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REGULAR ARTICLE

Climatic conditions, soil fertility and atmospheric nitrogen deposition largely determine the structure and functioning of microbial communities in biocrust-dominated Mediterranean drylands

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Abstract
Background and aims Nitrogen (N) deposition and climate change are a threat to the structure and function of drylands, where biocrust-dominated communities are prevalent. We aimed at evaluating the influence of N deposition, climate and edaphic properties of semiarid areas of Spain on soil microbial communities and N cycling.
Methods We quantified soil bacteria, fungi, ammonium oxidizing bacteria and archaea, estimated the abundance of autotrophic organisms (soil pigment content) and measured a wide array of variables related to the N cycle.
Results Local climatic conditions and soil fertility were main drivers of soil microbial communities and N cycling. In particular, cyanobacteria were favored in colder sites with lower soil fertility. Higher precipitation at high-fertility sites favored green algae. Soil N availability was negatively related to MAT. Increased N deposition (4.3–7.3 kg N ha^{-1} $yr.^{-1}$) reduced the abundance of soil bacteria and fungi, a response partially attributed to N-driven soil acidification, whereas it favored green-algae and increased available N in soil, contributing to a net ecosystem eutrophication.
Conclusions Changes in soil microbial community structure and nutrient cycling in response to N deposition and climate change will affect the overall functioning of semiarid Mediterranean ecosystems, which may have important implications in terms of long-term soil C sequestration.

Keywords Acidification · amoA genes · Climate · Eutrophication · Fertility · N deposition · Organic and inorganic N · Soil microorganisms

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Introduction

Many studies have shown negative impacts of atmospheric nitrogen (N) deposition on terrestrial ecosystems worldwide in terms of reduced native biodiversity and ecosystem services (Bobbink et al. 2010; Phoenix et al.

2006; Sala et al. 2000). This atmospheric pollution is the result of unprecedented rates of food production and energy consumption (Bobbink et al. 2010; Gruber and Galloway 2008). Other impacts of enhanced N deposition are increased soil eutrophication and acidification (Stevens et al. 2011), increased base cation leaching and concentration of toxic metals in soil (Horswill et al. 2008) and increased export of nitrate (NO_3^-N) and dissolved organic N (DON) and carbon (DOC), causing eutrophication in streams and rivers (DeForest et al. 2005; Pregitzer et al. 2004).

Nitrogen is, after water, the most important factor limiting net primary production and organic matter decomposition in drylands (Robertson and Groffman 2007). In low-productivity semiarid Mediterranean sites, recent studies have shown how increased N deposition can reduce both the accumulation of organic matter in soils (Ochoa-Hueso et al. 2013a) and productivity of certain local plant species (Ochoa-Hueso et al. 2013b), which may be partially attributed to the negative effects of excessive ammonium (Ochoa-Hueso et al. 2013b). An increase in N deposition may also promote alterations in the availability of different organic and inorganic N forms (Schimel and Bennett 2004), which may affect competitive interactions among species due to their different preferences for different N forms (Nordin et al. 2001; Warren 2009).

Other factors that are currently altering ecosystem structure and function in Mediterranean areas include land use and climate change (Sala et al. 2000). Future scenarios of climate change in Mediterranean regions have been related to a decrease in precipitation, increased temperatures and increased recurrence of extreme events (Gao and Giorgi 2008; Miranda et al. 2011). Despite this, the potential interactions between increased N deposition and climate change and its ecological consequences still remain unclear. Increased N deposition can modify the structure and composition of soil microbial communities (e.g., (van Diepen et al. 2010, 2011; Zechmeister-Boltenstern et al. 2011; Zhang et al. 2011)). These communities regulate soil biogeochemical cycles, and thus their modification by ecosystem N enrichment should alter nutrient cycling, particularly the N and C cycles (Phoenix et al. 2012), and the ability of soils to store these elements in the form of organic matter (DeForest et al. 2005; Treseder 2008). In this sense, increased and/or decreased N mineralization rates (Biudes and Vourlitis 2012; Sinsabaugh et al. 2015), reduced N fixation (DeLuca et al. 2007), altered function of C-degrading enzymes (Zak et al. 2011) and augmented concentrations of different forms of dissolved organic and inorganic N in soils (Phoenix et al. 2012) are expected consequences of increased N deposition mediated by soil microorganisms.

Low-productivity semiarid Mediterranean ecosystems, such as native shrublands, woodlands and tussock grasslands, can be dominated by biological soil crusts (biocrusts), a highly diverse consortium of soil microorganisms, including bacteria and cyanobacteria, microalgae, lichens and mosses (Belnap et al. 2008; Maestre et al. 2011). These communities, considered as a good model system in ecosystem ecology (Bowker et al. 2014), play an important role regulating the water, C and N cycles (Castillo-Monroy et al. 2010; Li et al. 2012; Maestre et al. 2011) and increasing soil stability (Hawkes 2003). For example, biocrusts contribute to C and N fixation in soils (Belnap 2002; Li et al. 2012), and thus increase soil fertility, while they can also modulate N transformation rates (e.g. nitrification) in dryland soils (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2013; Sinsabaugh et al. 2015). However, little is known on how N deposition and climate variability may simultaneously affect the abundance and physiology of both heterotrophic and autotrophic communities associated with biocrusts in drylands at the global scale, where any anthropogenic negative impacts on these communities would likely be followed by a reduction in the amount and quality of goods and services supplied by them (Maestre et al. 2012; Reed et al. 2012). This knowledge will be particularly relevant for dryland ecosystems from the Mediterranean Basin and California, where the interactive impacts of N deposition and climate change are predicted to be particularly high (Ochoa-Hueso et al. 2011a).

In this study, we investigated if overlapping natural and anthropogenic environmental gradients (local climatic and edaphic conditions and atmospheric N deposition) can explain variables related to the structure and function of soil microbial communities from semiarid Mediterranean ecosystems (soil pigment composition and total bacterial and fungal abundance, and abundance of ammonia-oxidizing bacterial and archaeal genes; hereafter AOB and AOA). We hypothesized that chronic N deposition can significantly alter soil microbial communities and the N cycle in semiarid Mediterranean ecosystems from Spain, although to a lesser extent than climate and local edaphic factors (Gaudnik et al. 2011; Limpens et al. 2011). We

specifically predicted that N deposition would be negatively related to total soil bacterial and cyanobacterial abundance, as evidenced in multiple fertilization studies (Belnap et al. 2008; Eisenlord and Zak 2010; Tian et al. 2014; Treseder 2008; Wei et al. 2013), and positively related to organic and inorganic N availability in soil (Fang et al. 2009; Phoenix et al. 2012; Waldrop and Zak 2006; Wei et al. 2013). We also predicted both AOA and AOB to increase along with N deposition and temperature as a consequence of an increase in substrate availability, with AOB being more favored than AOA along the N gradient (Tian et al. 2014). Similarly, we hypothesized a relative increase of bacteria in relation to fungi along the N deposition gradient (Demoling et al. 2008; Wei et al. 2013). Finally, we predicted an overall acidification of soils as a consequence of increased N deposition, particularly in the case of gypsiferous soils, which are naturally less buffered than calcareous soils (Phoenix et al. 2012; Stevens et al. 2009).

Materials and methods

Study sites and atmospheric N deposition gradient

A total of 16 study sites were sampled along the atmospheric N deposition gradient found in central, southern and eastern semiarid Mediterranean Spain (ranging between 4.3 and 7.3 kg N ha^{-1} $yr.^{-1}$). Nitrogen deposition estimates were obtained from the CHIMERE model applied for 2009 and for a domain covering the Iberian Peninsula and the Balearic Islands at a 0.1° horizontal resolution (Vivanco et al. 2009) and are representative of the N deposition loads in this area (García-Gómez et al. 2014). Based on our modelling data, oxidized N deposition in the area dominates over the reduced N fraction (1.9 times higher). We sampled low-productivity semiarid shrublands ($n = 4$), tussock grasslands ($n = 10$) and woodlands ($n = 2$) located below 1000 m a.s.l., with annual rainfall ranging between 300 and 650 mm, and with the presence of a well-developed biocrust community; soils were derived from either calcareous ($n = 7$) or gypsiferous ($n = 9$) parent material (see Bowker et al. 2011; Ochoa-Hueso et al. 2013a).

Soil sampling and analyses

Three independent soil samples (0–1 cm depth) were collected at all sites in May-September 2008 and May-August 2009, as described in Bowker et al. (2011). This sampling depth was selected because most soil organisms associated with biocrusts are concentrated in the first centimeter (Belnap et al. 2008). Samples were transported to the laboratory in cool conditions, where they were sieved (2 mm mesh) and separated into two fractions. One fraction was immediately frozen at −80 °C for pigment and molecular analyses and the other was air-dried for 1 month for analyses of variables of the C and N cycles. Soil biochemical properties are hardly affected by air-drying in semiarid Mediterranean soils, which otherwise are under dry conditions most of the year (Delgado-Baquerizo et al. 2014; Zornoza et al. 2009).

For soil pigment determinations, 1 g of defrosted soil was initially ground with 0.5 ml of deionised water; then another 4 ml of HPLC-acetone was added and the grinding was repeated. The samples were then transferred to test tubes and the final volume was brought to 10 ml (final concentration of 95 % acetone). The headspace of each tube was filled with helium, sealed with Parafilm, and refrigerated at 8 °C overnight. 24 h later, samples were filtered through GF/F filter paper and condensed to a 3 ml volume using helium. Soil pigments were separated by HPLC according to the method of Val et al. (Val et al. 1994) slightly modified as described in Martínez-Ferri et al. (Martínez-Ferri et al. 2000). After passing through a 0.45 μm nylon filter, 25 μL of the extract was injected into a C18 column (ACE 5 C18-AR, ACE, Scotland). The mobile phase rate was 1.2 mL min^{-1} and the elution time lasted 30 min. Solvents for HPLC analysis were degassed before use by bubbling helium. The Waters HPLC system was equipped with a Waters 996 photodiode array detector (Waters, USA). For peak identification and quantification, pure commercial standards (VKI, Hørsholm, Denmark) of neoxanthin, violaxanthin, diadinoxanthin, myxoxanthophyll, anteraxanthin, lutein, zeaxanthin, cantaxanthin, chlorophylls *a*, *b* and c_2, echinenone and β-carotene were used. Because scytonemin was not commercially available, it was estimated from its peak area at 436 nm as described in Bowker et al. (Bowker et al. 2002).

To quantify the abundance of total bacterial, fungal, AOB and AOA genes in the studied sites, soil DNA was extracted from 0.6 g of defrosted soil using the MoBio Powersoil DNA Isolation Kit (Carlsbad, USA) according to the instructions provided by the manufacturer. We performed quantitative PCR reactions in triplicate using

96-well plates on a AB 7300 Real-Time PCR (Life Sciences Technologies, Carlsbad, California, USA). The Bacterial 16S, fungal 18 s, AOB and AOA genes were amplified with the *Eub 338-Eub 518*, *ITS 1-5.8S (*Evans and Wallenstein 2011*)*, *amoA1F-amoA2R* (Rotthauwe et al. 1997) and *Arch-amoAF-ArchamoAR* (Francis et al. 2005) primer sets, respectively. The 25 μl reaction mixture contained: 12.5 μL FastStart Universal SYBR Green Master (Rox), 1.25 μL (10 mM) each primer, 1 μL BSA, 1-10 ng template DNA and ultra-clean water to volume. The cycling conditions were 95 °C for 10 min, followed by 35 cycles of 95 °C 60 s; 53 °C 30 s and 72 °C 60 s, for both the fungal and bacterial primer sets, and 95 °C for 10 min, followed by 35 cycles of 95 °C 60 s; 55 °C 45 s and 72 °C 60 s, for both the AOB and AOA 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. 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 *Eub 338-Eub 518*, *ITS 1-5.8S*, *amoA1F-amoA2R* and *Arch-amoAF-ArchamoAR* primer sets were used to amplify the Bacterial 16S, Fungal 18 s, AOA and AOB genes from DNA extracted from soil samples. In parallel, the four PCR products were cloned into *E. coli* using a TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. Plasmid DNA was extracted with a Plasmid Mini Kit (Invitrogen), and the insert was sequenced. The results were compared to known the respective genes in the GenBank database (http://www.ncbi.nlm.nih.gov) using the BLAST application. BLAST analysis showed that the sequences were >99 % similar to known fungal, bacterial, AOA and AOB genes, respectively.

Soil organic C was estimated using the Yeomans and Bremmer (1989) method, whereas soil organic N was measured using a SKALAR San^{++} Analyzer (Skalar, Breda, The Netherlands) after digestion in sulfuric acid. Because the response of organic C and total N to increased N deposition has already been studied in detail by Ochoa-Hueso et al. (2013a), soil organic C (2.6 % on average) and N (0.46 % on average) are considered here only as general indicators of soil fertility (first component of a principal component analysis (PCA) explaining 86.5 % of the total variance and positively related to both C and N). For the rest of the measured variables related to the N cycle, soil samples were extracted with K_2SO_4 0.5 M in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm for 1 h at 20 °C and filtered to pass a 0.45-μm Millipore filter (Jones and Willett 2006). The filtered extract was kept at 4 °C until colorimetric analyses, which were conducted within the 24 h following the extraction. Sub-samples of each non-incubated (air-dried) extract were taken for measurements of amino acids and proteins according to Chantigny et al. (Chantigny et al. 2006). Ammonium, nitrate and DON were measured following Delgado-Baquerizo et al. (2011). Total available N was calculated as the sum of ammonium, nitrate and DON. Soil pH was measured in all the soil samples with a pH meter, in a 1:10 mass:volume soil and water suspension.

Statistical analyses

Two PCAs were conducted with soil microbial (hereafter PCA_Micro) and N cycle (hereafter PCA_Nitro) variables, respectively, to identify common patterns of response of microbial community attributes and N cycling-related processes to environmental gradients. Then we followed a multi-model selection approach and chose the model with the lowest AIC value (Burnham and Anderson 2002). We evaluated all possible linear regression models (main effects only, no interactions) containing the different descriptors of the soil microbial community evaluated (components of the PCA_Micro) and biogeochemical variables (components of the PCA_Nitro) and the following independent variables: atmospheric N deposition gradient, mean annual temperature (MAT) and rainfall (MAP), and soil fertility and pH. Fertility was not considered in the analysis of the PCA_Nitro. In our dataset, rainfall and N deposition were weakly and negatively correlated (logarithmic regression; $r^2 = 0.336$; $P < 0.05$) and so effects of both were often difficult to separate from each other, a pattern commonly observed in other studies (Allen et al. 2007). In contrast, temperature and N deposition were not significantly correlated in our data set. To help elucidate some of the details of the patterns detected in the multimodel analysis and also to better understand the potential implications of N deposition, further linear and non-linear bivariate regression analyses were conducted to independently relate our dependent variables to the atmospheric N deposition gradient

studied. In order to check for the potential confounding role of vegetation type in the response of our studied variables to the environmental gradients evaluated, we used ANOVAs to compare between the values of both dependent and independent variables at sites dominated by tussock grasses and sites dominated by woody vegetation.

Multimodel analyses were conducted with SAM 4.0 whereas the rest of the statistical analyses were carried out with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

None of the environmental drivers considered in this study (climate, fertility and N deposition), nor any of the dependent variables measured, with the exception of soil protein content, differed significantly ($P > 0.05$) between sites dominated by tussock grasses and sites dominated by woody vegetation (shrublands + woodlands). For this reason, all results reported hereafter refer only to the 16 study sites as a whole.

Soil pigments and N cycling

Scytonemin, a photoprotective pigment exclusively found in cyanobacteria, was the most abundant pigment in the studied sites, followed by chlorophyll *a* and *b* (Table S1). Overall, we found pigments typical for cyanobacteria (e.g., scytonemin, zeaxanthin, cantaxanthin, echinenone), green algae (e.g., violaxanthin, chlorophyll *b*, lutein) and diatoms (e.g., diadinoxanthin, present in 7 out of 16 sites). Chlorophyll *a* and β-carotene were, in contrast, not exclusively attributable to any major group of soil microorganisms. Chlorophyll c_2 (diatoms) and myxoxanthophylls (cyanobacteria) were not found in any soil sample. In addition, bacteria were clearly more abundant in soil than fungi, whereas the nitrifying soil community was dominated by archaea (Table S1). Organic N availability dominated over the inorganic fraction in the studied soils; within the inorganic fraction there was a slight dominance of NH_4^+–N over NO_3^-N (Table S1).

Multivariate analysis of soil microorganisms and response to environmental drivers

Principal component analyses with soil microbial variables resulted in six main components with eigenvalues >1 that explained 86.7 % of the total variance found in our data (Table S2). Our analyses showed that the first component, highly associated with photoprotective pigments (scytonemin and β-carotene) and therefore possibly related to the light environment experienced by soil microorganisms, was best explained by the combination of temperature and fertility (Table 1; Fig. 1a). The second component (explained by chlorophyll *b*, zeaxanthin and lutein contents and therefore linked to green algal abundance) was positively related to MAP and N deposition (Table 1), suggesting that despite being correlated with one another, these two variables explain complementary portions of the total variance found for soil microbes. The third component, positively associated with total bacterial abundance, fungal abundance, and cyanobacterial pigments (echinenone and canthaxanthin) and negatively with green algal pigments (lutein and neoxanthin), was negatively related to the atmospheric N deposition gradient and soil fertility and positively related to soil pH (Table 1; Fig. 1f). This component was not significantly related to MAP. The fourth component, highly associated with AOA and diatoms, was explained by fertility, whereas the fifth component (associated with AOB and diatoms) was explained by MAT (Table 1). Finally, the sixth component, negatively associated with green algae, was negatively related to soil fertility.

Multivariate analysis of N cycling and response to environmental drivers

Principal component analyses conducted with all the variables related to the N cycle resulted in a main component explaining 56.1 % of the total variance (Table S3). Our multimodel analysis showed that this component, associated with an overall ecosystem eutrophication, was negatively related to MAT but not to N deposition (Table 1).

Effects of N deposition on soil microorganisms and N cycling

Univariate regressions showed significant relationships between modeled atmospheric N deposition and soil echinenone content, bacterial abundance, DON content, total available N, DON/DIN ratio and pH (Fig. 2). The significant decrease in soil pH with increasing N deposition was largely due to the influence of gypsum soils, whereas the relationship between soil pH and N

Table 1 Multimodel analyses with 16 locations for PCA components from the PCAs with soil microbial community-related indicators and N cycling-related indicators. See Tables S2 and S3 for the loading factors of each component. *AOB* ammonia-oxidizing bacteria; *AOA* ammonia-oxidizing archaea. Only best model is shown and the sign of each effect is shown between brackets

Microbial community	Variables	R^2
Component 1 – cyanobacterial and photoprotective pigments	Temperature(↓) + Fertility(↓)	0.423
Component 2 – green algae	Rainfall(↑) + **Nitrogen deposition(↑)**	0.720
Component 3 – bacteria and fungi	**Nitrogen deposition(↓)** + pH(↑) + Fertility(↓)	0.626
Component 4 – AOA and diatoms	Fertility(↓)	0.157
Component 5 – AOB and diatoms	Temperature(↑)	0.461
Component 6 – green algae	Fertility(↓)	0.233
Nitrogen cycle		
Component 1 – eutrophication	Temperature(↓)	0.222

deposition in calcareous sites was non-significant, despite the negative trend observed (Fig. 2f). Contrary to our predictions, we did not observe any shift in the AOA:AOB ratios along the N deposition gradient which, in contrast, decreased exponentially with increasing soil C:N values ($R^2 = 0.65$; $P < 0.01$). Also in contrast to our predictions, the bacteria:fungi ratio was negatively and positively correlated with pH ($R^2 = 0.29$; $P < 0.05$) and DON ($R^2 = 0.29$; $P < 0.05$), respectively, but not with the N deposition gradient. We found additional significant correlations between the third component of the PCA_Micro and the N x pH interaction (logarithmic negative regression; $r^2 = 0.33$; $P < 0.05$). Linear and non-linear relationships between bacterial abundance, total available N and soil pH (indicators of ecosystem eutrophication and acidification, respectively) and the rest of the environmental variables studied, including MAP, were not significant ($P > 0.05$ in all cases; data not shown), confirming the strong influence of atmospheric N deposition on them.

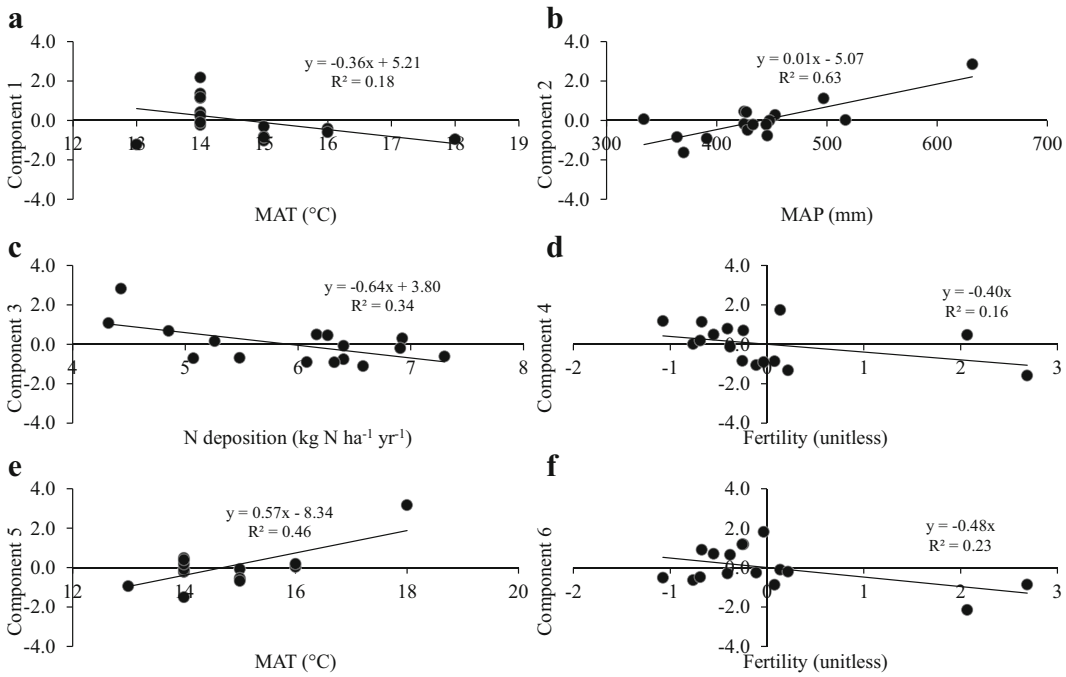

Fig. 1 Relationships between the main components of the PCAs and their main environmental predictors. Microbial community: component 1 = cyanobacterial and photoprotective pigments (**a**); component 2 = green algae (**b**); component 3 = bacteria and fungi (**c**); component 4 = AOA and diatoms (**d**); component 5 = AOB and diatoms (**e**); component 6 = green algae (**f**). *MAT* mean annual temperature; *MAP* mean annual precipitation

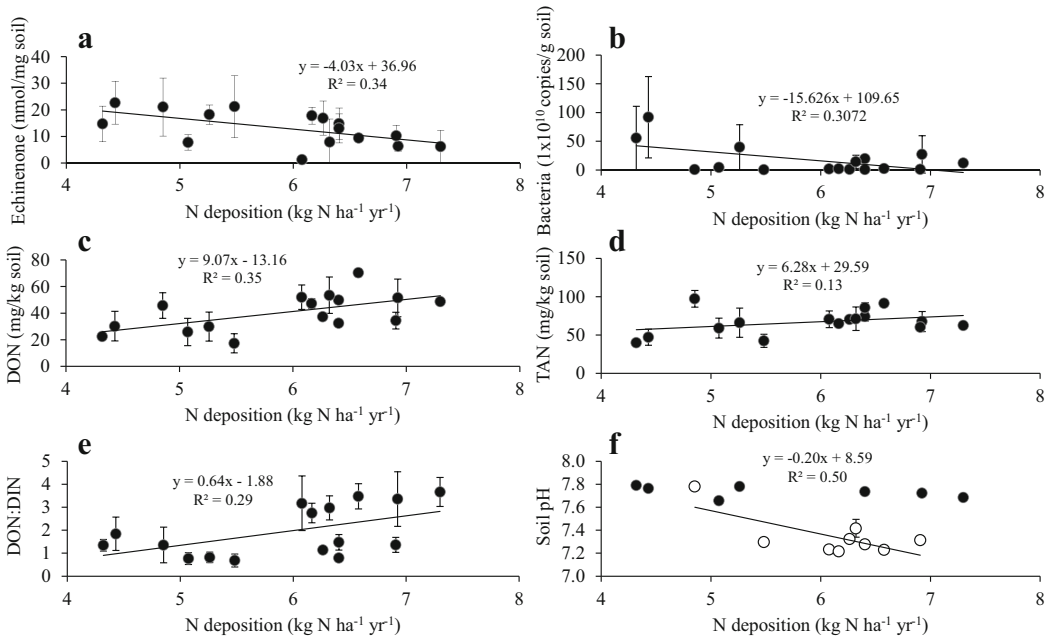

Fig. 2 Relationships between atmospheric N deposition and the concentration of echinenone (**a**), total bacterial abundance (**b**), dissolved N content (**c**), total available N (**d**), DON/DIN ratio (**e**) and soil pH (**f**). Data on the y axis are means ± SE, where $n = 3$ except in a few cases due to analytical constraints (5 missing samples out of 48 for total bacteria) or to unavailability of enough soil material (7 missing samples out of 48 for pH)

Discussion

Accumulated experimental and observational data from multiple sources suggest that Mediterranean ecosystems in Europe are currently being impacted by increased atmospheric N deposition (Bonanomi et al. 2006; Ochoa-Hueso et al. 2011a; Rodà et al. 2002). In the present study, we are showing how moderately low levels of N deposition (4.3–7.3 kg N ha^{-1} $yr.^{-1}$) are affecting microbial communities and soil properties linked to the global biogeochemical cycles in a semiarid Mediterranean region. However, impacts of N deposition on microbial communities and the N cycle were often secondary when compared to the unequivocally important role exerted by a wide array of local climatic and edaphic factors (e.g., (Allen et al. 2007; Hessen et al. 2009; Rao et al. 2011)), as initially predicted.

Impacts of environmental drivers on soil microorganisms

Scytonemin was the most abundant pigment in our study sites, indicating that cyanobacteria are the most dominant photosynthetic microbial group present in their soils, which is in agreement with other studies conducted in drylands (Belnap et al. 2008; Bowker et al. 2002). Together with cyanobacterial pigments, a wide array of pigments characteristic of other photosynthetic organisms (e.g., green microalgae and diatoms) were also present in the studied soils, highlighting their role as a highly valuable and often neglected reservoir of biodiversity (Dominati et al. 2010). In agreement with other experimental studies conducted in a variety of ecosystem types, including low litter-input semiarid Mediterranean ecosystems (Hueso et al. 2012) and northern high litter-input forests (Zechmeister-Boltenstern et al. 2011), bacteria dominated over fungi (at least in terms of number of DNA copies g $soil^{-1}$) in soils from the study sites.

Role of climate and soil fertility

Most of the soil pigment variability was explained by climate (MAT and MAP) and soil fertility. The negative relationship of component 1, highly associated with photoprotective cyanobacterial pigments typically found in open interspaces, with MAT and fertility suggests that it is probably the degree of canopy closure (putatively higher at high fertility, high MAT sites), and

thus competition with vascular plants, the main factor determining the abundance of soil photosynthetic microorganisms (Berkeley et al. 2005; Dougill and Thomas 2004). The positive relationship between component 2 and MAP is consistent with the evidence that demonstrates that water availability is a main limiting factor for dryland biota, and thus a primary driver of primary productivity and function in Mediterranean ecosystems (e.g., (Cox and Allen 2008; Ochoa-Hueso and Manrique 2010)), whereas the relationship between the abundance of AOB genes with MAT further supports the important role of climate on N cycling processes (Yun et al. 2011) and, consequently, on the availability of different N forms (Aranibar et al. 2003; Austin and Sala 2002; Griffiths et al. 2009). The role of temperature on N cycling processes was additionally supported by the significant relationship between this climatic factor and the first component of the PCA_Nitro, which was negatively associated with the availability of different forms of organic (particularly amino acids) and inorganic N. Taken together, these results suggest the profound implications that a reduction in mean rainfall, an increase of recurrence of drought events, and an increase in mean and maximum temperatures, as forecasted by climatic models, can have on soil microbial communities and nutrient cycling in arid and semiarid regions (Miranda et al. 2011; Zelikova et al. 2012). In particular, decreased rainfall, especially at high-fertility sites, may reduce green algal dominance, whereas higher temperatures may result in lower abundance of cyanobacteria and less N availability, particularly inorganic N. In support of this, Delgado-Baquerizo et al. (Delgado-Baquerizo et al. 2014) found a decrease in total N under increasing aridity conditions (higher temperatures and lower rainfall), which suggests that reduced N availability could be a real long-term consequence of climate change. However, given that N availability is the result of organic matter depolimerization and mineralization processes, which has been typically shown to increase along with temperature, and N uptake, which in turn depends on the structure and composition of the plant community and the amount of water available, our results could also just be an indirect indication of the relationship between higher N demand and use at warmer sites.

Role of N deposition

Reduced soil bacterial abundance (Eisenlord and Zak 2010; Treseder 2008) and the alteration of the relative dominance between soil bacteria and fungi (e.g., (Cusack et al. 2011; Demoling et al. 2008; Zechmeister-Boltenstern et al. 2011)) have frequently been observed in N-manipulation experiments conducted in a variety of habitats. The reduction in bacterial abundance along the N deposition gradient in this study was also associated with a significant decrease in echinenone content, a soil pigment exclusive of cyanobacteria, which can be also indicating an overall decrease in the abundance of this particular soil group with increased N deposition (Ramirez et al. 2012). In fact, reductions in soil cyanobacterial pigment concentration, including echinenone and scytonemin, have already been reported under N addition experiments (Belnap et al. 2008), although the mechanisms behind this significant decrease are poorly understood. This significant decrease in the abundance of bacteria adds up top recent findings that suggest that certain low-productivity semiarid Mediterranean sites might not able to store extra C in soil as part of (living and/or dead) organic matter under increased N deposition (Ochoa-Hueso et al. 2013a). The potential reduction in soil organic matter accumulation in semiarid Mediterranean ecosystems contrasts with the evidence from northern hardwood and boreal forests, where a higher accumulation of partially decomposed organic matter in soil as a consequence of reduction in litter decay has been described in parallel with increased simulated N deposition (Maaroufi et al. 2015; Zak et al. 2011). Contrasting results may be attributed to the relative importance of litter inputs to organic matter accumulation and turnover in soil and also to the quality of such organic matter inputs (Ochoa-Hueso et al. 2014; Zak et al. 2011). Whereas litter inputs are high in northern forests, such inputs are much lower in semiarid Mediterranean ecosystems. These alterations in soil organic matter have been described in parallel with alterations in the regulation of soil enzymes related to the main biogeochemical cycles (Zak et al. 2011) and with a reduction of soil respiration rates and microbial biomass (Treseder 2008). In contrast to the decrease in soil cyanobacterial abundance reported in this study, Ochoa-Hueso et al. (2013a) found a significant increase in cyanobacterial richness associated with N fertilization in a semiarid Mediterranean shrubland in central Spain. This response was attributed to a proliferation of facultative or non-N-fixers able to thrive under high soil N levels (i.e., copiotrophic taxa sensu; Ramirez et al. 2012). However, this overall increase in cyanobacterial

richness could also be coupled with a significant decrease in its total abundance, which makes these results compatible with those presented here. In contrast to bacterial and cyanobacterial abundance, our analyses showed that green micro-algae are favored by N additions (loading factors of component 2 and 3 of PCA_Micro), which is in agreement with other studies that have shown a proliferation of green algae in response to ecosystem eutrophication (Poikolainen et al. 1998).

Based on our experimental evidence, we suggest that soil acidification could be playing an important role in soil bacterial, and possibly cyanobacterial, community response to N deposition, as suggested by the significant correlations between the third component of the PCA_Micro and the N x pH interaction. This soil acidification is attributed to altered N mineralization rates, increased N uptake by plants and soil organisms and increased nitrate leaching, which also contributes to drain base cations as counter-ions (Horswill et al. 2008; Phoenix et al. 2012). Nitrogen deposition effects in basic soils are often predicted to operate mainly through ecosystem eutrophication, while acidification has been predicted to play only a minor role (Ochoa-Hueso et al. 2011a; Stevens et al. 2011). However, cumulative experimental evidence from an N manipulation experiment conducted in central Spain suggests that small variations in soil pH in basic soils could also be playing a strong role in the response of different biological groups that form part of the local soil communities (e.g., early and late successional biocrusts; Ochoa-Hueso et al. 2011b, 2013c).

Similar to the response of soil microorganisms, the positive relationships between N deposition and DON and total N availability suggest that important alterations in the N cycle as a consequence of increased atmospheric N deposition are currently taking place. Increased DON and DOC concentrations in soils have frequently been reported after N fertilization or along N deposition gradients (DeForest et al. 2005; Fang et al. 2009; Pregitzer et al. 2004), a response that has been attributed to a reduction in the activity of certain oxidative and hydrolytic soil enzymes, such as phenol oxydase and β-glucosidase (Waldrop and Zak 2006; Ochoa-Hueso et al. 2013a). This increase in DON and DOC contents after increased N deposition has been related to increased DON and DOC export down into the soil profile with rainfall events (e.g., (DeForest et al. 2005; Fang et al. 2009; Pregitzer et al. 2004)) and, therefore, high DON, DOC and NO_3^-N concentrations are usually reported in streams and rivers from N-polluted sites as a consequence of increased N leaching and a loss of N retention capacity (DeForest et al. 2005; Pregitzer et al. 2004). In arid and semiarid ecosystems, the characteristic decoupling between peaks of N availability and N demand by local plant and microbial communities also makes them more susceptible to N saturation and N leaching (McCulley et al. 2009). However, the preferential accumulation of DON with increased N deposition may also affect the N uptake by plants and microorganisms because of their different preferences for inorganic and organic N forms (Nordin et al. 2001; Warren 2009), thus altering the structure and composition of local communities (Zak et al. 2011). In contrast to northern forests, the observed increase in DON and DOC with increasing N deposition may be associated with a reduction in the ability of terrestrial ecosystems to store extra C and N in soil, and thus to mitigate the negative effects of climate change (Ochoa-Hueso et al. 2013a). This hypothesis needs to be explored more extensively.

Conclusions

As initially hypothesized, local climatic and edaphic factors were main explanatory variables of microbial community structure and function, and also of biogeochemical variables related to the N cycle. In particular, MAP and MAT were the most important predictors of microbial community structure and of variables related to the N cycle, respectively. Superimposed on all of these natural gradients, the anthropogenic N gradient evaluated unequivocally caused both ecosystem eutrophication and acidification. In this study, gypsum sites, less buffered than calcareous sites, seemed more susceptible to suffer soil acidification, whereas the eutrophication process was widespread. Such increases in N availability and soil acidification have altered soil microbial communities. Specifically, soil bacterial and cyanobacterial abundance were significantly reduced by increased N deposition, probably via soil acidification, which adds up to the existing evidence on the low ability of low-productivity semiarid ecosystems to store extra C and N in soil under increased N deposition scenarios. In contrast, green algae, usually considered as bioindicators of ecosystem eutrophication, increased along with N deposition. Dissolved organic N

concentration in soil was also increased by N deposition, which means that this fraction could be more easily leached down from soils during and after rainfall events, thus contributing to a significant net ecosystem N loss and stream water eutrophication. Finally, and given the cumulative evidence of negative impacts of current levels of N deposition on semiarid terrestrial ecosystems, we recommend an urgent implementation of political and technological strategies to avoid as much as possible the future consequences of this undesirable environmental threat.

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