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REGULAR ARTICLE

Biocrusts control the nitrogen dynamics and microbial functional diversity of semi-arid soils in response to nutrient additions

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Abstract
Aims Human activities are causing imbalances in the nutrient cycles in natural ecosystems. However, we have limited knowledge of how these changes will affect the soil microbial functional diversity and the nitrogen (N) cycle in drylands, the biggest biome on Earth. Communities dominated by lichens, mosses and cyanobacteria (biocrusts) influence multiple processes from the N cycle such as N fixation and mineralization rates. We evaluated how biocrusts modulate the effects of different N, carbon (C) and phosphorus (P) additions on theN availability, the dominance of different available N forms and the microbial functional diversity in dryland soils.

Methods Soil samples from bare ground (BG) and biocrust-dominated areas were gathered from the center of Spain and incubated during seven or 21 days under different combinations of N, C and P additions (N, C, P, N+C, N+P, P+C, and C+N+P).
Results The relative dominance of dissolved organic N (DON) and the microbial functional diversity were higher in biocrust than in BG microsites when C or P were added. Changes in the C to N ratio, more than N availability, seem to modulate N transformation processes in the soils studied. In general, biocrusts increased the resilience to N impacts (N, C+N, N+P, C+N+P) of the total available N, ammonium, nitrate and DON when C was present.
Conclusions Our results suggest that biocrusts may buffer the effects of changes in nutrient ratios on microbial functional diversity and DON dominance in dryland soils. Thus, these organisms may have an important role in increasing the resilience of the N cycle to imbalances in C, N and P derived from human activities.

Keywords Mineralization · Dissolved organic N · Shannon-Weaver diversity index · Carbon · Phosphorus

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Introduction

Human activities are changing the ratios of nitrogen (N), carbon (C) and phosphorus (P) in natural ecosystems at an unprecedented rate in the history of Earth (Finzi et al.

2011). For instance, an increase in the C and N to P ratio is currently happening in most terrestrial ecosystems because of C and N fertilization derived from human activities (Peñuelas et al. 2012). However, our knowledge of how these changes modulate key soil processes in arid, semi-arid and dry-subhumid ecosystems (drylands hereafter) is still scarce (Hooper et al. 2005; Schimel 2010; Finzi et al. 2011), even when this biome covers 41 % of Earth's land surface and supports over 38 % of the global human population (Reynolds et al. 2007).

After water, N is the most important factor limiting net primary production and organic matter decomposition in drylands (Schlesinger 1996; Robertson and Groffman 2007). The N cycle includes "narrow" processes carried out by specific groups of microorganisms, such as nitrification, and "aggregated" procedures such as depolymerization (production of dissolved organic N [DON]) and ammonification, which are carried out by a larger and more diverse group of microorganisms (Wall et al. 2005; Schimel et al. 2005; Cookson et al. 2006). Some authors have found that augments in N and C availability decrease and increase microbial diversity, respectively, while increases in P had negligible effects on this variable (Sharma et al. 1998; Coleman and Whitman 2005; Schimel et al. 2005). Thus, a decrease in functional diversity may limit N transformation processes such as depolymerization in soils (Robertson and Groffman 2007). Very few studies have evaluated how simultaneous changes in C, N and P availability affect the dominance of N forms (NH_4^+, NO_3^- and DON), particularly in N-poor ecosystems such as drylands (Cookson et al. 2006; Qiu et al. 2008; Delgado-Baquerizo et al. 2011; Delgado-Baquerizo and Gallardo 2011). A shift in the dominance of N form derived from changes in the C, N and P ratio may affect N uptake by plants due to their different preferences for different N forms (Nordin et al. 2001; Warren 2009; Paulding et al. 2010). For example, while early-successional species show preference by nitrate, the uptake of ammonium and organic N is quantitatively more significant for late-successional species (Chapin et al. 1993; Houlton et al. 2007).

Of special consideration when studying drylands are communities of mosses, lichens, and cyanobacteria living on the soil surface (biocrusts hereafter), which occupy open spaces located between plant canopies. Biocrusts are considered key players in the N cycle in drylands, as they affect N fixation (Belnap 2002), nitrification (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2013a), and gaseous N losses (Barger et al. 2005). In addition, biocrusts can also affect the abundance and diversity of soil organisms such as bacteria and fungi (Yeager et al. 2007; Bates et al. 2010; Castillo-Monroy et al. 2011a). However, much less is known on how biocrusts and changes in nutrient ratios jointly impact the functional diversity of heterotrophic microbial communities. Biocrusts can also confer physical protection to the soil (e.g. Belnap 2006), and increase the resistance of specific ecosystem functions, like N mineralization, to global change impacts such as changes in temperature and soil water content (Delgado-Baquerizo et al. 2013a; Reed et al. 2012). Biocrusts have also shown to enhance processes such as C fixation, atmospheric N fixation and phosphatase activity, suggesting an important role in the stoichiometry of the C, N and P cycles in drylands (Castillo-Monroy et al. 2011a; Bowker et al. 2011; Maestre et al. 2012; Elbert et al. 2012).

Given the multiple roles that biocrusts play in N cycling, and the large areas covered by these organisms (Belnap and Lange 2003), explicitly considering them when evaluating N transformation processes can greatly improve our knowledge on the N cycle in drylands. In this study, we evaluated the effects of biocrusts on the response of N availability, the relative dominance of N forms (ammonium, nitrate and DON), and the functional diversity of microbial communities to multiple N, C and P additions (N, C, P, N+C, N+P, P+C, and C+N+P) in soils from a semi-arid site located in Central Spain, where strong effects of biocrusts on different aspects of the N cycle have already been observed (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010, 2013a, b).

We tested the following hypotheses: i) at high C availability, biocrusts may promote DON dominance and increase the soil microbial functional diversity, because of their association with heterotrophic communities that carry out organic matter decomposition (Bates et al. 2010); ii) the C:N ratio modulates N dynamics regardless the N availability. At the same N concentration, but different C availabilities, the N form dominance will shift from nitrate (when only N is added) to ammonium and DON (when both C and N are added; Cookson et al. 2006); iii) the heterotrophic functional diversity of biocrust-dominated soils may be limited by C because N fixation is frequently associated with biocrust-forming organisms (Belnap 2002), and most C sources coming

from biocrusts, particularly from lichens, are recalcitrant forms of C (Cornelissen et al. 2007); iv) biocrusts will confer resilience to the N cycle facing imbalances in the C:N:P ratio. We expect this because biocrusts have been shown to enhance the availability of C, N and P in soils where present (Castillo-Monroy et al. 2011a; Bowker et al. 2011; Maestre et al. 2012; Elbert et al. 2012), and consequently may help to maintain the functional diversity of soil microbial communities and the dominance of processes that control the N cycle.

Methods

Sampling design

Soils for this study were collected from the Aranjuez experimental station, located at the center of the Iberian Peninsula (40°02′ N – 3° 37′W; 590 m a.s.l.; 8° slope facing SE). The climate is Mediterranean semi-arid, with an average annual rainfall and temperature of 388 mm and 14 °C, respectively. Perennial plant cover is lower than 40 %, and is dominated by the perennial grass *Stipa tenacissima* L. Open areas between plant patches contain a well developed biocrust community dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt (see Castillo-Monroy et al. 2010 for a full species checklist). The soil is classified as Xeric Haplogypsid (USDA 2003), and its main properties are shown in Table S1.

Soil sampling was carried out during the spring of 2010. Five soil samples from the top 4 cm of the mineral soil profile were collected under each of two microsites: well-developed biocrusts dominated by *Diploschistes diacapsis*) and bare ground areas devoid of vascular vegetation and visible biocrust components (cover of mosses and lichens <5 %; BG hereafter). Soil samples were taken at distances higher than 5 m. Previous studies conducted at our study area shown a small-scale spatial dependence (lower than 20 cm) for N in biocrust microsites (Delgado-Baquerizo et al. 2013b). As such, the samples collected are believed to be independent. Soils were transported to the laboratory and air-dried at room temperature for four weeks. Previous studies have found that soil biochemical properties are hardly affected by air-drying in semiarid Mediterranean soils (Zornoza et al. 2009), which otherwise are under dry conditions most of the year (e.g., Maestre et al. 2002; see also Castillo-Monroy et al. 2011b for moisture data for our study area).

Nutrient treatments, N availability and relative dominance of N forms

Soil samples (2.5 gr of air-dried soil) from BG and biocrust-dominated microsites were preconditioned before the addition of nutrients. To recover their microbial activity, we incubated them at 20 °C and 60 % of water holding capacity during 7 days (Qiu et al. 2008). These soils were then treated with 0.5 ml of an amendment solution, which contained alone or in combination, the following ingredients: 100 mg N kg^{-1}, 2.323 mg C kg^{-1} and 20 mg P kg^{-1} (Qiu et al. 2008). The nutrients amended were within the range concentrations of available soil N, C and P typically found in drylands (Jalali 2007; Vu et al. 2008; Delgado-Baquerizo et al. 2013a). Thus, soil samples were incubated at 20 °C under different nutrient additions in a factorial design (Control, N, C, P, C+N, N+P, C+P, and C+N+P), and analyzed after 7 and 21 days. The last period was chosen to make sure that the initial effect of the added ammonium on the N transformation processes and dominance forms had disappeared. Samples were mixed by vibration after nutrient additions, and soil containers were covered with gas-permeable thin plastic film prior to incubations.

Incubated (Control, N, C, P, C+N, N+P, C+P, and C+N+P) and non-incubated soil samples were extracted with K_2SO_4 0.5 M in a 1:5 ratio. 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 2 °C until colorimetric analyses were conducted, which took place within the 24 h following the extraction. Sub-samples of each non-incubated (air-dry) extract were taken for measurements of glucose (Chantigny et al. 2006) and P (Allen et al. 1986). Ammonium (NH_4^+-N), Nitrate (NO_3^--N) and DON concentrations were also measured for each non-incubated air-dried, K_2SO_4 extract subsample. The availability of N was estimated from K_2SO_4 extracts as the sum of DON, NH_4^+-N and NO_3^--N before and after the 7 and 21 days incubation period for each nutrient treatment evaluated (Delgado-Baquerizo et al. 2011). The relative dominance of N forms (DON, NH_4^+-N and NO_3^--N) were calculated after the 7 and 21 days

incubation period as described in Delgado-Baquerizo et al. (2013a). All results were expressed on a dry soil basis.

Microbial functional diversity

The functional diversity of soil heterotrophic microbial communities was analyzed with the MicroResp® technique (Campbell et al. 2003). This method is based on obtaining community level physiological profiles (CLPP) using 15 carbon sources that differ in structural complexity, and has been successfully used with dryland soils (Oren and Steinberger 2008; Garcia-Palacios et al. 2011). We used amino acids (L-alanine, L-lysine, arginine, L-cysteine and N-acetyl-glucosamine), carbohydrates (D-fructose, D-galactose, D-glucose, L-arabinose and D-trehalose), and carboxylic acids (citric acid, L-malic acid, oxalic acid and amino butyric acid) for MicroResp® analyses. This method does not provide information on the taxonomical or phylogenetical diversity of the soil microbial community, but is commonly used to interpret changes in heterotrophic functional diversity because different carbon sources correspond to the catabolic attributes of diverse soil microbial functional groups (Zak et al. 1994; Øvreås 2000; Garcia-Palacios et al. 2011). Prior to MicroResp® analyses, soil samples were incubated at 50 % SWC and 20 °C under the different treatments using two incubation periods (7 and 21 days). MicroResp® plates were set up following Garcia-Palacios et al. (2011). They were incubated for 6 h and read at 570 nm. Then, the results were calculated on the basis of the 16th substrate (water), which represents the basal respiration. In this study we compared the samples from the same soil type and were interested in the relative differences between treatments more than in the absolute CO_2 rates; thus we expected any artifacts promoted by the emission of abiotic CO_2 to similarly affect all the treatments evaluated (Garcia-Palacios et al. 2011). The Shannon-Weaver Diversity Index (H') was calculated to determine heterotrophic microbial functional diversity by using the CO_2 responses to the different C sources as (Shannon and Weaver 1963):

$$H' = -\sum_{i=1}^{S} pi \cdot \ln pi$$

where pi is the ratio of the activity of a particular C substrate and the sum of activities of all C substrates (Zak et al. 1994).

Statistical and numerical analyses

To evaluate the effects of the different nutrient additions, we calculated the absolute increment (A_i) in the total available N, the relative dominant of N forms (NH_4^+-N, NO_3^--N and DON) and the microbial functional diversity (H') for each treatment (C, N, P, C+N, N+P, C+P and C+N+P) relative to the control (incubated soils with no nutrients addition) in both biocrust and BG microsites for the 7 and 21 days incubation periods. As our data did not follow ANOVA assumptions (normality and homogeneity of variances), the effects of the incubation period (TI: 7 and 21 days), microsite (MI: BG and biocrust soils) and nutrient treatment (TR: N, C, P, C+N, N+P, C+P, C+N+P), on these increments were tested by using the semi-parametric PERMANOVA approach (Anderson 2001), with all these factors being fixed. When significant interactions between TI and TR factors were found, we conducted separate PERMANOVA analyses for the different TI levels. We also tested the differences between microsite and incubation periods in the concentration of ammonium, nitrate and DON for the controls (non-nutrient treatment) and between biocrust and BG microsites in the different forms of N (ammoniun), C (glucose) and P (phosphate) before nutrient additions by using PERMANOVA, with biocrust presence/absence as a fixed factor. 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).

We also evaluated how biocrusts affected the resilience of the soil N variables to N additions (N, CN, NP and CNP) using the Orwin and Wardle (2004) resilience index. This index was calculated for N availability, ammonium, nitrate and DON using the following equation:

$$RL = \frac{2 \cdot |Do|}{(|Do| \cdot |Dx|)} - 1$$

where Do is the difference between the control and the disturbed (extracted N in the control + added N) soil at the end of the disturbance and Dx is the difference between the control and the disturbed soil

at the time point chosen to measure resilience (7 and 21 days). This index has the advantage to be standardized by the control, being bounded between −1 (less resilience) and +1 (maximal resilience); it remains bounded even when extreme values are encountered (Orwin and Wardle 2004).

Finally, we checked how the A_i in microbial functional diversity relates to A_i in N availability, the dominant N forms and to the N resilience by using Spearman's correlation coefficient. Correlation analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Before incubation, the amounts of hexoses and NH_4^+ (which were used as C and N sources for the amended soils) in biocrust and BG soils had similar values (Table S1). However, biocrust soils had a higher PO_4^{3-} concentration than BG soils (P=0.03; Table S2).

After incubation, neither the microsite nor the incubation period generated differences in NH_4^+-N nor NO_3^--N in the control treatments (incubated soils without nutrients addition; Table 1; Table S3). A TR×TI interaction (P<0.01; Table 1; Table S2) was observed while analyzing DON data. This variable was higher under BG after 7 days of incubation (P=0.01; Table 1; Table S2) but no differences in DON concentrations were found between microsites after 21 days of incubation (P>0.05; Table S3). Microbial functional diversity was lower in biocrust than in BG soils (P<0.001), but significant differences in this variable between incubation periods were not observed (P=0.51, Table 1, Table S3).

In general, the immobilization of N was higher in biocrust than in BG soils (Fig. 1). Significant differences between biocrust and BG microsites were only observed after 7 days of incubation (P=0.01; Table S4). Overall, the addition of C promoted a decrease in the total amount of available N (Fig. 1). Differences between nutrient addition levels on this variable were observed for both incubation periods, despite the significant TR×TI interaction found (P<0.001; Table S4).

The A_i in DON dominance was higher in biocrust than in BG soils (P=0.02; Table S4; Fig. 2). Significant differences between biocrust and BG soils were not observed either for the A_i in NH_4^+-N or NO_3^--N dominance (P>0.05; Table S4; Fig. 1). The A_i in DON dominance was higher in C (C and C+P) treatments for both incubation periods, despite the significant TR×TI interaction observed (P<0.001; Table S4). Similarly, the A_i in NH_4^+-N dominance was highest in the C+N treatment in both incubation periods regardless of the significant TR×TI interaction observed (P<0.001; Table S4; Fig. 2). Nitrate was the dominant N form for both BG and biocrust microsites in N (N and N+P) treatments after only 21 days of incubation (Fig. 2). Despite the significant TR×TI interaction observed (P<0.001; Table S4; Fig. 2), significant differences in A_i in the NO_3^--N dominance were not found between treatments in both incubation periods (P<0.01, Table S4). The dominance of nitrate dominance was higher in C (C, C+P) treatments for BG soils in the seven day incubation period (P<0.001; Table S4; Fig. 2), but differences between microsites were not observed after 21 days of incubation (P= 0.19; Table S4; Fig. 2).

Table 1 Concentration of inorganic (ammonium and nitrate) and organic N forms and microbial functional diversity index (H') in the control treatment for both biocrust and bare ground (BG) microsites after the different incubation periods (seven [T7] or 21 [T21] days)

	T7		T21		PERMANOVA results		
	BG	Biocrust	BG	Biocrust	TI	MI	Mi x TI
NH_4^+	2.62 (0.61)	4.75 (0.96)	2.4 (0.49)	1.87 (0.01)	0.88 (0.34)	2.66 (0.12)	0.55 (0.49)
NO_3^-	15.31 (2.12)	19.11 (0.2)	16.39 (3.79)	15.91 (1.41)	0.07 (0.78)	1.44 (0.24)	0.02 (0.90)
DON	12.09 (3.78)	3.85 (1.42)	5.54 (0.6)	6.28 (0.23)	0.01 (0.97)	4.56 (0.06)	11.94 (**<0.001**)
Available N	31.06 (5.48)	29.08 (1.31)	24.33 (3.82)	29.3 (3.50)	0.37 (0.56)	0.02 (0.89)	1.28 (0.28)
H' (*bits*)	2.41 (0.04)	1.71 (0.30)	2.34 (0.08)	1.90 (0.10)	0.49 (0.50)	20.08 (**<0.001**)	0.01 (0.99)

Units for all variables are mg kg^{-1} soil, except when indicated. Data represent means (SE), n=5. Differences between microsites (BG and biocrust) and incubation periods (T7 and T21) were analysed by using a two-way PERMANOVA. *MI* microsite, *RES* residuals, *TI* period of incubation. PERMANOVA results are the pseudo-F (*P value*). *P* values below 0.05 are in bold

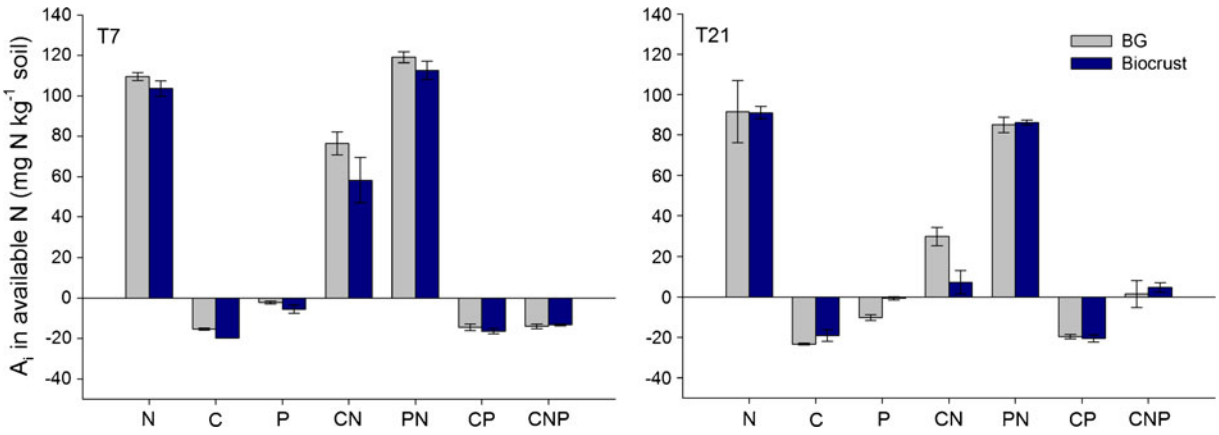

Fig. 1 Increment (A_i) in the available N (measured as the sum of NH_4^+-N, NO_3^--N and DON) under the different nutrient treatments for biocrust and bare ground soils. T7 and T21 indicate results obtained 7 and 21 days after the beginning of incubation

Nutrient additions always decreased H' in BG soils, with the exception of the C+N+P treatment (Fig. 3). However, in biocrust soils, the addition of C increased H' for all cases after 1 week; after 3 weeks such an increase was observed only in the C, C+N+P and N+P treatments (Fig. 3). A significant TR×TI interaction was found when evaluating the A_i in the microbial functional diversity (H'; Table S4). Even so, significant differences between treatments were observed in this variable for both incubation periods ($P<0.001$, Table S4).

Biocrust soils had a higher DON resilience than BG soils after N additions ($P<0.01$; Fig. 4). Regarding total available N, differences between microsites were only observed after 21 days of incubation ($P=0.03$; Table S5), where biocrust soils showed a higher total available N resilience than BG soils in the C+N treatment (Fig. 4). Non-significant differences between BG and biocrust soils were found when analyzing the resilience of nitrate and ammonium to nutrient additions. However, a trend to increase the resilience of both ammonium and nitrate was observed in the biocrust soils amended with C+N (Fig. 4; Table S5). A significant TR×TI interaction was found when evaluating the resilience of total available N (Table S5). Nonetheless, this variable was highest in the C+N+P treatment at both incubation periods (Fig. 4). The highest resilience of the DON was found in the C+N+P treatment for both microsites ($P<0.01$; Fig. 4). Significant differences between incubation periods were not observed for this variable ($P>0.05$; Table S5). The highest resilience in both NH_4^+-N, and NO_3^--N was found for the C+N+P treatment in BG and biocrust microsites (Fig. 4). Significant differences between microsites were not observed for any incubation period ($P>0.05$, Table S5). A significant TR×TI interaction was found when evaluating the resilience of ammonium and nitrate (Table S5). Even so, significant differences between treatments were observed when evaluating the resilience of NH_4^+-N, and NO_3^--N ($P<0.01$, Table S5).

The A_i in H' was positively related to the dominance of NH_4^+-N in the BG microsite after 21 days of incubation. However, the A_i in H' was negatively related to the dominance of NO_3^--N in both microsites for the seven day incubation period, a response that was observed only in BG soils after 21 days of incubation (Table 2). The A_i in H' was negatively and positively related to N availability and DON dominance, respectively, in both microsites, but only after 7 days of incubation (Table 2). In general, the A_i in H' was positively related to the total available N, ammonium and nitrate resilience in both microsites, but only a positive trend was observed when analyzing the relationship with the resilience of DON (Table 2).

Discussion

The addition of N had a negative impact on the microbial functional diversity of biocrust and BG microsites. However, when C and P were added, biocrust soils showed a higher increase in the dominance of DON and microbial functional diversity compared to BG soils. An increase in the availability of C may promote the activity of heterotrophic communities, which play an important role in the decomposition and depolymerization

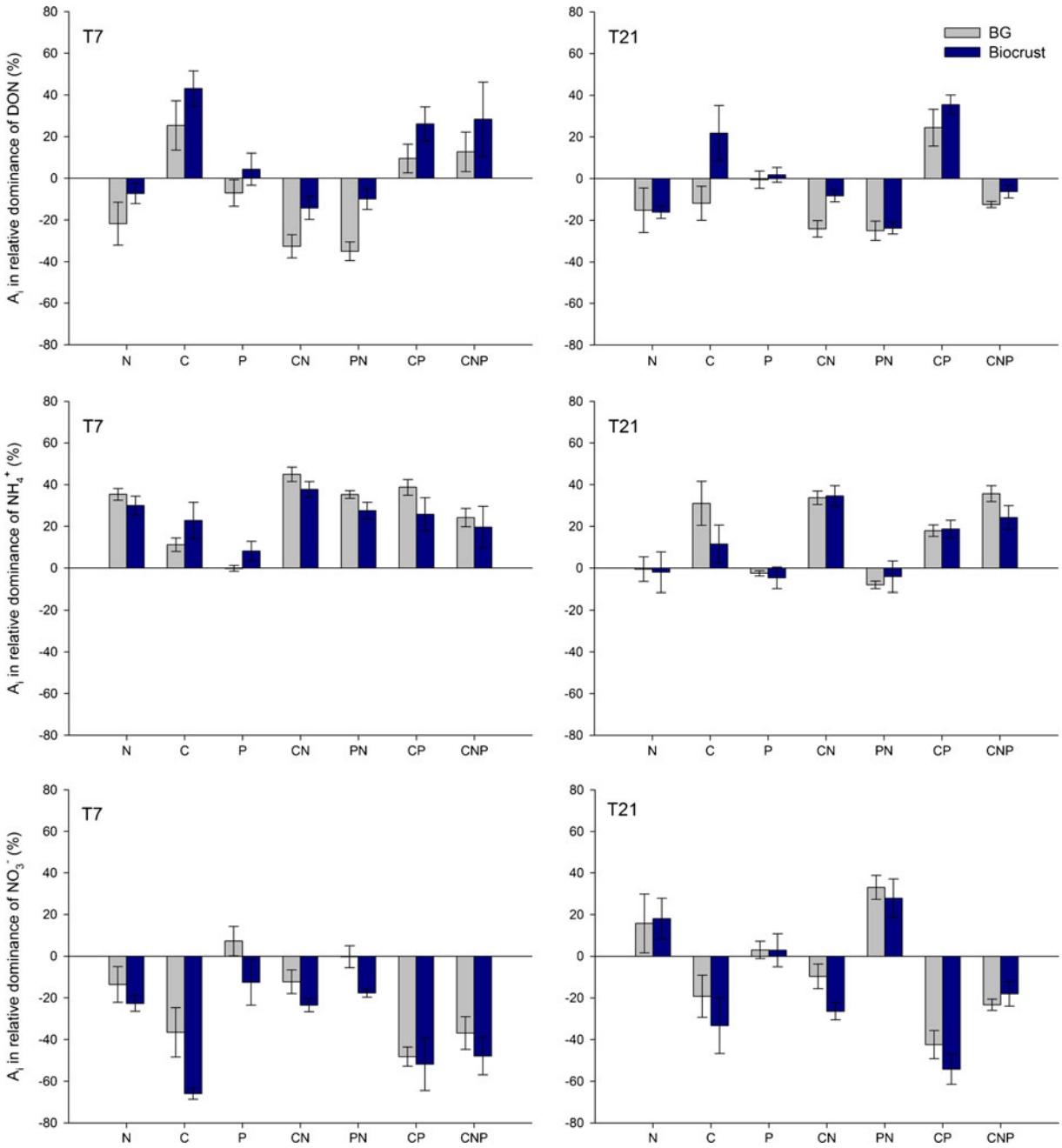

Fig. 2 Increment (A_i) in the relative dominance of different N forms (DON, ammonium and nitrate), calculated as the difference in the DON, NH_4^+-N and NO_3^--N concentrations between the seven (T7) and 21 (T21) days of incubation, under the different nutrient treatments for biocrust and bare ground soils

processes, increasing the dominance of DON under biocrust soils (Schimel and Bennett 2004; Cookson et al. 2006; Robertson and Groffman 2007). In addition, the A_i in H' was positively and negatively related to the A_i in DON and nitrate dominance in both microsites, respectively. These results support the idea that "aggregated" processes such as depolymerization may require a larger and more diverse group of heterotrophic microorganisms to be carried out, so the lack of diversity in some microbial groups (e.g. fungi), may limit organic

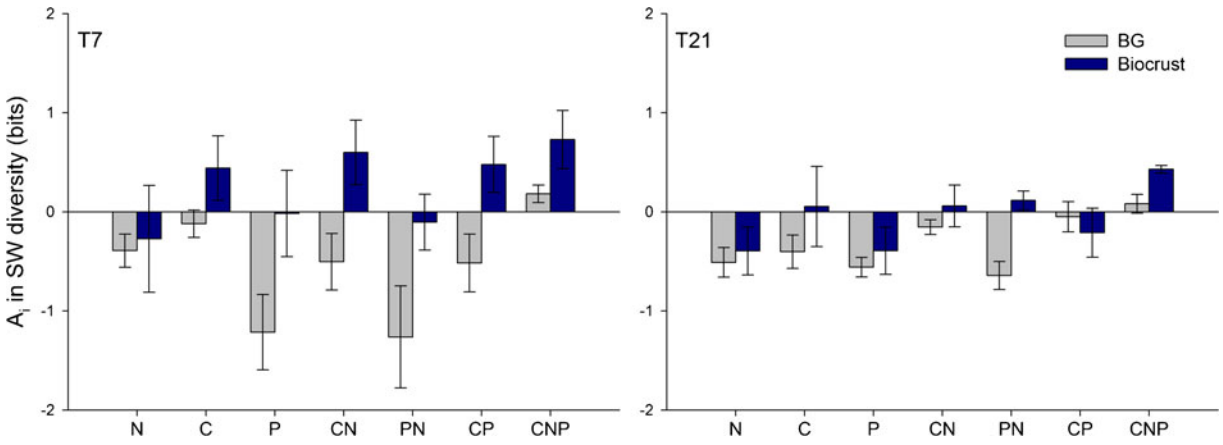

Fig. 3 Increment (A_i) in the Shannon-Weaver (SW) diversity index obtained from the Microresp results for the different nutrient treatments for biocrust and bare ground soils. T7 and T21 indicate results obtained 7 and 21 days after the beginning of the incubations

matter decomposition and N transformation processes (Schimel et al. 2005; Cookson et al. 2006). Biocrust maintain abundant and rich fungal communities underneath them (Bates et al. 2010), which are linked to the decomposition of soil organic matter (Austin et al. 2004), and thus may explain their higher functional diversity and DON production when C is available (Table S1; Bates et al. 2010; Delgado-Baquerizo et al. 2013c).

The increase in nitrate dominance was matched with a lower microbial functional diversity when N was added in both microsites. Hence, an increase in the N deposition derived from human activities (Finzi et al. 2011; Peñuelas et al. 2012) may result into a lower functional diversity under biocrusts, decreasing the dominance of "aggregated" processes such as depolymerization and increasing the dominance of "narrow" processes such as nitrification (Schimel et al. 2005; Cookson et al. 2006). This fact may derive into a more inorganic control of the N cycle (Schlesinger et al. 1990), which may affect the uptake of organic N by plants (Warren 2009). The addition of P resulted in a lower impact than that of N and C on the functional diversity of biocrust soils, also favoring the DON dominance comparing to the BG microsite. Our results suggest that P may be an essential nutrient in biocrust-dominated soils, limiting highly energetic processes such as organic matter decomposition or N-fixation, where ATP, and therefore P, works as currency (Belnap and Lange 2003; Bottomley and Myrold 2007). The higher microbial functional diversity observed in biocrust compared to BG soils for most of the nutrient treatments evaluated here suggests that biocrusts may confer higher protection to microbial communities against changes in ratios C, N and P derived from human activities such as fertilization and atmospheric N deposition.

Changes in the labile C: N ratios (from glucose and ammonium, respectively), more than N availability itself, seem to modulate N transformation processes in both biocrust and BG microsites. Residual ammonium from the N treatment (addition of N alone) was still present after incubating the soils for 7 days, influencing the dominance of N forms. However, after incubating the soils for 21 days, NO_3^--N was the dominant N form in the N and N+P treatments, NH_4^+-N was dominant in the C+N and C+N+P treatments, and DON was the dominant N form in the C and C+P treatments. Besides, a decrease in the total available N was observed when C was added, and while there was an increase in the microbial functional diversity, suggesting that the addition of C may promote the immobilization of N by heterotrophic microbial communities. All these facts support the hypothesis that the availability of C relative to that of N (Cookson et al. 2006; Robertson and Groffman 2007), more than N availability by itself (Schimel and Bennett 2004), may be modulating the N dominance form in drylands when nutrients are in easily

Fig. 4 Resilience in the N variables studied: available N, ammonium, nitrate and DON to the different N treatments (N, C+N, C+P and C+N+P) for biocrust and bare ground soils. T7 and T21 indicate results 7 and 21 days after the beginning of incubation ▶

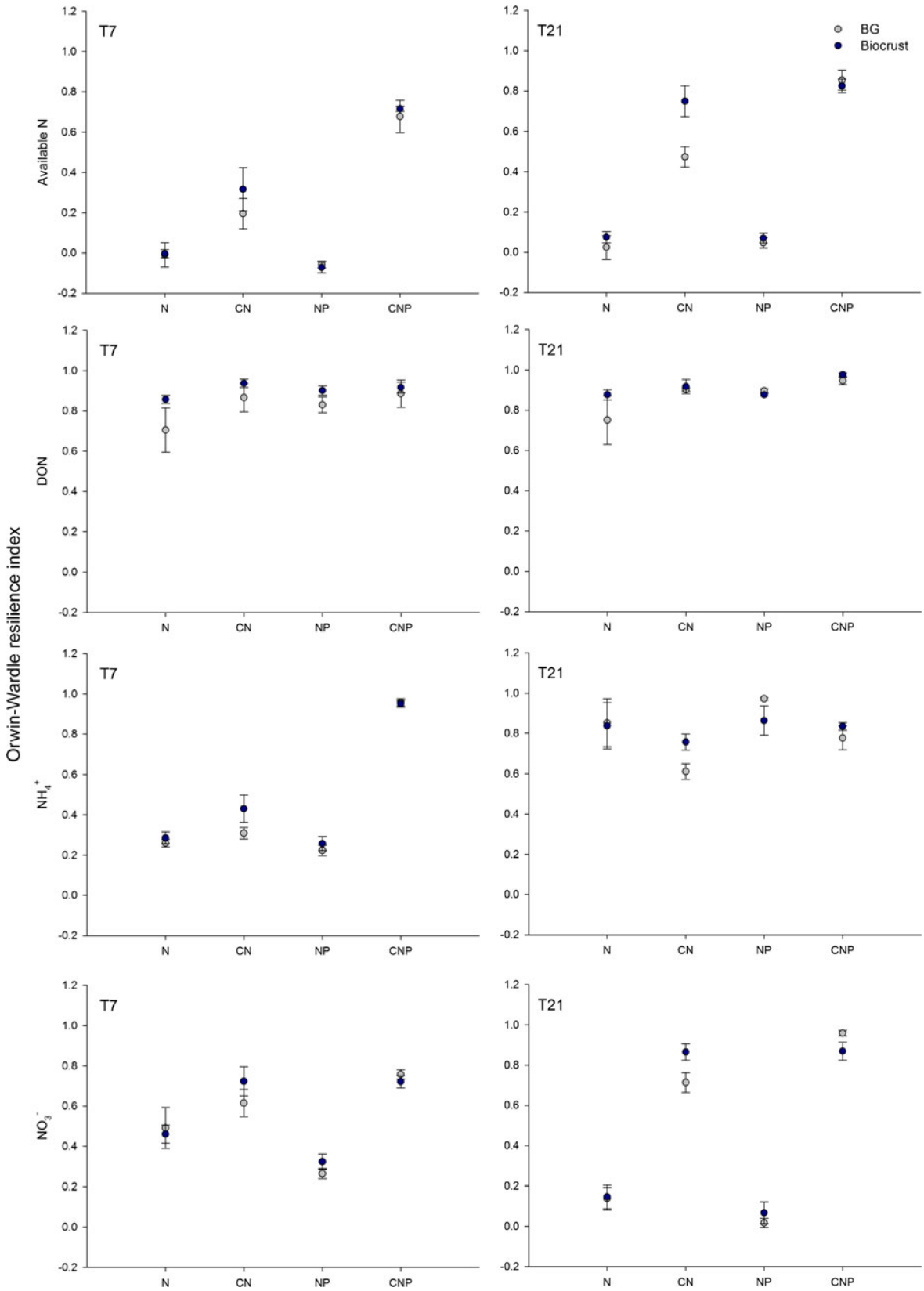
Orwin-Wardle resilience index
Available N
DON
NH_4^+
NO_3^-
T7
T21
BG
Biocrust
N
CN
NP
CNP
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2

Table 2 Correlation coefficient (Spearman's ρ) between the absolute increment (A_i) in microbial functional diversity (Shannon's diversity index; H') and the A_i in avalable N (n=35), in the dominance of N forms (DON, NH_4^+ and NO_3^-; n=35) and in the resilience of total available N, DON, NH_4^+ and NO_3^- facing N additions (N, CN, NP and CNP; n=20) for both biocrust and bare ground (BG) microsites in the two incubation periods (seven [T7] or 21 [T21] days)

	T7		T21	
	Biocrust	BG	Biocrust	BG
Available N	−0.377*	−0.324*	0.014	−0.27
Relative dominance of DON	0.332*	0.343*	0.096	−0.116
Relative dominance of NH_4^+	−0.179	0.026	0.149	0.495**
Relative dominance of NO_3^-	−0.349*	−0.384*	−0.214	−0.319*
Total available N resilience	0.502*	0.417*	0.363	0.762**
DON resilience	0.302	0.38	0.302	0.267
NH_4^+ resilience	0.525*	0.685**	0.083	−0.33
NO_3^- resilience	0.415*	0.335	0.22	0.620**

Significance levels are as follows: *p<0.05 and **p<0.01

available forms for plants and microorganisms. In this way, the nitrate accumulation typically found in drylands may be due to the low C:N ratio characterizing these systems (Hook and Burke 1995; Cookson et al. 2006; Castillo-Monroy et al. 2010).

We also evaluated how biocrusts modulate the resilience of N variables to N additions (N, C+N, N+P and C+N+P). Total available N and DON were more resilient to joint additions of N, C+N, N+P and C+N+P in biocrust soils than in BG soils. This fact may have important implications at the global scale (Elbert et al. 2012), since biocrusts may provide a higher resilience than bare ground areas to changes in the nutrient ratios, and terrestrial ecosystems are currently facing an increase in the C: P and N: P ratios derived from human C and N fertilization (Peñuelas et al. 2012). In general, a higher microbial functional diversity, which was linked to C treatments and to the presence of biocrusts, was matched with a higher resilience in the N variables studied. This fact suggests that by increasing heterotrophic microbial diversity, biocrusts may provide soils with a higher capacity to recover from processes such as N deposition. Overall, the resilience of the N cycle to N additions was always higher in the C+N+P treatment, suggesting that any limitation in these nutrients may affect the ability of the system to absorb N impacts such as N deposition (Ochoa-Hueso et al. 2011; Peñuelas et al. 2012). However, we must explicitly highlight that extrapolations from controlled experiments such as that employed here to the field should be made with caution given the complexity of biogeochemical processes in natural environments colonized by biocrusts. Even when considering the limitations of our experimental approach, our results highlight the important role of biocrusts in the response to human-induced nutrient inputs in drylands, and pave the way for future field studies aiming to understand how these organisms will modulate nutrient cycling responses to ongoing global change.

Concluding remarks

Our results suggest that biocrusts modulate soil N dynamics and the functioning of the microbial communities in response to the changes in the availability of C, N and P. Thus, biocrusts promoted an increase in the DON dominance and microbial functional diversity when C or P was added. Changes in the ratios of labile C to N, more than N availability, seems to modulate nitrification processes in the dryland soils studied. Biocrusts may have an important role in increasing the resilience of the N cycle to C:P and N:P imbalances derived from C and N human fertilization, such as N deposition and increases in atmospheric CO_2 concentration.

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