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REGULAR ARTICLE

Biological soil crusts modulate nitrogen availability in semi-arid ecosystems: insights from a Mediterranean grassland

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Abstract Biological soil crusts (BSCs) greatly influence the N cycle of semi-arid ecosystems, as some organisms forming them are able to fix atmospheric N. However, BSCs are not always taken into account when studying biotic controls on N cycling and transformations. Our main objective was to understand how BSCs modulate the availability of N in a semi-arid Mediterranean ecosystem dominated by the tussock grass *Stipa tenacissima*. We selected the six most frequent soil cover types in the study area: *S. tenacissima* tussocks (ST), *Retama sphaerocarpa* shrubs (RS), and open areas with very low (BS), low (LC) medium (MC) and high (HC) cover of well developed and lichen-dominated BSCs. The temporal dynamics of available N dynamics followed changes in soil moisture. Available NH_4^+-N did not differ between microsites, while available NO_3^--N was substantially higher in the RS than in any other microsite. No significant differences in the amount of available NO_3^--N were found between ST and BS microsites, but these microsites had more NO_3^--N than those dominated by BSCs (LC, MC and HC). Our results suggest that BSCs may be inhibiting nitrification, and highlight the importance of this biotic community as a modulator of the availability of N in semi-arid ecosystems.

Keywords Biological soil crusts · Semi-arid ecosystem · Nitrogen availability · Nitrification potential · *Stipa tenacissima* · Nitrogen cycle

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Introduction

Nitrogen (N) cycling is a fundamental ecological process that regulates the structure and function of many ecosystems (Vitousek et al. 2002). Compared to other ecosystems, the N cycle in arid and semi-arid ecosystems is characterized by a relatively low N availability because of low atmospheric deposition inputs and low N_2-fixation rates by microorganisms (Peterjohn and Schlesinger 1990, 1991). Despite their low overall N availability, it is estimated that 30% of the total gaseous N- emissions (N_2, N_2O, NO_x, NH_3) from the global N-cycle come from arid and semi-arid ecosystems (Bowden 1986), highlighting its importance for the global N cycle.

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By acting as a 'gatekeeper' determining whether local N pools remain reduced or are oxidized, the transformation of ammonium into nitrate is a key process in the overall N cycle (Harmsen and Van Schreven 1955; Aber and Melillo 2001). Most of the studies on nitrification in arid and semi-arid regions have been conducted in the United States, with additional work carried out in Australia, Israel and South America (e.g., Mazzarino et al. 1991; Schlesinger et al. 1996; Eldridge and Greene 1994; Zaady et al. 1996). Surprisingly, little research has been conducted in areas such as the Mediterranean Basin and Africa (but see Bastida et al. 2009; Aranibar et al. 2004), despite semi-arid areas are common there. Expanding current research efforts to characterize the dynamics and rates of nitrification in semi-arid ecosystems from these regions is of paramount importance to improve our understanding of the global N cycle.

Arid and semi-arid ecosystems are characterized by marked patchiness in the distribution of vegetation, with discrete patches of vascular plants and "open" areas devoid of vascular vegetation (Valentin et al. 1999). The latter are commonly dominated by biological soil crusts (BSCs), assemblages of lichens, fungi, cyanobacteria, and mosses that colonize the soil surface, which can represent up to 70% of the living cover in these areas (Belnap and Lange 2001). Biological soil crusts exert a great influence on ecosystem functioning by affecting soil stability, by fixing CO_2, by modulating water fluxes through their effects on runoff and infiltration, by influencing the establishment and performance of vascular plants, and by serving as habitats for a large number of arthropods and microorganisms (West 1990; Eldridge and Greene 1994; Belnap and Lange 2001).

The role of plant patches in the spatio-temporal N dynamics in arid and semi-arid ecosystems has been acknowledged for a long time, as the spatial pattern of soil resources is largely determined by the accumulation of soil nutrients beneath plant canopies (García-Moya and Mckell 1970; Smith et al. 1994; Schlesinger et al. 1996). BSCs play also key roles in the N cycle, as N-fixing lichens and free-living, heterotrophic bacteria forming part of BSCs are able to fix substantial amounts of atmospheric N (Evans and Ehleringer 1993; Belnap 2002). However, BSCs are not always taken into account when studying biotic controls on N cycling and transformations in semi-arid ecosystems (Peterjohn and Schlesinger 1990, 1991; Schlesinger et al. 1996). In this regard, the extent and importance of other N transformations in BSC-dominated ecosystems remains understudied or controversial (Belnap 2002; Evans and Lange 2001).

Improving our knowledge of the dynamics of N transformations in BSC-dominated areas is crucial to improve our understanding of nutrient cycling in arid and semi-arid environments, to balance the importance of these areas for the global N cycle, and to accurately predict how they will respond to the ongoing global environmental change and the increases in N inputs associated to it (Norby 1998). To advance in this direction, we evaluated the effects of both vascular plants and BSCs on the availability of ammonium and nitrate, and on microbial biomass-N and depolymerization rates, in a semi-arid Mediterranean grassland dominated by *Stipa tenacissima* L. Soil microorganism may act both as source and sink of nutrients, and thus is crucial to consider them when studying N transformations in soil (Singh et al. 1989, Wardle 1992). Depolymerization rate is emerging as a key process in the nitrogen cycle (Schimel and Bennet 2004). Our main objective was to understand the effects of BSCs on the availability of N in this ecosystem. To achieve this objective, we quantified the spatio-temporal variability in N availability among the dominant plant- and BSC microsites, measured seasonally over a two-year period, and evaluated the relative importance of BSCs as contributors to soil N dynamics at the ecosystem level.

Given their role as N fixers (Belnap 2002), we hypothesize that BSCs should increase N availability compared to adjacent bare ground soil, and that this effect should be maximized with increases in BSC cover.

Materials and methods

Site description

This research was conducted in the Aranjuez Experimental Station, in the centre of the Iberian Peninsula (40º02' N – 3º 37'W; 590 m a.s.l.; 8º slope facing

Table 1 Main soil characteristics for the different microsites sampled

	0–4 depth				0–10 depth		
	BS	LC	MC	HC	ST	BS	RS
pH	7.2±0.06	7.2±0.05	7.2±0.03	7.4±0.07	7.7±0.05	7.4±0.08	7.4±0.04
OC ($mg \cdot g^{-1}$ soil)	9.0 ± 1.1^{A}	13.0 ± 1.9^{A}	12.0 ± 1.3^{A}	14.0 ± 1.1^{A}	15.0 ± 2.1^{a}	10.0 ± 3.1^{a}	32.0 ± 0.9^{b}
TN ($mg \cdot g^{-1}$ soil)	0.8 ± 0.08^{A}	1.4 ± 0.17^{B}	1.4 ± 0.12^{B}	1.4 ± 0.20^{B}	1.8 ± 0.32^{a}	1.6 ± 0.31^{a}	4.0 ± 0.17^{b}
Silt (%)	38.0±1.09	32.4±1.16	32.0±2.28	30.0±2.75	29.2±0.97	28.4±0.8	28.4±0.74
Clay (%)	6.3±0	6.3±0	6.3±0	6.3±0	6.7±0.48	7.1±0.4	7.9±0.40
Sand (%)	55.7±1.09	61.3±1.16	61.7±2.28	63.7±2.75	64.1±0.80	64.5±0.97	63.7±0.63

OC organic carbon, *TN* total nitrogen, *ST Stipa tenacissima*, *RS Retama sphaerocarpa*, *BS* Bare soil, *LC* low biological soil crust (BSC) cover, *MC* medium BSC cover, *HC* high BSC cover

Soil samples correspond to the Autumn-2008 sampling. Data represent means ± SE ($n=5$). Different superscripts in organic carbon and total nitrogen indicate significant differences between microsites after one-way ANOVA (Stuart-Newman-Keuls post-hoc test; $P < 0.05$)

SE). The climate is Mediterranean semi-arid, with a 30-year average rainfall and temperature of 388 mm and 13.8°C, respectively. There is a pronounced dry season from June to September, with only small amounts of rain. The soil is derived from gypsum outcrops, and is classified as Xeric Haplogypsid (Soil Survey Staff, 1994). The main soil properties of the study area are shown in Table 1. Perennial plant cover is below 40%, and is dominated by the tussock grass *S. tenacissima* (18% of total cover) and the N-fixing shrub *Retama sphaerocarpa* (L.) Boiss (6% of total cover). The open areas between perennial plants are colonized by well-developed BSCs dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt, *Toninia sedifolia* (Scop.) Timdal, and *Psora decipiens* (Hedw.) Hoffm. (see Appendix A for a full species checklist). Bare soil and BSC-dominated areas cover 28% and 32% of the study site, respectively.

Experimental design

A stratified random design was employed for this study. In January 2007, we randomly placed 12 50× 50 cm plots in each the six most frequent soil cover types in the study area: *S. tenacissima* tussocks (ST), *R. sphaerocarpa* shrubs (RS), and open areas with very low (< 5%; hereafter BS), low (5–25%; hereafter LC) medium (25–75%; hereafter MC) and high (above 75%; hereafter HC) cover of well developed and lichen-dominated BSCs (Appendix B). Plots located in the ST microsite were placed under the north side of *S. tenacissima* canopies; this microsite is characterized by permanent shade conditions, high litter accumulation, and presence of mosses on the soil surface (mostly *Pleurochaete squarrosa* [Brid.] Lindb. and *Tortula revolvens* [Schimp.] G. Roth). Those located in RS microsites shrubs were placed under the canopy of *R. sphaerocarpa*; this microsite is characterized by moderate shade conditions, high litter accumulation and cover of annual plants, particularly in Spring, and the occasional presence of mosses. The different BSC-dominated microsites do not have perennial vascular plants.

Field measurements and laboratory analyses

We monitored climatic (rainfall and temperature) conditions using an on-site meteorological station (Onset, Pocasset, MA, USA). We also measured soil moisture and temperature between January 2007 and December 2008 in five microsites (ST, RS, BS, MC and HC, $n=3$ per microsite) by using ECH_2O humidity sensors (Decagon Devices Inc., Pullman, USA) and protected diodes, respectively. Soil moisture measurements were continuously recorded at a depth of 0–5 cm every 150 minutes.

Environmental conditions registered at every season differed during the two years of study (Appendix C).

Spring rainfall was much higher in 2007 (136 mm) than in 2008 (28 mm). The opposite pattern was found in Autumn (113 and 14 mm in 2007 and 2008, respectively). In general, average temperatures during each sampling period did not vary substantially between the studied years. Soil moisture was higher in RS than ST microsites, and was lower in HC than BS microsites during most of the study period (Appendix C). The availability of ammonium and nitrate was measured in situ in all the evaluated microsites using ion-exchange membranes (IEMs; Subler et al. 1995). We selected this technique because they generate minimal disturbances to the soil superficial communities, and because they allow intensive sampling over multiple time period at the same spatial localization. One anion and cation IEMs (types I-100 and I-200, Electropure Excellion, Laguna Hills, California) per plot and microsite were placed seasonally for two consecutive years (2007-2008). They were first subjected to expansion treatment by submerging them in distilled water at 82–90°C for 48 h. After this, IEMs were cut into 2.5 × 2.5 cm squares, attached to a plastic rod with acrylic glue, and inserted into the soil at a 0.5–3 cm depth. During each sampling, IEMs were incubated in the field during 23–25 days. After removal, the IEMs were taken to the laboratory and dried at ambient temperature. They were carefully separated from the plastic rod, brushed to remove soil particles, and placed into 125 ml flasks for extraction with 25 ml of 2 M KCL by orbital spinning (1 h at 200 rpm). The extracts were analyzed for NH_4^+-N, NO_3^--N and total mineral N (the sum of NH_4^+-N and NO_3^--N) by the indophenol blue method using a microplate reader (Sims et al. 1995).

In parallel with the measurements of in situ ammonium and nitrate availability, we collected additional soil samples for measuring the N in soil microbial biomass, potential net N mineralization (both nitrification and ammonification rates) and depolymerization rates. Depolymerization rate is defined as the production of organic monomers from soil organic matter; this variable can give us an idea the net balance between decomposition and mineralization rates (Schimel and Bennet 2004). Two independent samplings were carried out for this aim. In the first, we identified three sources of spatial heterogeneity: ST, RS and open areas (without differentiating any BSC cover). Five randomly selected soil samples (0–10 cm depth) were seasonally collected from each microsite during 2007 and 2008 using a circular soil corer (5 cm diameter × 10 cm height), coinciding with IEMs measurements. In the second sampling, five samples from the top 4 cm of soil were collected from randomly selected LC, MS, HC and BS microsites seasonally during 2008 using a circular soil corer (5 cm diameter × 4 cm height). Soil samples were stored after field collection at 4°C and processed as soon as possible. Large (visible) roots and BSC fragments were removed from soils by hand before processing.

Microbial biomass-N was determined using the fumigation-extraction method proposed by Brookes et al. (1985). Twenty grams of fresh soil subsamples were fumigated with chloroform for 5 days. Non-fumigated replicates were used as controls. Fumigated and non-fumigated samples were extracted with 100 ml of K_2SO_4 0.5 M and filtered through a 0.45-μm Millipore filter. The extracts were digested with potassium persulphate ($K_2S_2O_8$) in an autoclave at 121°C over 55 min and then incubated with Devarda alloy overnight (Sollins et al. 1999). Total N in the digested extracts was determined by colorimetry (indophenol blue method) using a microplate reader (Sims et al. 1995). Microbial biomass-N concentration was estimated as the difference between total N of fumigated and unfumigated digested extracts divided by a Kn (fraction of *biomass* N extracted after the $CHC1_3$ treatment) of 0.54 (Brookes et al. 1985).

Potential net N mineralization and depolymerization rates were estimated from the Spring 2008 sampling, the most biologically active season. Soil subsamples (20 g) were incubated in dark condition at 30°C and 80% of water holding capacity during 14 days. Initial and final soil subsamples were extracted with 0.5 M K_2SO_4, and analyzed for mineral- and total N following the procedure described above. The concentration of dissolved organic N (DON) was calculated as the difference between total- and mineral N in the soil extracts. Potential net N mineralization and depolymerization rates were estimated as the difference between initial and final NO_3^--N, NH_4^+-N and DON, concentrations, respectively.

Statistical analyses

Seasonal measurements of in situ availability of NO_3^--N and NH_4^+-N obtained from the IEMs were analyzed by two-way (Microsite and Time) ANOVA, with repeated measures of one of the factors (Time). When significant ($P<0.05$) Microsite effects were found, the Student-Newman-Keuls (SNK) post-hoc test was employed to evaluate differences between microsites. Data from the IEMs were tested prior to these analyses for assumptions of normality and homogeneity of variances, and were log-transformed when necessary. Potential ammonification, potential nitrification and potential depolymerization rates obtained in Spring 2008 were analyzed by one-way ANOVA. The same analysis was used to analyze organic carbon and total nitrogen obtained in Autumn 2008. Differences between the microsites were further assessed with the SNK test. Separate analyses were carried out for the two depths evaluated.

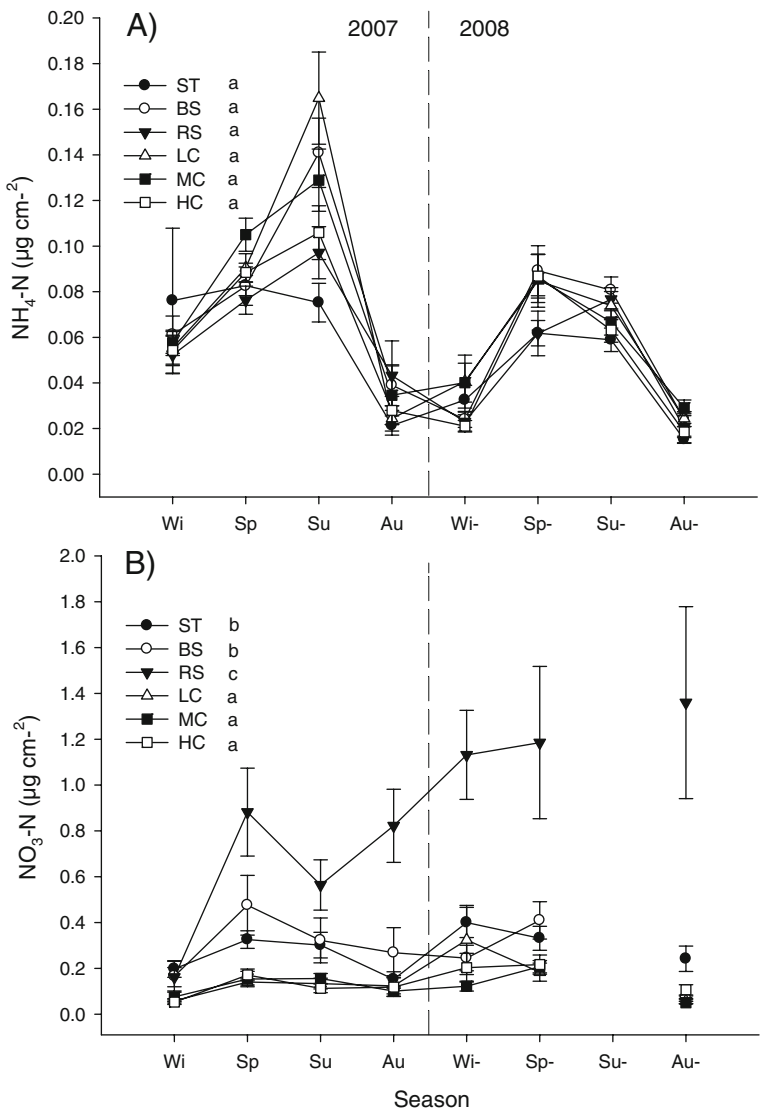

Fig. 1 Changes in NH_4^+-N (A) and NO_3^--N (B) availability between January 2007 and December 2008. Data represent means ± SE ($n=12$). Different letters indicate significant differences between microsites after repeated measures ANOVA ($P<0.05$). ST = *Stipa tenacissima* tussocks; RS = *Retama sphaerocarpa* shrubs; BS = Bare soil; LC = low biological soil crust (BSC) cover; MC = medium BSC cover; and HC = high BSC cover. Note the differences in scale in the y-axis

Microbial biomass N data did not follow ANOVA assumptions (normality and homogeneity of variances), even after data transformation. Thus, the semi-parametric PERMANOVA approach developed by Anderson (2001) was used to evaluate the effects of Time and Microsite on the data collected at 0–10 cm depth, and those of Microsite on the data collected at 0–4 cm depth. In these analyses, both factors are considered fixed. PERMANOVA uses permutation tests to obtain *P* values, does not rely on the assumptions of traditional parametric ANOVA, and can handle experimental designs such as employed here. PERMANOVA analyses were carried out using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+for PRIMER statistical package (PRIMER-E Ltd., Plymounth Marine Laboratory, UK). All the ANOVA analyses were carried out with the SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA).

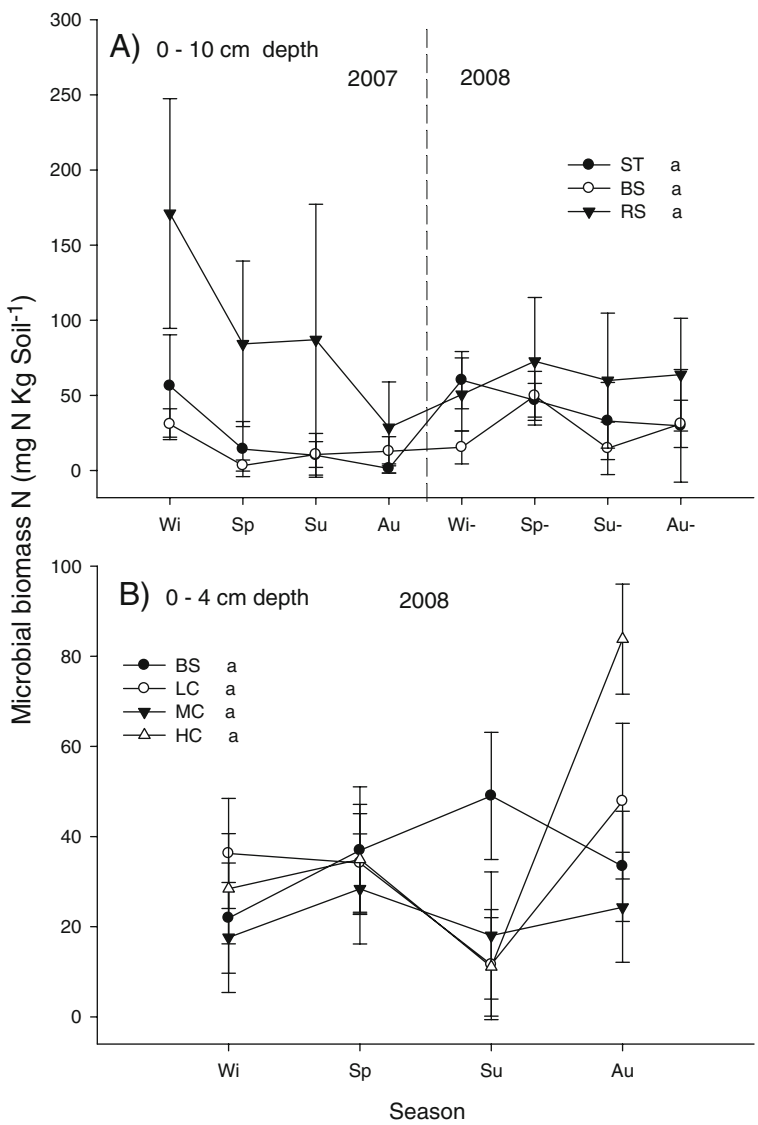

Fig. 2 Microbial biomass N measured at 0-10 cm (A) and 0-4 cm (B) depth. Data represent means ± SE (n=5). Different letters indicate significant differences between microsites after the semi-parametric PERMANOVA (P<0.05). Rest of legend as in Fig. 1

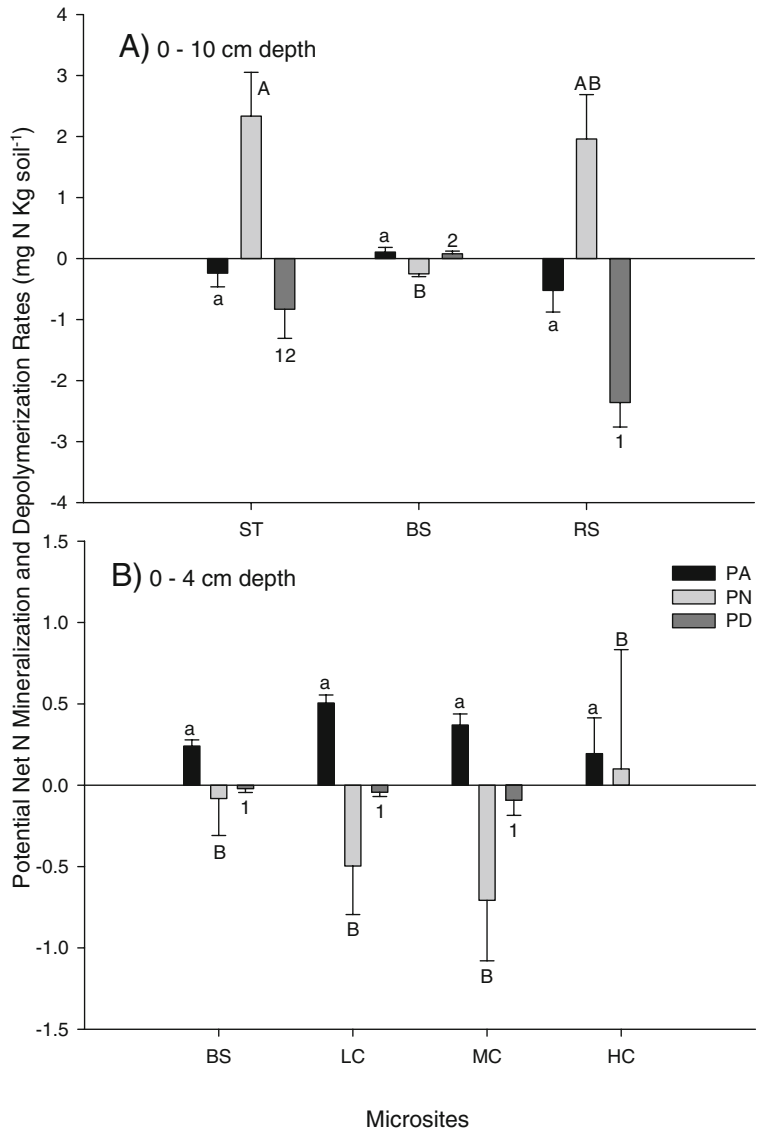

Fig. 3 Mean values for potential ammonification (PA), potential nitrification (PN) and potential depolymerization (PD) measured at 0-10 cm (A) and 0-4 cm (B) depth. Data represent means ± SE (n=5). Different letters (PA and PN) and numbers (PD) indicate significant differences between microsites after a one-way ANOVA (P<0.05). Rest of legend as in Fig. 1

Results

Spatio-temporal variation of available nitrogen

The dynamics of available N followed changes in soil moisture, rather than those in temperature; during the dry season the available N decreased substantially (Fig. 1, Appendix C). Available NH_4^+-N was lower than available NO_3^--N throughout the studied period (Fig. 1). Peaks in available NH_4^+-N were always found in Spring and Summer in all microsites (Fig. 1a). We did not find any significant differences in this variable between microsites when analyzing all the data gathered during the studied period (repeated measures ANOVA, F_{Time}= 1125.13, df=6,402, P<0.001; $F_{Microsite}$=2.26, df= 5,67, P=0.058; $F_{Time\ x\ Microsite}$=1.35, df=30,402, P =0.103).

During the Summer of 2008, no rainfall was registered during the incubation of the IEMs, and thus all NO_3^--N data were close to zero (Fig. 1b). Available NO_3^--N was substantially higher in the RS than in any other microsite. No significant differences in the amount of available NO_3^--N were found between ST and BS microsites, but these microsites had more NO_3^--N than those dominated by BSCs (LC, MC and HC microsites; repeated measures ANOVA, F_{Time}= 38.94, df=6,402, P<0.001; $F_{Microsite}$=23.43, df=5,67, P<0.001; $F_{Time\ x\ Microsite}$=4.59, df=30,402, P< 0.001).

Spatio-temporal variation in microbial biomass N

We found a significant Season x Microsite interaction ($F_{Season\ x\ Microsite}$=3.10, df=6,95, P=0.007) when analyzing microbial biomass N collected at BS, RS and ST microsites during 2007 and 2008 (Fig. 2a). This was mostly promoted by the increase in microbial biomass N observed at the RS microsite during most of 2007. During 2008 we found no significant differences in microbial biomass N between areas of different BSC cover (Fig. 2b; $F_{Microsite}$=0.53, df=3,45, P=0.854; F_{Season}=2.32, df=3,45, P=0.023; $F_{Season\ x\ Microsite}$= 0.88, df=9,45, P=0.621).

Potential N mineralization

Significant differences were found between microsites when analyzing potential nitrification and depolymerization rates at 0–10 cm depth (Fig. 3a; ANOVA, $F_{Nitrification}$=5.57, df=2,9, P=0.027; $F_{Depolimerization}$= 9.19, df=2,9, P=0.001). Potential nitrification rate was higher in ST than in BS microsites, and potential depolymerization rate was substantially lower under RS that in BS, suggesting the complete mineralization of DON under the canopy of *R. sphaerocarpa*. These differences were not found when analyzing potential ammonification rate (ANOVA, $F_{2,10}$=1.98, P=0.188). No significant differences were found between microsites in any variable analyzed at 0–4 cm depth (Fig. 3b; ANOVA, $F_{Amonification}$=0.93, df=3,13, P=0.45; $F_{Nitrification}$=0.84, df=3,13, P=0.495; $F_{Depolymerization}$ =0.75, df=3,13, P=0.539).

Discussion

Spatio-temporal variation in N availability has been described in a wide variety of semi-arid and arid ecosystems around the world (e.g. Mazzarino et al. 1991; Bernard-Reversat 1982; Stubbs and Pyke 2005). However, it has rarely been reported in semi-arid Mediterranean ecosystems (but see Zaady et al. 1996 and Monokrousos et al. 2004 for examples from Israel and Greece). Our results show that N availability in semi-arid *S. tenacissima* grasslands is highly heterogeneous in space and time, and that BSCs largely contribute to create such heterogeneity.

The distribution of soil N in arid and semi-arid zones is related to the occurrence of resource "hotspots" under plant canopies (García-Moya and Mckell 1970; Schlesinger et al. 1996; Goberna et al. 2007). *Retama sphaerocarpa* is a N-fixing shrub, and thus it is not surprising to find that the highest mineralization rates and values of available N are located under its canopy. RS and ST microsites present high accumulation of inorganic N in the study area, mainly during the growing season (120 and 85 mg NO_3^--N·kg^{-1} soil, respectively; Delgado-Baquerizo et al. 2010). However, we did not find greater NO_3^--N availability under the canopy of *S. tenacissima*, which showed no significant differences between adjacent bare ground soil (Fig. 1b). Despite the important differences in environmental conditions between ST and BS microsites (Appendix C), we did not find differences in microbial biomass N and N availability (NH_4^+-N and NO_3^--N) between them, as well as in organic carbon and total nitrogen (Table 1). Although higher values of total N have been reported under the canopy of *S. tenacissima* compared to adjacent areas of bare ground in calcareous grasslands of SE Spain (Maestre et al. 2001), Goberna et al. (2007) failed to find significant differences in microbial activity between these microsites in similar grasslands of the same area. It is important to note that *S. tenacissima* is a perennial grass tussock with slow growth rates, relatively small root systems and low litter quality (Maestre et al. 2007). In N-poor ecosystems where litter inputs,

decomposition and N cycling processes are slow, such as the study area, plants could prefer organic N rather than inorganic N (Neff et al. 2003; Schimel and Bennet 2004). Indeed, it has been found that organic N contributes significantly to the annual N take up of species of the genus *Stipa* (*S. aliena*; Xu et al. 2006). Thus, the concentration of mineral N in soils might be regulated by competition for DON between plants and soil microorganisms more than by organic matter and total N content. Such competition could explain a lower N availability than expected in ST microsites (as compared with BS microsites), suggesting a breakdown of the N cycle under *S. tenacissima* canopies. This possibility must be, however, confirmed by future work, as direct take up of organic N by this species has not been measured yet.

Although we expected higher nitrogen availability under BSCs due to their role as N fixers, we found a reduced N availability in BSC-dominated areas. Biological soil crusts can fix atmospheric N only when they are dominated by cyanobacteria, contain cyanolichens such as *Collema sp.* or hold free-living soil cyanobacteria. We have not measured the amount of cyanobacteria present in our study area, but cyanolichens are not very common there (Appendix A). Our experimental design and measurements cannot provide a mechanistic understanding for the results observed, but we speculate with two alternative, but not mutually exclusive, mechanisms to explain them. First, allelophatic effects of BSC-forming lichens on the microbial communities responsible for nitrification processes could be occurring in our study area. Secondary substances produced by lichens can be leached out to the soil during rainfall events, remaining in the soil for long periods of time (Malicki 1965; Dawson et al. 1984). These compounds can significantly inhibit the activity of soil microbes (Hyvärinen et al. 2002; Sedia and Ehrenfeld 2005). Although 350 secondary metabolites are known from lichens (Tay et al. 2004), little is known about of the effect of these substances on the activity of soil microbes under field conditions. Akpinar et al. (2009) observed in a field study an inhibition effect of *Cladonia* sp. on soil bacteria. However, Stark and Hyvärinen (2003) found that the leaching of usnic acid did not change the amount of microbial biomass in soil and, in a more recent study, Stark et al. (2007) did not find antimicrobial effects of secondary substances from *Cladonia* sp. Our results are consistent with those of by Stark and colleagues, as we did not find differences in microbial biomass N between BSC-dominated microsites and bare ground soil. However, we cannot exclude the possibility of allelophatic effects on the nitrifier community, since other microbial populations may be unaffected and show a parallel increase in biomass. Furthermore, we found strong evidence that both the available NO_3^--N and the potential nitrification rate are reduced under BSC (Figs. 2 and 3). Species such as *Cladonia convoluta* (Lam.) Anders are present at our study site but are not very abundant (Appendix A). Future studies are needed to determine if allelopathic interactions are responsible for the results obtained, and if so, to identify which BSC species and substances are driving such responses.

Another mechanism that could explain the results obtained is direct take up of NO_3^--N by BSCs. While lichens are generally thought to take up atmospheric nutrient sources rather than substrate sources, recent research suggests that this may be overstated in the case of those lichens living closely attached to their substrate (St. Clair et al. 2002). Several investigations have shown that intact lichens can directly take up NH_4^+-N and NO_3^--N (Dahlman et al. 2004, Gaio-Oliveira et al. 2005). It is also known that a wide range of soil bacteria, including cyanobacteria associated to BSCs, have the ability to take up nitrate by keeping high levels of the enzyme nitrate reductase (Lin and Stewart 1998). Thus, there may be a buildup of microbially-mediated N in the bare soil due to the low take up by vascular plants in these areas, compared with substantial incorporation of crust-produced N within the areas covered by BSCs.

Denitrification could be another alternative mechanism to explain the lack of NO_3^--N observed under BSCs. The rates of this process may be high in arid and semi-arid ecosystems due to the variable precipitation regimes and extreme wetting and drying

cycling at soil surfaces (Peterjohn and Schlesinger 1991). We found that under aerobic conditions, the amounts of NO_3^--N underneath BSCs are lower than other microsites. Therefore, we do not believe that denitrification is playing a key role in our study area. Our results agree with those from other semi-arid ecosystems dominated by BSCs, who failed to detect denitrification (Johnson et al. 2007).

Understanding the factors controlling the availability of N is critical to manage arid and semi-arid ecosystems (Peterjohn and Schlesinger 1990). While the role of BSCs as N fixers has long been recognized (e.g., Mayland and McIntosh 1966; Zaady et al. 1998, Belnap 2002), little is known about their effects on other pathways of the N cycle. Barger et al. (2005) estimated that approximately 3-7% of the N inputs via N fixation by the BSCs were lost by nitric oxide emissions, and Maestre et al. (2005) found that attributes of BSC communities such as species evenness and diversity influenced the amount of total N in the soil. To our knowledge, the results presented here are the first showing a strong effect of BSCs on the in situ availability of N in semi-arid ecosystems. Although the reason why BSCs inhibit nitrification can only be speculated upon, our study has value as a reference point for future investigations aiming to unravel this apparent nitrogen paradox. Our results also contribute to our knowledge of the role of BSCs in the N cycle, and indicate that crusts may have important and unexpected roles in moderating of N availability in semi-arid environments.

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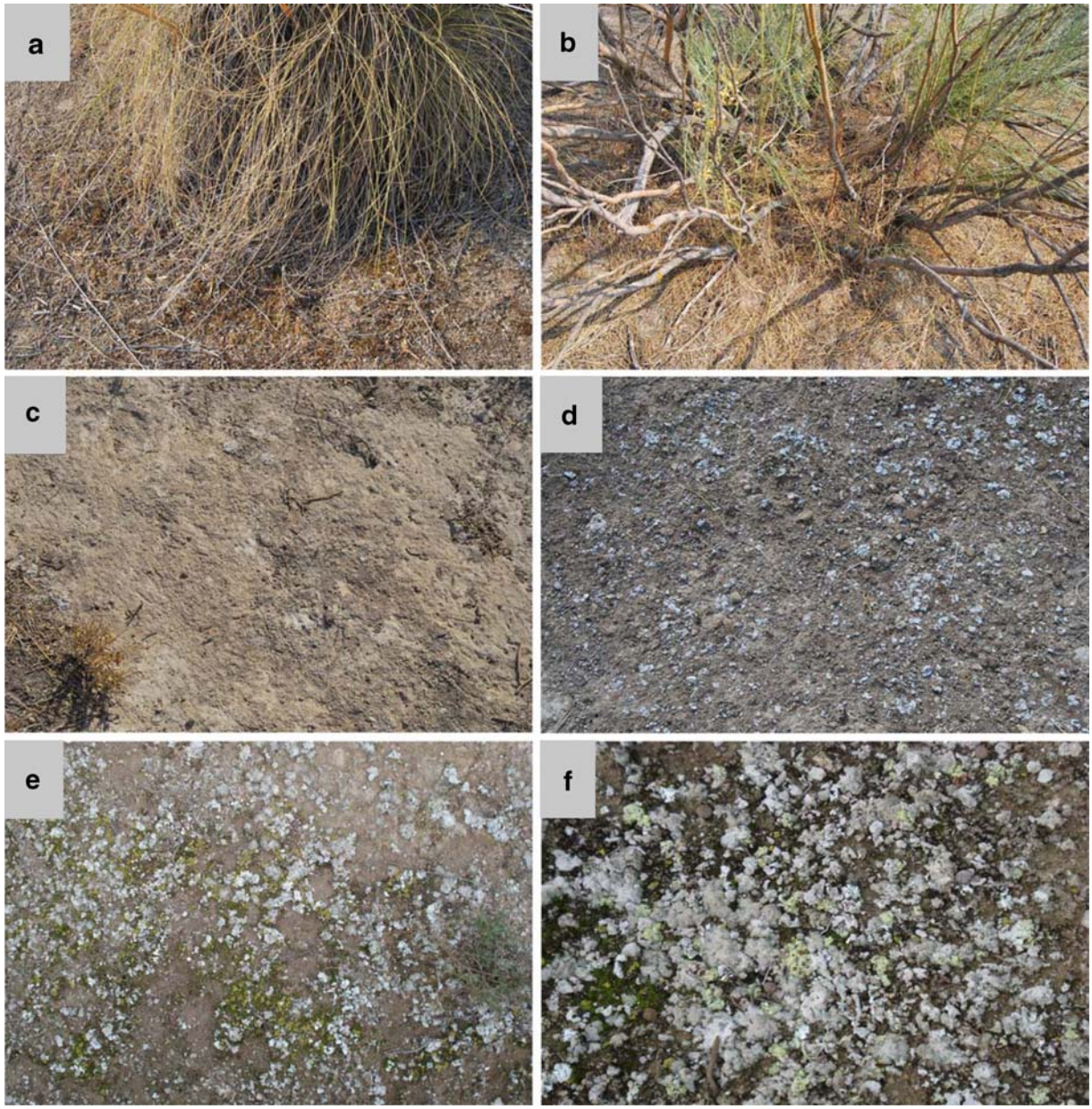
Appendix A

Checklist and frequency (in %) of lichen and mosses in the different microsites sampled

Species	ST	RS	LC	MC	HC	BS
Pleurochaete squarrosa	100.0	73.3	0.0	0.0	6.7	6.7
Syntrichia papillosissima	33.3	33.3	0.0	0.0	0.0	0.0
Didymodon acutus	0.0	0.0	26.7	20.0	20.0	26.7
Tortula revolvens	0.0	13.3	26.7	46.7	46.7	13.3
Weissia sp.	6.7	6.7	0.0	0.0	0.0	0.0
Acarospora reagens	6.7	0.0	33.3	40.0	40.0	33.3
Buellia epipolium	0.0	0.0	13.3	13.3	13.3	0.0
Buellia zoharyi	0.0	0.0	0.0	0.0	6.7	0.0
Cladonia convoluta	46.7	6.7	6.7	6.7	13.3	0.0
Collema crispum	6.7	13.3	20.0	0.0	20.0	6.7
Diploschistes diacapsis	6.7	6.7	53.3	40.0	73.3	66.7
Endocarpon pusillum	0.0	0.0	6.7	0.0	0.0	0.0
Fulgensia subbracteata	0.0	6.7	66.7	53.3	73.3	53.3
Placidium pilosellum	0.0	6.7	0.0	0.0	20.0	0.0
Placidium squamulosum	0.0	0.0	20.0	0.0	6.7	0.0
Psora decipiens	0.0	0.0	46.7	46.7	60.0	13.3
Psora savizcii	0.0	0.0	20.0	33.3	13.3	0.0
Squamarina cartilaginea	0.0	0.0	0.0	0.0	6.7	0.0
Squamarina lentigera	6.7	6.7	46.7	60.0	66.7	46.7
Toninia sedifolia	0.0	6.7	6.7	26.7	33.3	6.7

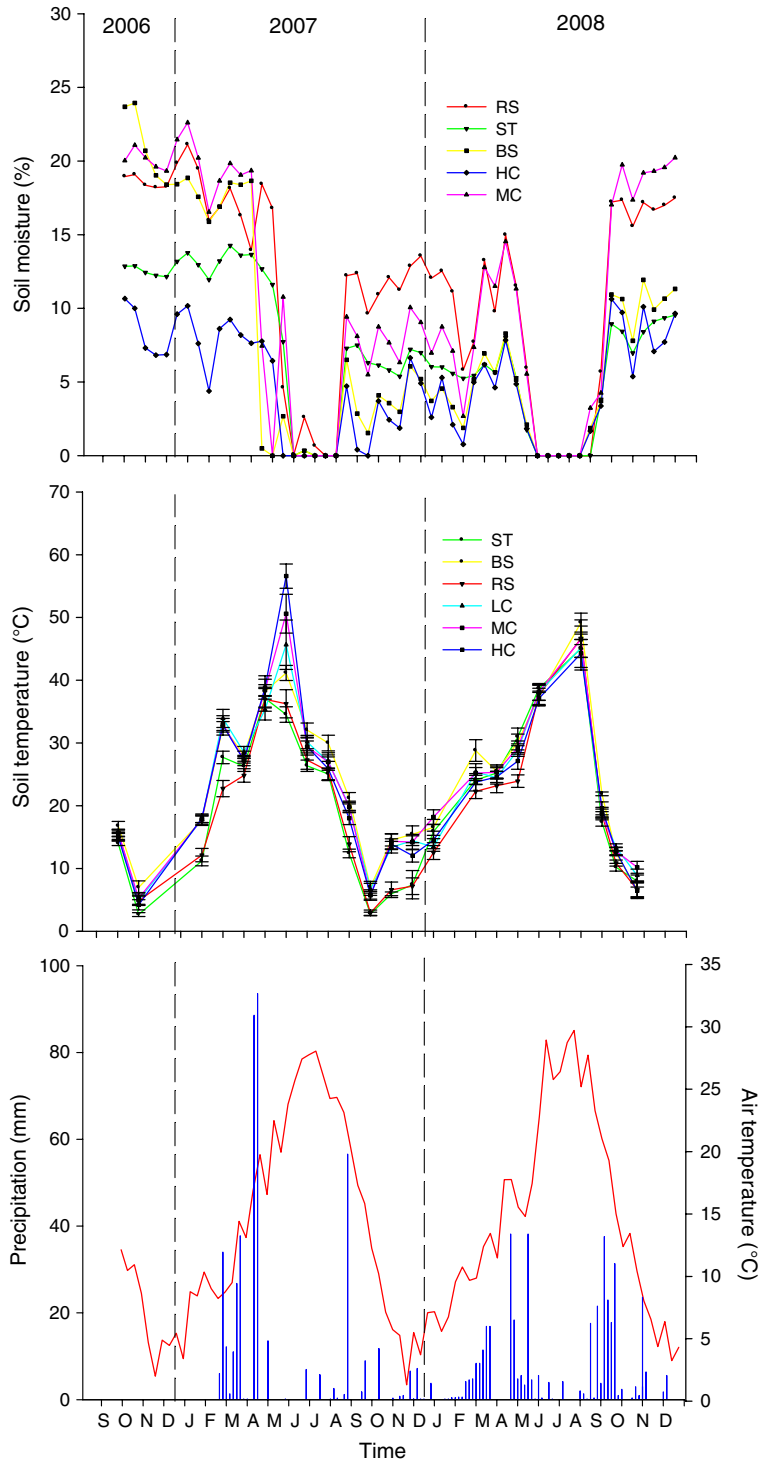
ST Stipa tenacissima, *RS Retama sphaerocarpa* L., *BS* Bare soil, *LC* low cover of biological soil crust (BSC), *MC* Medium cover of BSC, *HC* high cover of BSC

Appendix B



View of the different microsites sampled. A = *Stipa tenacissima* tussocks; B = *Retama sphaerocarpa* shrubs; C = Bare Soil; D = low biological soil crust (BSC) cover; E = medium BSC cover; and F = high BSC cover

Appendix C



Changes in soil moisture (upper graph) and temperature (middle graph) in different microsites, and precipitation and air temperature (bottom graph) registered in the study area between January 2007 and December 2008. The lack of rainfall data between January and February 2007 was due to the fact that the meteorological station was installed in March 2007. Soil moisture represents values obtained at 0-5 cm depth; soil temperature is obtained at 2 cm depth. ST = *Stipa tenacissima* tussocks; RS = *Retama sphaerocarpa* shrubs; BS = Bare soil; LC = low biological soil crust (BSC) cover; MC = medium BSC cover; and HC = high BSC cover. Data represent means ± SE for soil temperature (n=12). Data for soil moisture represent the average value of three sensors per microsite (the SE of these measures is omitted for clarity)

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