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Nitrification and ammonia-oxidizing bacteria shift in response to soil moisture and plant litter quality in arid soils from the Patagonian Monte

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ABSTRACT

We aimed to evaluate the effects of both plant litter quality, characteristic of sites with different histories of grazing disturbance, and soil water content on nitrification in soils from an arid ecosystem of Patagonia. To reach this goal, soil microcosms covered by plant litter of different quality and subjected to different soil water conditions were sampled at different times to analyze: (i) the nitrifying enzyme activity; (ii) the concentration of inorganic forms of nitrogen; and (iii) the abundance of bacterial (AOB) and archaeal (AOA) *amoA* genes. Soil water enhanced nitrifying activity in average 16% during the period of highest nitrification rates, and nitrate concentration in average 733% after 70 days of incubation. Microcosms amended with high litter quality showed the highest ammonium and the lowest phenolics concentrations, and higher or equal nitrification rates than microcosms amended with poor litter quality. After one week of incubation, the combination of both high litter quality and soil water significantly enhanced *amoA* gene abundance from AOB ($p < 0.05$). The AOB:AOA *amoA* genes ratio ranged from 12 to 3170. Altogether, our results suggest that high soil water and litter quality exerted positive effects over the nitrifying activity and the abundance of AOB but not AOA in these arid soils.

1. Introduction

Although scarce attention is paid to drylands compared to agricultural lands or forests, these are globally important ecosystems that cover a large area (more than 41%) of the Earth's terrestrial surface (Niemeijer et al., 2005). In arid ecosystems, with spatially and temporally discontinuous distribution of resources, water inputs are the main factor controlling biological processes (Noy-Meir, 1973). However, nitrogen is, after water, the most important nutrient controlling primary productivity in arid and semiarid lands (Belnap, 1995), and hence it is of major importance to understand the processes that regulate its abundance and dynamics in soils. Nitrification, the biological oxidation of ammonia to nitrate *via* nitrite (Haynes, 1986), is a critical process in the N cycle of terrestrial ecosystems, because it regulates the form and mobility of inorganic nitrogen in soils and its availability for plants (Austin et al., 2006; Yao et al., 2011).

The rate-limiting step in the aerobic nitrification is the oxidation of ammonia (Wong-Chong and Loehr, 1975), a reaction that is usually studied by targeting the *amoA* marker gene, which encodes the α subunit of the ammonia monooxygenase enzyme. This reaction has for long been attributed to a group of chemo-lithoautotrophic β and γ-proteobacteria named AOB (Koops et al., 2006). In the last decade, it was found that some AOA are also capable of ammonia oxidation and that archaeal genes encoding the enzymes responsible for ammonia oxidation are globally distributed (Norton and Stark, 2011). However, it is still unclear whether it is the bacteria or the archaea that have predominant roles in ammonia oxidation in soils, and although several studies indicated predominance of AOA (Adair and Schwartz, 2008; Chen et al., 2008; Leininger et al., 2006; Zhang et al., 2012), there has also been evidence of the opposite (Banning et al., 2015; Jia and Conrad, 2009; Jung et al., 2011; Wu et al., 2011).

Abbreviations: AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea; H, site under high grazing pressure; L, site under low grazing pressure; HL, microcosms amended with plant litter from H; LL, microcosms amended with plant litter from L; CTRL, control microcosms without litter.

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Vegetation in arid and semiarid lands is composed of shrubs and grasses, which display different growth, defense, and nutrient conservation strategies, influencing the chemistry of their green and senescent tissues (Mazzarino et al., 1998). Grass litter supply a more labile C-rich substrate for decomposers than woody plant litter (Aerts and Chapin, 2000; Bosco et al., 2015; Carrera et al., 2005). In addition, because of differences in N-resorption efficiency from senescent leaves, leaf litter produced by these plant groups has different N concentration and C:N ratio (Campanella and Bertiller, 2008; Carrera et al., 2009). Concentrations of C and N in plant litter (C:N ratios) as well as the quality of C compounds influence microbial activity and litter decomposition (Carrera et al., 2009). Shrubs also present lignified stems and high concentration of other secondary metabolites (*e.g.*, phenolic compounds, including tannins), which function as defenses against UV-B damage, water stress, pathogens and herbivory (Aerts and Chapin, 2000; Mazid et al., 2011). Unlike labile C substrates from grass litter, secondary compounds are recalcitrant to microbial degradation, particularly polyphenols may also inhibit decomposition (Hättenschwiler and Vitousek, 2000; Reyes-Reyes et al., 2003).

Livestock grazing induces several changes in ecosystems through their direct and indirect impacts on vegetation, soil nutrients and on the soil conservation state. For example, grazing may change the spatial distribution of nutrients in soil through urine and feces deposition (Shand and Coutts, 2006). In infertile ecosystems with low herbivore densities, the positive effects of nutrient return from animal excreta are highly localized and may not be able to produce significant effects at the ecosystem scale (Bardgett and Wardle, 2003; Wardle et al., 2004). In addition, herbivores may change the soil bulk density through trampling (Le Roux et al., 2003). Regarding their impact on vegetation, livestock grazing reduces total plant cover and produces changes in the species composition of plant patches (Bertiller and Bisigato, 1998). One of the most conspicuous effects of long term grazing disturbance is the reduction of the cover of the most preferred plant species (perennial grasses and some deciduous shrubs) and the increase of the absolute or relative cover of most evergreen shrubs. In the Patagonian Monte, some evergreen shrubs such as *Larrea divaricata*,*Nassauvia fuegiana*, and *Junellia seriphioides* increase their relative cover, while perennial grasses such as *Nassella tenuis,* and *Poa ligularis* decrease their relative cover under heavy grazing disturbance (Bisigato and Bertiller, 1997; Bertiller and Bisigato, 1998; Larreguy et al., 2014). These changes in plant species in heavily grazed sites compared to lightly or non-grazed sites result in low quality (more recalcitrant) plant litter and in turn, influence the input of nutrients into the soil matrix (Carrera and Bertiller, 2013; Olivera et al., 2014). Moreover, grazing-induced changes in the size and composition of plant patches were associated with increments in soil erosion and degradation and low soil organic C concentration (Bertiller and Bisigato, 1998; Larreguy et al., 2014). Previous studies in the Patagonian Monte showed that plant litter with high concentration of lignin and soluble phenolics from heavily grazed sites slows down decomposition (Carrera and Bertiller, 2013; Vargas et al., 2006), has a negative effect on soil enzyme activities and microbial biomass (Olivera et al., 2014), and also affects soil bacterial diversity (Olivera et al., 2016). However, little is known about the specific effect that changes in plant litter quality produce on the soil nitrifying microbiota.

The potential influence of plant litter quality on nitrification might also vary with soil water content, through its impact on litter decomposition and nutrients release (Liu et al., 2006). Water availability controls nitrification through its effect on nitrifier accessibility to substrates, metabolism and physiology (Norton and Stark, 2011). Under dry conditions, water films in soil pores become thinner, restricting the mobility of substrates to nitrifiers (Stark and Firestone, 1995). Alternatively, nitrification also declines in soils that remain flooded for long periods, probably due to oxygen limitation (Norton and Stark, 2011). Furthermore, water availability controls microbial activity, causing physiological stress to microorganisms under very dry conditions (Schimel et al., 2007). In the Patagonian Monte, precipitations are low and may predominate during autumn and winter when, coupled with low temperatures, determine wetter conditions than in summer (soil water >10% during winter and 5–6% in summer, Coronato and Bertiller, 1997). A seasonal study in the same region showed that soil water content in combination with microbial biomass-C and total soil-N were the best predictors of soil protease activity, suggesting that dry periods with low humidity and low N-input may restrict soil proteolysis which in turn, is associated with ammonium release and N availability (Olivera et al., 2014). As a consequence, nitrification in this environment may be restricted during the dry season through low microbial activity, low proteolysis and limited accessibility of nitrifiers to substrates.

The aim of this research was to test under controlled conditions the effect of plant litter quality and soil water content on nitrification in arid soils from Patagonia. Plant litter was representative of two sites with different histories of grazing disturbance, and hence differed in C and N concentrations, C:N ratio, and concentration of labile *vs*., recalcitrant C compounds. Soil water contents tested were representative of those found in dry and wet seasons in the study area. We evaluated: (i) the nitrifying activity, (ii) the concentration of inorganic forms of N (nitrate and ammonium), and (iii) the abundance and ecological relevance of AOB and AOA in soils from an arid ecosystem. We hypothesized that both low water availability and plant litter quality slow down the nitrifying activity and decrease the abundance of the ammonia oxidizing microorganisms.

2. Material and methods

2.1. Sampling and microcosm set up

Sampling was performed at the field “La Esperanza” (42°12′S, 64°58′W), covering an area of about 6975.7 ha in NE Chubut province, within the Argentinean Patagonia. This area belongs to the southern part of the Monte Phytogeographic Province, where vegetation corresponds to a shrubland of *L. divaricata* Cav. and *Stipa* spp. (León et al., 1998). Plant cover is scarce and distributed in patches of shrubs and grasses separated by bare soil (Bisigato and Bertiller, 1997). This patchy organization of vegetation has important implications in the distribution of soil resources, in the creation of sheltered areas with favorable microclimatic conditions for seeding emergence and plant establishment, and in the protection of soils from erosion and nutrient losses (Bisigato et al., 2005). Mean annual temperature is $13.6 \pm 0.7\,^{\circ}C$ (32-year average, data from Automatic Weather Station, Climatology Laboratory, CENPAT) and mean annual precipitation is 188 mm, mainly concentrated in autumn and winter (Barros and Rivero, 1982). Soils are a complex of Typic Petrocalcids-Typic Haplocalcids (del Valle, 1998; Soil Survey Staff, 1998). This ecosystem has been exposed to continuous sheep grazing since the beginning of the last century in paddocks of *ca.* 2500 ha with a single watering point (Ares et al., 2003). Under these grazing conditions, grazing pressure is higher in places nearby watering points compared to sites distantly located from them (Bisigato et al., 2005). With the conversion of “La Esperanza” into a wildlife refuge in 2003, the sheep stocking rate of the field was reduced, and after five years all sheep were removed (Bär Lamas et al., 2013). However, signs of different grazing pressures in soil and vegetation characteristics within paddocks persist nowadays. We selected two sites with

contrasting vegetation states resulting from high (*H*) and low (*L*) past grazing pressures. These contrasting vegetation states differ in total and perennial grass cover ($H < L$), upper soil organic C concentration ($H < L$), and litter recalcitrance ($H > L$) (Bär Lamas et al., 2013; Bosco et al., 2015; Larreguy et al., 2014). We collected 12 upper soil samples (0–5 cm) associated with vegetated patches from each site using a 10 cm diameter core in September 2013. Soil samples were transported to the laboratory at 4 °C, pooled in a single composite sample, homogenized and sieved through a 2 mm mesh. We used a pooled soil sample since our objective was to test the individual and the combined effect of litter and soil water on the nitrification process, controlling the influence of other soil properties. Aliquots of the composite sample were used to assess moisture, pH, and to perform chemical analyses. Besides, we collected recently fallen plant litter samples (without signs of deterioration) underlying the same plant patches at both sites. After removing soil particles, small rocks and animal feces, litter samples from each site were pooled and homogenized in a single composite sample per site. Then, such samples were dried at 45 °C for 48 h and aliquots used for chemical analyses.

Soil microcosms containing 100 g of composite soil covered by 1 g of plant litter from H or L, and control microcosms without litter (HL, LL and CTRL, respectively) were subjected to two different soil water conditions (5 and 15% soil water content). These soil water conditions represent the usual water content of these soils in dry and wet periods (Coronato and Bertiller, 1997), and were shown to influence soil enzymes and microbial activity in a seasonal study in this region (Olivera et al., 2014). Soil water contents of 5 and 15% correspond to soil water potentials of approximately −5 and −0.3 MPa, respectively (Bisigato and Bertiller, 1999). Soil water holding capacity of the study soil is *ca.* 25% (Bisigato and Bertiller, 1999). Twenty one replicates of each treatment (combination of soil water content and litter quality) were prepared, totalizing 126 soil microcosms. Every two days during the whole incubation period, microcosms were weighted to calculate soil water content and watered with distilled water to maintain the respective soil water condition. Incubation was performed at constant temperature of 25 °C, which is the generally optimum temperature of cultured ammonia oxidizers from soils (Norton and Stark, 2011), and three replicates of each treatment were destructively sampled at different times to analyze: (i) the nitrifying enzyme activity; (ii) the concentration of ammonium and nitrate; and (iii) the abundance of bacterial and archaeal *amoA* genes in soil.

2.2. Soil and plant litter chemical analyses

The concentration of organic C and total N in the composite soil were assessed by wet combustion (Nelson and Sommers, 1982) and by the semi-micro Kjeldahl technique (Coombs et al., 1985), respectively. In addition, the semi-micro Kjeldahl technique was used to assess total N concentration in plant litter, while organic C concentration was assessed by ashing the plant tissue in a muffle furnace at 550 °C as previously described (Larreguy et al., 2014), assuming that half of the ash-free mass is considered carbon mass (Larreguy et al., 2014). The concentration of total soluble phenolics in plant litter was measured by the Folin–Ciocalteu method (Waterman and Mole, 1994), using tannic acid as a standard for quantification. Soil and plant litter chemical properties are reported in Table 1.

The concentration of extractable ammonium and nitrates + nitrites was assessed in the composite soil before microcosms set up (time 0) and in microcosm soil samples at different incubation times. Since the concentration of nitrites in soils is seldom detectable (Keeney and Nelson, 1982), the determination of nitrates + nitrites can be used as an estimation of the concentration of nitrates. Briefly, ammonium and nitrates were extracted from 8 g of wet soil with 40 ml of a 1 M solution of KCl, and the extracts were stored at −20 °C until analysis. The concentrations of N-NH_4^+ and N-NO_3^- + N-NO_2^- in the extracts were measured colorimetrically, using a San^{++} Continuous Flow Analyzer (Skalar, Breda, Netherlands).

2.3. Short-term nitrification activity

Nitrification activity in soil microcosms was measured according to Alef, 1995. Since no external ammonium was added to the soil, the accumulation of nitrite measured after the addition of an inhibitor of its oxidation to nitrate can be used as an indicator of the actual (instead of potential) rate of ammonium oxidation at the time of sampling (Berg and Rosswall, 1985). Briefly, 2.5 ml of a 75 mM potassium chlorate solution (an inhibitor of the oxidation of nitrite to nitrate) were added to 5 g of wet soil (*i.e.*, soil from microcosms at 5 or 15% soil water), and the suspension was incubated for 24 h at 25 °C. A second flask containing the same soil sample and potassium chlorate solution was frozen at −20 °C to determine the initial concentration of nitrites in the soil sample. After the incubation period, nitrites were extracted from the soil suspension by using a 2 M solution of KCl. The extracts were filtered and incubated at room temperature for 15 min with NH_4Cl buffer (pH 8.5) and reagent for nitrite determination, after which color intensity was measured at 520 nm in an Agilent 8453 UV–vis Spectroscopy System (Agilent Technologies, Inc., Santa Clara, CA, USA). A calibration curve constructed by diluting a sodium nitrite standard solution in the range of 0–1 μg NO_2^--N ml^{-1} was used to calculate the ammonium oxidation rate in soil microcosms, expressed as NO_2^--N g^{-1} dry soil day^{-1}.

2.4. Metagenomic DNA extraction

Metagenomic DNA was extracted and further used for *amoA* genes quantification from three replicates of each microcosm treatment (combination of soil water and plant litter condition) at dates 6, 14 and 70-days, plus three replicates from the original composite soil used for microcosms set up (time 0). DNA extractions were performed from 0.25 g of soil microcosm samples using the UltraClean® Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's instructions. In order to further purify the DNA, extracts were subjected to 0.8% (w/v) agarose gel electrophoresis, followed by the excision and melting

Table 1
Chemical properties of the composite soil and plant litter from the heavily grazed (*H*) and lightly grazed (*L*) sites (mean values ± standard deviation). Different lowercase letters indicate significant differences ($p < 0.05$) in plant litter organic C, total N, C:N ratio, soluble phenolics and polyphenols:N ratio between sampling sites, as determined by the Wilcoxon non-parametric test.

Sample	pH	Organic C (mg g^{-1} dry sample)	Total N (mg g^{-1} dry sample)	C:N	Soluble phenolics (mg g^{-1} dry litter)	Polyphenols:N
Composite soil	8.30 ± 0.06	8.69 ± 0.79	0.71 ± 0.03	12.24	n.a.	n.a.
Plant litter from H	n.a.	371.15 ± 3.31 a	7.95 ± 0.36 a	46.77 b	5.20 ± 0.26 b	0.66 b
Plant litter from L	n.a.	393.43 ± 10.20 b	10.02 ± 0.52 b	39.39 a	3.64 ± 0.24 a	0.36 a

n.a.: not applicable.

of the gel band and its purification with the illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, UK).

2.5. Quantification of amoA genes

Real-time PCR amplifications of bacterial and archaeal *amoA* genes were performed using the *amoA*-1F/*amoA*-2R (Rotthauwe et al., 1997) and Arch-amoAF/Arch-amoAR (Francis et al., 2005) primer sets, respectively. Three independent replicates of each microcosm treatment were analyzed per date. All qPCR reactions were carried out in a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), using the iTaq™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 0.3 μM of each primer and 1 or 2 μl of template DNA. Control reactions, were DNA was replaced by ultrapure water, were added to all runs. The amplification protocol for AOB *amoA* genes was: 5 min at 95 °C and then 44 cycles of 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C and a final step before fluorescence read of 40 s at 81 °C, to avoid primer-dimers quantification (Pfaffl, 2004). In addition, the thermocycling program for the quantification of AOA *amoA* genes was: 5 min at 95 °C and then 45 cycles of 45 s at 94 °C, 1 min at 53 °C, 1 min at 72 °C and a final step of 40 s at 80 °C before fluorescence read. Melting curves were run at the end of the amplification protocol to verify the specificity of the amplified fragments. Possible inhibition to the PCR was evaluated by spiking 1 μl of DNA from selected environmental samples with 10^4 copies of standard DNA, as previously described by Marcos et al., 2012. Standard curves were constructed by performing 1:10 serial dilutions of linearized plasmids containing the *amoA* gene from *Nitrosomonas europaea* or from uncultured archaea (clone E2), in the range of 10^8–10^2 *amoA* gene copies μl^{-1} ($r^2 > 0.99$). Reaction efficiencies were 76% for the *amoA* gene of AOB and 83% for that of AOA.

2.6. Statistical analyses

Because the ANOVA assumptions of normality and/or homoscedasticity were not met, the non-parametric Wilcoxon two-sample test (Wilcoxon, 1945) was performed to test for differences between the chemical properties of plant litter from H and L, and between the abundance of *amoA* genes from AOA *vs*., AOB within the same microcosm treatment. In addition, differences in: (i) nitrifying activity, (ii) ammonium and nitrate concentrations, and (iii) the (log transformed) *amoA* gene abundance between different soil microcosm treatments at each incubation time were tested using two-way ANOVA followed by the Scheffé interaction contrast test, which is robust to departures from normality and homoscedasticity (Sokal and Rohlf, 1995). Differences in the same variables among time points were tested separately at each microcosm treatment using one-way ANOVA.

3. Results

3.1. Nitrifying activity in soil microcosms

We found significant effects of soil water on the nitrifying activity in all treatments along the complete experiment ($p < 0.05$). During the first week of incubation, the highest nitrifying activity was in the 5% soil water condition (Fig. 1). However, this effect was reversed in the two following weeks, were nitrifying activity was on average 16% higher at the highest soil water. In addition, we observed significant effects of plant litter amendment on the nitrifying activity. Nitrification rate in LL was higher or equal to that of HL, but never lower (Fig. 1). Nitrification varied along time (LL-5% $F_{7,16} = 43.22^{***}$; LL-15% $F_{7,16} = 35.59^{***}$; HL-5% $F_{7,16} = 11.91^{***}$; HL-15% $F_{7,16} = 13.19^{***}$; CTRL-5% $F_{7,16} = 10.75^{***}$; CTRL-15% $F_{7,16} = 113.84^{***}$), with the highest values usually between days 10 and 35. At the end of the incubation period, the nitrification rate declined and there was a significant interaction between the effects of soil water and litter amendment ($p < 0.05$). In this period, plant litter had no effect on the nitrifying activity at 5% soil water, but higher nitrification rates in LL than in CTRL were observed at 15% soil water (Fig. 1).

3.2. Concentration of inorganic N in soil microcosms

We found significant effects of plant litter amendment on soil ammonium concentrations ($p < 0.05$). Ammonium concentration in LL was always higher than in HL, except for the microcosms at

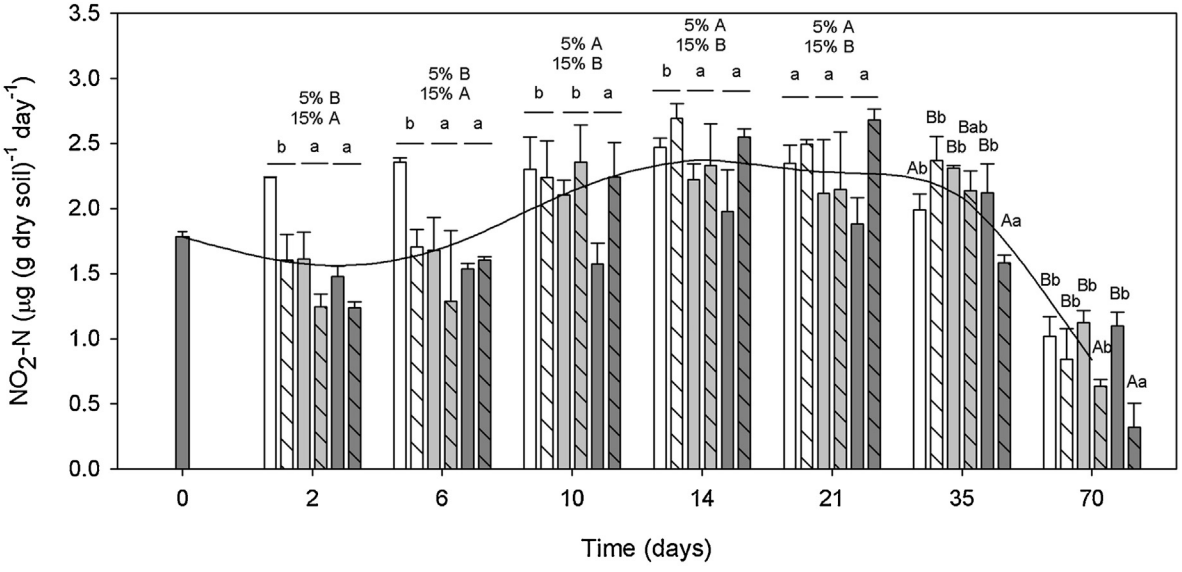

Fig. 1. Nitrifying activity in soil microcosms (mean values ± standard deviation, $n = 3$). Bar colors represent plant litter amendment (white, LL; light gray, HL; dark gray, CTRL), while shading patterns indicate soil water condition (not shaded, 5%; shaded, 15%). Identical letters on top of the bars indicate no significant differences between treatments (lowercase letters, comparisons among litter treatments; uppercase letters, comparisons between soil water conditions), according to Scheffé test ($p < 0.05$). In those cases where interaction was detected, a combination of lowercase and uppercase letters was used. Line: average nitrification rate among treatments.

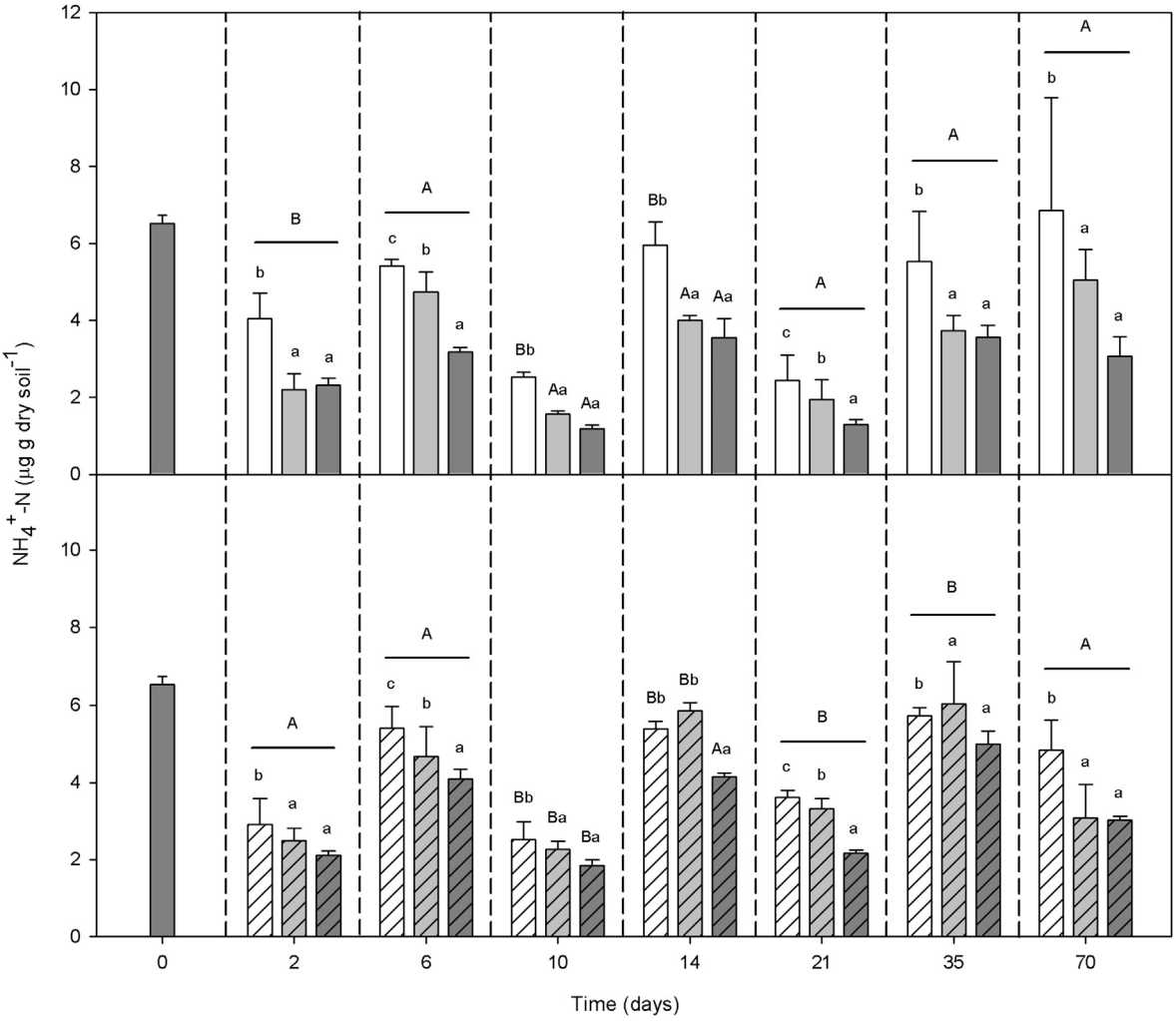

Fig. 2. Extractable ammonium concentration in soil microcosms (mean values ± standard deviation, $n = 3$) at 5% (upper figure) and 15% (lower figure) soil water. Bar colors indicate litter treatment (white, LL; light gray, HL; dark gray, CTRL). Shading patterns represent soil water condition (not shaded 5%; shaded 15%). Results of the Scheffé test ($p < 0.05$) were indicated with letters on top of the bars (lowercase letters, comparison among litter treatments; uppercase letters, comparison between soil water treatments). Combinations of lowercase and uppercase letters were used when interaction was detected.

15% soil water at date 14-days (Fig. 2). Moreover, the concentration of ammonium in LL was 32–92% higher than in CTRL. However, ammonium concentration in HL was frequently equal to CTRL, and occasionally higher. The effect of the soil water condition on the ammonium concentration was less clear. Higher ammonium concentrations at 5% soil water were observed at the beginning of the incubation time (day 2), while no differences between soil water conditions were detected on days 6 and 70, and higher concentrations at 15% soil water were observed at dates 21 and 35-days (Fig. 2). Ammonium differed along the incubation period (LL-5% $F_{7,16} = 6.12^{**}$; LL-15% $F_{7,16} = 8.04^{**}$; HL-5% $F_{7,16} = 46.08^{***}$; HL-15% $F_{7,16} = 24.42^{***}$; CTRL-5% $F_{7,16} = 92.80^{***}$; CTRL-15% $F_{7,16} = 231.98^{***}$), following a pulsed pattern.

In line with the results of nitrifying activity and ammonium concentration, we observed significant effects of plant litter condition on nitrate concentration. Nitrates in LL were always higher than or equal to HL, but never lower. Surprisingly, nitrate concentration in CTRL was frequently higher than in LL and HL (Fig. 3). In addition, we observed significant differences in nitrate concentration due to soil water. Also coincidently with the results of nitrifying activity, the concentration of nitrates was higher at 5% soil water during the first week of incubation, and higher at 15% soil water after the second week (Fig. 3). Additionally, we observed a temporal trend, in which nitrates increased in all treatments along the incubation period (LL-5% $F_{7,16} = 14.35^{***}$; LL-15% $F_{7,16} = 8.52^{**}$; HL-5% $F_{7,16} = 20.66^{***}$; HL-15% $F_{7,16} = 108.67^{***}$; CTRL-5% $F_{7,16} = 31.03^{***}$; CTRL-15% $F_{7,16} = 1799.79^{***}$), although at different rates depending on the soil water condition. In the 5% soil water condition nitrate concentration increment was relatively low, varying from 6.9 ± 0.2 µg NO_3^--N g soil^{-1} at the beginning of the experiment to 11.1 ± 0.9 µg NO_3^--N g soil^{-1} (average among all litter treatments) on day 70 (Fig. 3). On the contrary, nitrate concentration in the 15% soil water treatment increased in average 7-fold (733%) after 70 days of incubation. The maximum rate of nitrate production was in the 10–35-day period, coincidently with the highest rates of nitrification (Fig. 1).

3.3. *amoA* gene abundance

amoA genes from AOB and AOA were quantified in soil samples at the beginning of the experiment and after 6, 14 and 70 days of incubation. The abundance of the AOB *amoA* genes ranged from $2.3 \pm 0.1 \times 10^5$ to $1.7 \pm 0.9 \times 10^8$ gene copies g soil^{-1} (Fig. 4). We found significant effects of soil water (15% > 5%) on gene abundance in microcosms amended with LL at dates 14 and 70-days (day 14: $p = 2 \times 10^{-4}$; day 70: $p = 0.03$). This effect was markedly higher at

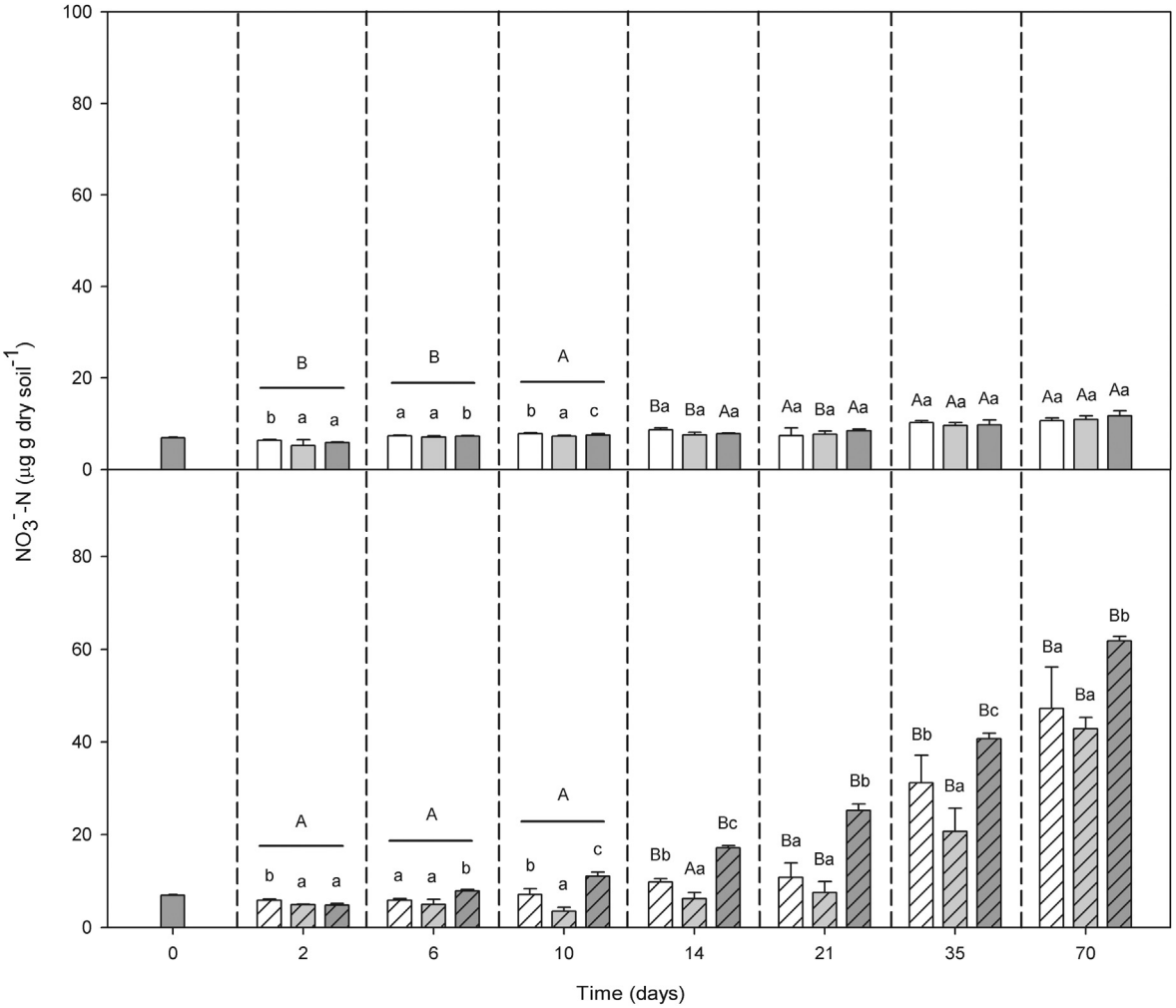

Fig. 3. Nitrate concentration in soil microcosms (mean values ± standard deviation, $n = 3$) at 5% (above) and 15% (below) soil water. Bar colors represent plant litter treatment (white, LL; light gray, HL; dark gray, CTRL), while shading patters indicate different soil water conditions (not shaded, 5%; shaded, 15%). No significant differences between treatments according to Scheffé test were indicated with identical letters on top of the bars (lowercase letters, comparisons among plant litter treatments; uppercase letters, comparisons between soil water conditions). Combinations of lowercase and uppercase letters were used when interaction was detected.

the 14th day of the incubation period, were gene abundance in LL microcosms at 15% soil water was more than 2 orders of magnitude higher than at 5% soil water (Fig. 4). The effect of plant litter on the bacterial *amoA* gene abundance was less clear. At the beginning of the experiment (day 6), gene abundance was lower in LL than in HL and CTRL ($p = 0.02$). In contrast, we found significant interaction effects between soil water and plant litter on gene abundance on dates 14 and 70-days (day 14: $p = 8 \times 10^{-3}$; day 70: $p = 0.045$). Gene abundance in soil microcosms amended with LL was equal (5% soil water) or higher (15% soil water) than in HL after 14 days of incubation, and lower (5% soil water) or equal (15% soil water) at the end of the experiment (Fig. 4). Bacterial *amoA* gene abundances varied along the incubation period in all the treatments except for HL-5% (LL-5% $F_{3,8} = 7.49^{**}$; LL-15% $F_{3,8} = 43.35^{***}$; HL-5% $F_{3,8} = 0.42^{ns}$; HL-15% $F_{3,8} = 68.49^{***}$; CTRL-5% $F_{3,8} = 26.78^{***}$; CTRL-15% $F_{3,8} = 22.22^{***}$).

In contrast to bacterial genes, the abundance of the AOA *amoA* genes ranged from $7.3 \pm 3.0 \times 10^3$ to $2.1 \pm 2.2 \times 10^5$ gene copies g soil^{-1} (Fig. 4). We found significant effects of plant litter on gene abundance after 14 days of incubation, where AOA *amoA* genes were at higher abundances in microcosms amended with LL than with HL or CTRL ($p = 1 \times 10^{-4}$). No soil water effects were observed in AOA gene abundance, with the only exception of microcosms amended with HL at the 70th day of the incubation period (5% > 15%, $p = 0.007$). Archaeal genes varied with time in all treatments except for LL-15%, usually decreasing at the end of the experiment (LL-5% $F_{3,8} = 5.21^{*}$; LL-15% $F_{3,8} = 2.50^{ns}$; HL-5% $F_{3,8} = 5.67^{*}$; HL-15% $F_{3,8} = 8.78^{**}$; CTRL-5% $F_{3,8} = 11.97^{**}$; CTRL-15% $F_{3,8} = 13.56^{**}$).

The abundance of *amoA* genes from AOB was always significantly higher than that of AOA ($p < 0.05$), and the AOB: AOA *amoA* genes ratio ranged from 12 to 3170 (Fig. 5). Furthermore, even assuming bacterial genomes have in average 2.5 *amoA* genes more than archaeal genomes (Norton et al., 2002; Walker et al., 2010), the higher abundances of AOB respect to AOA cells remains being significant ($p < 0.05$, Fig 5).

4. Discussion

In this study, we report the effects of soil water and grazing-induced changes in plant litter quality on the nitrifying activity, the concentration of inorganic forms of N, and the abundance and ecological role of ammonia oxidizers in soils from an arid ecosystem. Plant litter from sites H and L differed in their initial chemical characteristics (organic C, total N, C:N ratio, soluble phenolics and polyphenols:N ratio). A previous decomposition

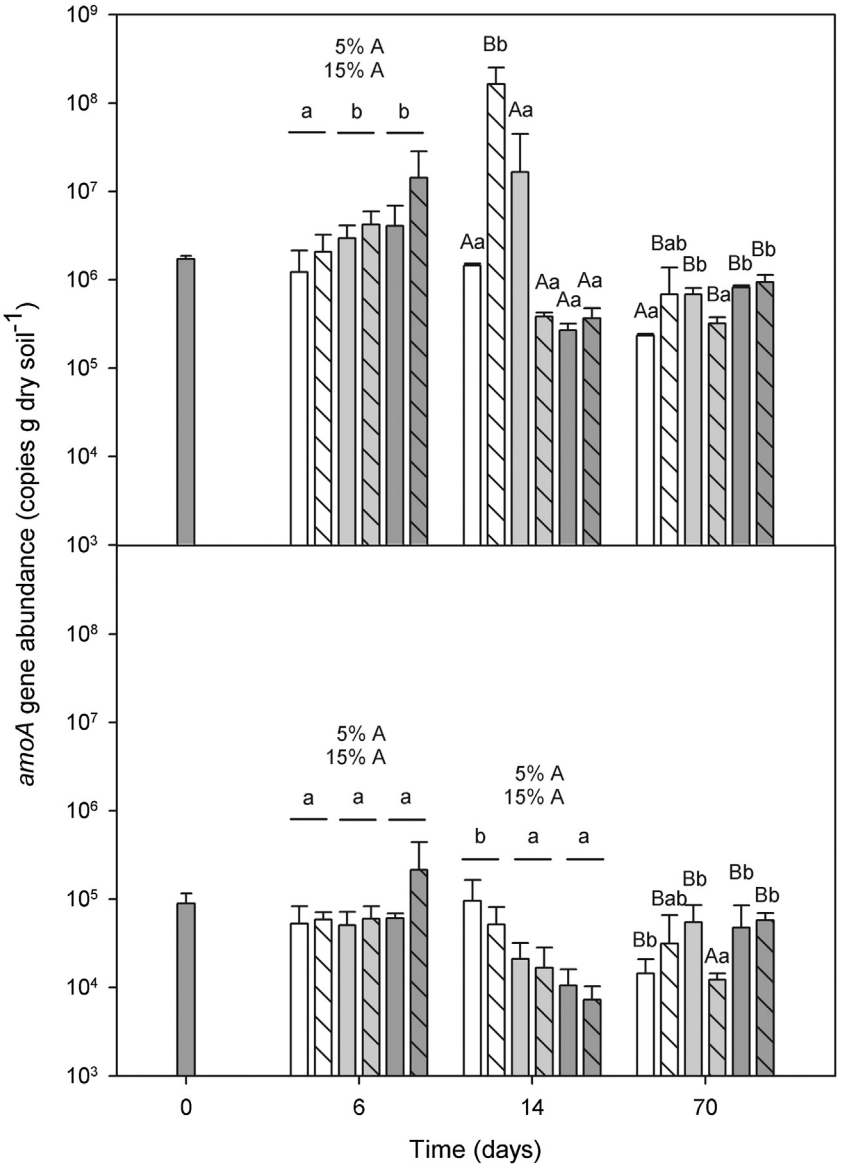

Fig. 4. Abundance of bacterial (upper figure) and archaeal (lower figure) *amoA* genes in soil microcosms (mean values ± standard deviation, $n = 3$). Bar colors indicate litter condition (white, LL; light gray, HL; dark gray, CTRL). Shading patterns illustrate soil water condition (not shaded, 5%; shaded, 15%). A combination of lowercase and uppercase letters on top of the bars was used to represent the results of a Scheffé test, where lowercase letters indicate comparisons among litter treatments and uppercase letters indicate comparisons between soil water conditions.

assay from the same study area demonstrated that differences between both litter types remained even after two years of incubation (Carrera et al., 2008). Most of the N contained in plant litter is usually immobilized and incorporated into soil organic matter, and there is a general consensus that N immobilization prevails over mineralization when soil N concentration is below 2% (Knops et al., 2002; Palm and Sanchez, 1991). This N immobilization is caused by differences between the C:N ratios of plant litter and soil organic matter (Knops et al., 2002). Our study of arid N-poor soils (total N: 0.07%, Table 1) agree with this notion, as no sharp increase in soil inorganic N concentration due to mineralization has been observed after the addition of plant litter (Figs. 2 and 3). However, there was litter induced mineralization along the experiment, and subtle although significantly higher ammonium concentrations were released in soil microcosms amended with LL than with HL (Fig. 2). This could be due to a higher concentration of polyphenolics in HL, since these compounds can form resistant complexes with organic N leading to lower inorganic N release (Hättenschwiler and Vitousek, 2000; Palm and Sanchez, 1991). In line with this, the higher polyphenolics:N and C:N ratios observed in HL compared to LL (Table 1) could be indicative of lower N release from plant litter, as has been previously shown (Palm and Sanchez, 1991; Seneviratne et al., 1999). High C:N ratios (*i.e.*, low N content in the litter mixture) induce low rates of organic matter decomposition, microbial activity and N mineralization, and hence could have negatively influenced the nitrification process (Carrera et al., 2009). Accordingly, plant litter from site L had lower C:N ratio and *ca.* 26% more N than plant litter from site H (Table 1), and induced in average 20% higher nitrification rates during the period of maxima nitrifying activity, although we cannot assure that all nitrifying activity was attributed to the N incorporated through litter. Furthermore, lignin could also be in high concentration in HL due to an increased proportion of shrub tissues in the litter mixture

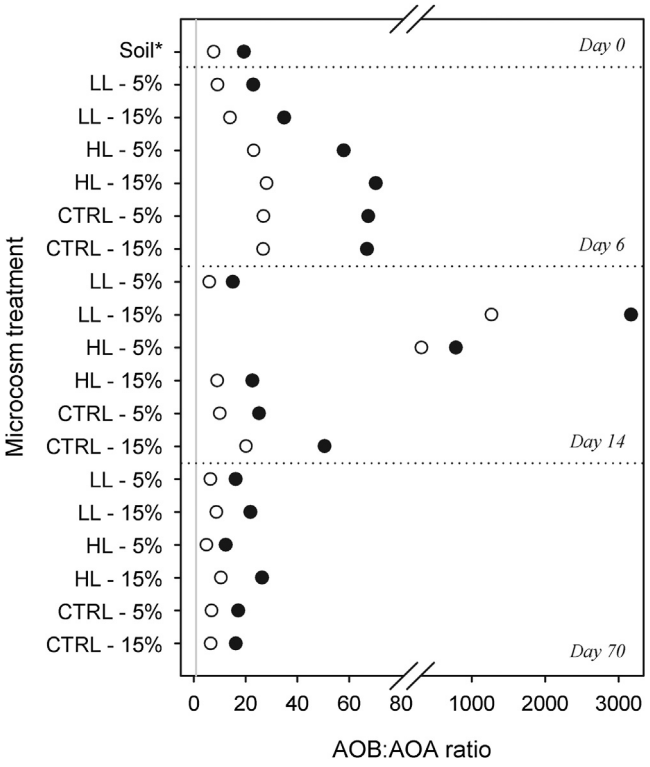

Fig. 5. AOB:AOA ratio in soil microcosms. Black circles: AOB:AOA *amoA* genes ratio, white circles: ratio between AOB and AOA cells, assuming 2.5 and 1 *amoA* genes per AOB and AOA genomes, respectively (Norton et al., 2002; Walker et al., 2010). Vertical gray line indicates AOB:AOA ratio = 1. Asterisk: composite soil used for microcosms' construction.

(Carrera et al., 2005). This polymer may restrict microbial access to N compounds, since it is recalcitrant to enzymatic degradation and represents a structural barrier that hinders microbial access to labile organic matter (Austin and Ballaré, 2010).

Besides (or more likely, as a result of) its effects on N immobilization, HL induced lower nitrification enzyme activities and nitrate concentrations than LL in many of the analyzed microcosms, suggesting negative effects of plant litter of low quality on nitrification in arid soils. Low litter quality has been found to negatively correlate with nitrification in north-western Patagonian forests, and has been proposed as one of the possible causes of reduced nitrification and N mineralization in grazed arid grasslands from Mongolia (Hirobe et al., 2013; Satti et al., 2003). In contrast to our results, nitrification was enhanced by grazing in semi-natural grasslands of France (Le Roux et al., 2003). In that study, grazing-induced changes on plant species composition were probably not the cause of nitrification shifts, which could be the result of soil trampling, nutrient recycling from animal excreta, and/or changes in nutrient uptake and release by defoliated plants (Le Roux et al., 2003). Unlike infertile ecosystems like the Patagonian Monte, productive ecosystems can support high herbivore densities and have high soil nutrient recycling from animal urine and dung (Wardle et al., 2004). On the contrary, unproductive ecosystems have low nutrient return into the soil from animal excreta and higher nutrient return as plant litter (Bardgett and Wardle, 2003). Therefore, it is probable that animal dejections only exert localized effects on the soil processes of this ecosystem and have a low impact at the landscape scale (Prieto et al., 2011). Further studies are needed to elucidate the *in situ* effects of grazing on nutrient recycling besides alterations of plant litter, including the potential inhibition of soil nitrification by plant root exudates (Subbarao et al., 2013).

Nitrification in soil microcosms was also influenced by soil water, as it is expected in arid ecosystems. In this study, we only tested the effects of two different soil water contents representative of dry and wet seasons, without further testing microbial stress imposed by multiple drying and rewetting events. Nitrification potentials were shown to increase with drying-rewetting events, probably as a consequence of low mortality of nitrifiers coupled with their ability to thrive after a release of ammonium during rewetting (Fierer and Schimel, 2002). It is possible, then, that values for *in situ* nitrification in the Patagonian Monte were higher than those in our microcosms experiment. During the first week of incubation, nitrification was higher at 5% soil water (Figs. 1 and 3), suggesting that the nitrifying community adapted to low soil water might have still not responded to the watering treatment. However, this effect was reversed in the following weeks and nitrification rates were higher at the 15% soil water treatment (Figs. 1 and 3). Negative effects of water deficiency on ammonia oxidation includes: limited ammonia diffusion to microbial cells, increased concentration of solutes that negatively impact microbial metabolism and growth, and low microbial activity due to low intracellular water potential, low enzyme activity or negative physiological effects produced by dehydration (Hu et al., 2015). In addition, soil water can lead to shifts in the abundance of nitrifying microorganisms, since ammonia oxidizers from arid regions may be drought resistant and thrive after soil rewetting (Gleeson et al., 2008). In accordance, the *amoA* gene abundance from AOB remarkably increased in response to the soil water treatment after the first week of incubation, but only when soil microcosms were amended with high quality litter, which in turn provided higher concentrations of ammonium than poor quality litter (Figs. 4 and 2). This increase in AOB in the 15% soil water LL treatment represents a generation time of 1.3 days, and is similar to those of other soil microcosm studies (1–2 days, Cavagnaro et al., 2008; Okano et al., 2004), and 7-fold smaller than *in situ* generation times of soil AOB (15 days, Okano et al., 2004). It is known that water facilitates the breakdown of surface litter and the leaching of its cellular content (Liu et al., 2006). Thus, not only the nutrients are released into the soil matrix, but also the compounds that bind to and immobilize organic N. It is possible then, that some microbial populations involved in the cycling of N (*e.g.*, AOB) in soils receiving high quality litter inputs are limited by soil water, while those under low quality litter are more sensitive to nutrient availability, as it has been previously proposed for decomposers (Liu et al., 2006). Further studies should be performed to confirm this hypothesis. Lastly, since microcosms are closed systems comparable to batch cultures, the drop in ammonia oxidizer abundance by day 70 was probably caused by the accumulation of waste products that are toxic to microbial cells. Nitrifiers would probably resume growth if transferred to fresh soil samples.

In our study of arid Patagonian soils, AOB were significantly more abundant than AOA in all the analyzed samples (Fig. 4). This was surprising, since we had expected that the low concentration of ammonium (<15 μg $NH_4^+ g^{-1}$ soil, Fig. 2) promoted AOA over AOB, as it has been shown in oligotrophic arid soils (Delgado-Baquerizo et al., 2013) and microcosm soil experiments (Di et al., 2010; Verhamme et al., 2011). However, higher bacterial than archaeal ammonia oxidizers were observed in oligotrophic semi-arid soils (Barton et al., 2013). In addition, it is possible that other environmental factors than ammonium concentration had a stronger effect in modulating AOA and AOB abundance in this ecosystem. For example, the alkaline pH of these soils (Table 1) could have favored AOB over AOA (Nicol et al., 2008; Yao et al., 2011). Another possibility is that the particular chemical properties

from vegetated patches of soil had affected the observed AOB:AOA ratios. Interestingly, Muema and coworkers recently found that litter quality had an effect on ammonia oxidizers abundance, and while only high quality litter (low C:N ratio and polyphenolics concentration) promoted the abundance of bacterial *amoA* genes, archaeal genes were promoted by litter with low polyphenolics concentration, independently of their C:N ratio (Muema et al., 2015).

If studies based on cultured ammonia oxidizers were representative of natural environments, the higher (approximately 10-fold) specific activity of cultured AOB compared to AOA would suggest that the latter should be at least 10 times more abundant than AOB to dominate ammonia oxidizing activity in soils (Prosser and Nicol, 2012). Accordingly, AOB were shown to dominate ammonia oxidation in agricultural soils even though their *amoA* gene abundance was similar to or lower than AOA (Di et al., 2009; Jia and Conrad, 2009). In this study, AOB cell abundance largely outnumbered AOA in all the analyzed dates, under different soil water conditions and litter amendments (based on an assumption of an average of 2.5 and 1 *amoA* genes per bacterial and archaeal genomes, respectively [Norton et al., 2002; Walker et al., 2010], the AOB:AOA cells ratio would range from 4.8 to 1268, Fig. 5). Overall, although *amoA* gene abundance may not necessarily reflect functional activity, and assumptions of ammonia oxidizing activity based on this measure should be cautious (Jia and Conrad, 2009; Prosser and Nicol, 2012), these results could indicate that AOB are stable members of the microbial communities from these arid soils, and they could be playing a more important role than AOA in the oxidation of ammonia in this environment.

5. Conclusions

The results from this work preclude us from rejecting our hypotheses and indicate that grazing-mediated changes in plant litter quality negatively influence nitrification in arid soils, by providing worse substrates with higher concentration of inhibitory compounds. In contrast, plant litter of high quality from lightly-grazed sites combined with high soil water promotes nitrification and AOB abundance, while AOA remain stable at lower abundances. This suggests that AOB could be playing a relevant role in the process of nitrification in this arid ecosystem. Furthermore, it suggests that AOB are adapted to arid conditions, and might be capable of rapidly shifting, particularly if high soil water and plant litter quality are available.

Conflict of interest

The authors declare there is no conflict of interests.

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