (aridity, abiotic-ax1, and nutrient-ax1); shaded cells indicate that the variable has been included in the model. AICc measures the relative goodness of fit of a given model; the lower its value, the more likely the model to be correct. ΔAICc values are difference between the AICc of each model and that of the best model. Wi means Akaike weights. BARE = models conducted with bare ground data. STIPA = models conducted with *Stipa tenacissima* data.

	Aridity	Abiotic-ax1	Nutrient-ax1	R^2	AICc	ΔAIC	Wi
BARE							
AOA							
		■	■	0.85	2.97	0.00	0.37
			■	0.81	3.56	0.59	0.28
■		■		0.84	4.44	1.47	0.18
■			■	0.86	5.78	2.81	0.09
■				0.82	6.27	3.29	0.07
AOB							
		■		0.11	21.00	0.00	0.34
			■	0.08	21.52	0.52	0.26
■				0.07	21.66	0.66	0.24
		■	■	0.11	24.66	3.56	0.06
■		■		0.11	24.56	3.60	0.06
AOA/AOB							
■		■		0.73	8.15	0.00	0.33
			■	0.64	9.01	0.87	0.21
		■	■	0.70	9.86	1.71	0.14
■				0.61	10.07	1.92	0.13
		■		0.59	10.99	2.84	0.08
STIPA							
AOA							
			■	0.42	15.10	0.00	0.45
		■		0.36	16.62	1.52	0.21
■				0.32	17.55	2.45	0.13
		■	■	0.42	17.47	3.37	0.08
■			■	0.42	18.71	3.61	0.07
AOB							
			■	0.22	15.83	0.00	0.38
		■		0.19	16.52	0.69	0.27
■				0.12	17.68	1.86	0.15
■			■	0.24	18.94	3.12	0.08
		■	■	0.22	19.38	3.56	0.06
AOA/AOB							
■				0.04	−16.47	0.00	0.32
			■	0.02	−16.18	0.29	0.27
		■		0.01	−15.92	0.55	0.24
■		■		0.05	−13.16	3.31	0.06
			■	0.06	−12.91	3.56	0.05

(Table S4). These results suggest that AOA may outcompete AOB microorganisms under oligotrophic conditions due to their high resistance to water and nutrient stress (Adair & Schwartz, 2008; Verhamme *et al.*, 2011), hence carrying out nitrification under conditions that have long been assumed to be unfavorable. As ammonium is the primary substrate for both AOA and AOB, we might expect that both groups would both respond positively to increases in ammonium. However, this may not be the case in natural systems because they have to compete for ammonium with heterotrophic microorganisms and plants. Previous studies have shown that AOB increase in response to ammonium fertilization (Verhamme *et al.*, 2011), but it has been unclear if this pattern translates to natural fertility gradients. We observed a trend of increasing AOB with increasing nutrient availability in Bare microsites, suggesting that AOB are able to compete

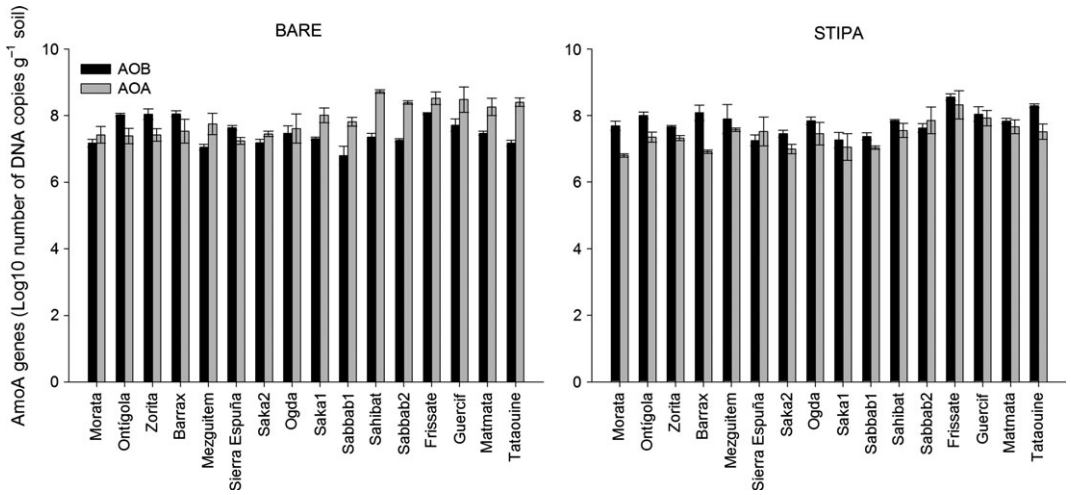

Fig. 3. *amoA* gene abundance (AOA and AOB) in both *Stipa tenacissima* (STIPA) and bare ground (BARE) areas for the different study sites sorted from less (left) to more aridity (right). Data represent means ± SE (n = 5).

for ammonium against heterotrophic microorganisms. Additionally, AOB were always more abundant than AOA in Stipa microsites (Table S6), suggesting that AOB are also better competitors for ammonium than AOA in the face of competition with plants. However, the negative relationship observed between both AOA and AOB and the nutrient-ax1 – highly related to organic C and phosphatase activity (Table S4) – in Stipa microsites suggests that both AOA and AOB can be outcompeted by heterotrophic microorganisms and plants as fertility increases. Thus, nitrification rates might decrease with increasing fertility despite the corresponding increase in substrate availability.

Other abiotic factors besides fertility might also affect the abundance of AOA and AOB and thus the potential for nitrification in any environment. In our study, AOA and AOB tended to increase with increasing pH in the Stipa microsites, although our study sites have limited and alkaline pH range (7.56–8.57). Other studies have shown that AOB increase and AOA decrease as pH increases (from pH 4.9 to 7.5; Nicol *et al.*, 2008). These contradictory findings might suggest that unique AOA ecotypes are found in dryland soils that are better adapted to high pH than those found in acid and neutral soils. Other factors such as sand content were positively related to the abundance of AOA (but not AOB) in both Bare and Stipa microsites. Similarly, Wessén *et al.* (2011) observed a decrease in AOA with increasing clay% (usually inversely related to sand content), but these authors did not find any relationship between clay and AOB abundance. Increasing sand content reduces the ability of soils to retain nutrients (FAO, 1989), but increases soil aeration, which may favor AOA growth. Electrical conductivity, highly linked to salinity, was negatively related to AOA, but not to AOB abundance. This result suggests that salinity concentration may modulate AOA growth, as suggested by others (Mosier & Francis, 2008; Moin *et al.*, 2009). In the Bare microsites, AOA were more abundant than AOB under the most arid conditions (Table S6). Overall, abiotic-ax1, together with aridity, was the most important factor modulating the AOA/AOB ratio, a response consistent with studies showing that abiotic factors such as pH, salinity, and sand content may be important factors distinguishing the niches of AOA and AOB microorganisms (Nicol *et al.*, 2008; Moin *et al.*, 2009; Wessén *et al.*, 2011).

While it is clear that both abiotic factors and substrate competition can affect the abundance of AOA and AOB, plants might also impact their abundance by altering abiotic conditions. Soils under *S. tenacissima* receive higher litter inputs and have lower temperature and higher moisture content than soils located in adjacent bare ground areas (Maestre *et al.*, 2001, 2003). Environmental changes induced by *S. tenacissima* may promote increased AOB dominance under its canopy, while AOA, more resistant to abiotic stress, dominate in Bare microsites (Valentine, 2007; Adair & Schwartz, 2008; You *et al.*, 2009). In addition, *S. tenacissima* may act as islands of fertility, where AOA may be outcompeted by AOB. These results are consistent with previous reports of differences in the abundance of different microbial groups between Stipa and Bare microsites (Maestre *et al.*, 2009). Thus, the nitrate accumulation reported in this study (Table S2) and observed in many other arid and semi-arid ecosystems (Hook & Burke, 1995; Bennett & Adams, 1999; Cookson *et al.*, 2006) could be the result of the activity of AOA and AOB in different microsites (vegetated and bare ground areas). In a previous study under controlled

conditions, Verhamme *et al.* (2011) related nitrification rates to AOA and AOB abundance in soils. The generally weak relationship observed in our soils between nitrate and both AOA and AOB abundance suggests that other nitrogen transformations, such as denitrification, could be taking place at our sites, making it difficult to interpret the actual contribution of AOA and AOB to standing pools of nitrate. The modulating effects of *S. tenacissima* tussocks on the relative dominance of two physiologically different groups of microorganisms, such as AOA and AOB, suggests that the microsites typically found in these ecosystems (e.g. vegetated patches and bare ground areas) may provide different niches to other physiologically contrasting groups of microorganisms, which may be involved in diverse processes that affect the overall functioning of these ecosystems. Given the importance of both abiotic and plant-mediated drivers of AOA and AOB abundance, climate change could have a strong impact on nitrification rates. The increase in aridity predicted for the Mediterranean Basin (Gao & Giorgi, 2008) could affect the AOA/AOB ratio by increasing the abundance of AOA, potentially relegating AOB organisms to the microsites provided by *S. tenacissima*. In addition, increasing aridity may lead to an increase and decrease in the sand content and nutrient availability, respectively (FAO, 1989), affecting the abundance of AOA, hence the AOA/AOB ratio in drylands.

In conclusion, we showed that increases in overall soil fertility inhibit AOA abundance in arid and semi-arid Mediterranean grasslands, where low nutrient concentrations are typical. Thus, AOA may only be competitive under oligotrophic conditions because of their high resistance to low nutrient conditions. Abiotic factors such as aridity and pH modulate the relative dominance of AOA genes, but their influence is ultimately determined by local-scale environmental changes promoted by perennial vegetation, which result in different niches for microorganisms within a given site. Although the actual contribution of AOA and AOB microorganisms to the nitrification remains unknown, this study showed that in spatially heterogeneous ecosystems such as drylands, there is a mutual exclusion and niche division between these microorganisms, suggesting that they may be functionally complementary. Thus, the amount of nitrate observed in this study in the less fertile conditions suggests that the basal nitrate accumulation reported in many arid and semi-arid ecosystems may be related to the relative abundance of AOA and AOB in different microsites.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Examples of *Stipa tenacissima* grasslands in Spain (A, B), Morocco (C, D) and Tunisia (E, F) shrubs, showing the dominance of *Stipa* tussocks.

Table S1. Location, texture and % of C and N in the studied sites.

Table S2. Values used in this study for the climatic (AI: Aridity index, MAP: Mean annual precipitation, MAT: Mean annual temperature), abiotic (SAC: Sand content; pH; CON: Electrical conductivity), carbon (C: Organic-C; HEX: Hexoses; BGL: Activity of b-glucosidase), nitrogen (NH_4^+; NO_3^-; DON, total available N) and phosphorus (PO_4^{3-}; Activity of phosphatase) variables.

Table S3. DNA sequences from AOA and AOB selected clones by using generic M13F and M13R primers.

Table S4. Pearson correlations coefficients between abiotic-ax1 and nutrient-ax1 with the original abiotic and nutrient variables respectively, for the bare soil Bare and Stipa microsites.

Table S5. Pearson correlations coefficients between ammonium oxidising bacteria (AOB), archaea (AOA) and the AOA : AOB ratio with the climatic (aridity), abiotic (sand content, pH, and electrical conductivity) and nutrient (organic-C, hexoses: HEX, activity of b-glucosidase: BGL, ammonium: NH_4^+, nitrate: NO_3^-, dissolved organic N: DON, total available N, phosphate: PO_4^{3-} and activity of phosphatase: FOS) variables for the bare soil Bare and Stipa microsites.

Table S6. Summary results of the three-way ANOVA analyses carried out with AMO organisms (AOA and AOB), and of the two-way ANOVA analyses conducted with the AOA : AOB ratio.