Species identity of biocrust-forming lichens drives the response of soil nitrogen cycle to altered precipitation frequency and nitrogen amendment

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ABSTRACT

Biological soil crusts (biocrusts) are fundamental components of drylands worldwide, and are of great importance for the regulation of ecosystem functioning. However, little is known on the role of species identity of biocrust-forming lichens in mediating the response of nitrogen (N) cycling to concurring global environmental change. Here, we conducted a microcosm study to evaluate how the species identity of biocrust-forming lichens (*Diploschistes thunbergianus*, *Psora crystallifera* and *Xanthoparmelia reptans*) regulate key processes of N cycling in response to simulated changes in rainfall frequency and N addition. We explicitly considered both direct and indirect effects (i.e. driven via microbial diversity and abundance) of global changes on N availability and losses using structural equation models. Our results showed that species of biocrust-forming lichens differentially mediated effects of N amendment and altered rainfall frequencies on belowground nitrate availability and N₂O flux rate. For instance, soils under *P. crystallifera* species showed the highest increase in nitrate content in response to N amendment under low rainfall frequency. Moreover, soils under *D. thunbergianus* showed the highest N₂O flux under high rainfall frequency without N addition. Interestingly, soils under *X. reptans* showed lowest and highest resistance in nitrate availability and N₂O flux, respectively, in response to N addition regardless of different rainfall frequencies. Strikingly, we only found an indirect impact of either rainfall frequency or N amendment on the nitrate availability (but not N₂O flux) driven via the ammonia-oxidizing community under *X. reptans*. Our results provide evidence that the species identity of biocrust-forming lichens modulates the response of N cycling to global change drivers. These findings have implications for predicting the potential consequence of altered rainfall patterns and environmental N inputs in dryland ecosystems.

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1. Introduction

Dryland ecosystems cover 41% of earth's land surface and support over 38% of the total global population (Reynolds et al., 2007;

Maestre et al., 2012). These ecosystems are highly vulnerable to ongoing global environmental changes (Schlesinger et al., 1996; Reynolds et al., 2007; Maestre et al., 2012; Dai, 2013; Delgado-Baquerizo et al., 2013a). Climate change models predict major changes in rainfall amounts and patterns in drylands worldwide during the second half of this century (Solomon, 2007). In parallel to climate change, environmental nitrogen (N) inputs resulting from anthropogenic activities is changing the N cycle in terrestrial ecosystems (Vitousek et al., 1997; Cui et al., 2013), affecting ecosystem processes (Phoenix et al., 2012; Concilio and Loik, 2013). Despite global change drivers are known to interact in their impacts

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on ecosystem services, we have limited knowledge on how the interaction between important factors such as decreasing rainfall frequency and increasing N inputs will affect ecosystem functioning in drylands (e. g. N cycle) and influence microbial communities; which carry out vital ecosystem functions (Fay et al., 2008; Delgado-Baquerizo et al., 2014). In drylands, water and N are the most important factors limiting resources for plant and microbial activity (Hooper and Johnson, 1999; Austin, 2011). Thus, understanding the underlying mechanisms that control effects of water (i.e. precipitation frequency) and N (N amendment) on nutrient availability is particularly important for managing soil fertility and ecosystem productivity in dryland ecosystems (Robertson and Groffman, 2007; LeBauer and Treseder, 2008).

Biological soil crusts (biocrusts hereafter) are photosynthetic, diazotrophic communities of bacteria, fungi, algae, lichens and moss that colonize the surfaces of dryland soils and are prominent surface features in all natural drylands (Belnap, 2003). Biocrusts are important to the stability and productivity of dryland ecosystems where plants are typically sparse (Eldridge and Greene, 1994; Belnap et al., 2008; Lindo and Gonzalez, 2010; Zelikova et al., 2012). Previous studies have demonstrated that biocrusts play crucial roles in mediating key processes of N turnover, such as N fixation, nitrification and denitrification (Belnap, 2003; Johnson et al., 2007; Strauss et al., 2012; Abed et al., 2013; Delgado-Baquerizo et al., 2013c; Kidron et al., 2015). Much less, however, is known about the importance of biocrusts on the diversity and abundance of particular microbial communities such as those related to N processes. Aboveground components of biocrusts have been reported to influence the structure of associated soil bacterial communities (Maestre et al., 2013; Maier et al., 2014), as well as functional groups related to N transformation such as ammonia-oxidizing prokaryotes (Marusenko et al., 2013; Delgado-Baquerizo et al., 2014, 2015). The availability of N for plants and microbes is predominantly mediated by particular microbial communities that carry out important processes such as nitrification and denitrification (Robertson and Groffman, 2007). These processes ultimately control N inputs and losses in terrestrial ecosystems. Thus, complex interactions between biocrusts and microbial communities may synchronously mediate effects of climate change (i.e. altered rainfall patterns) and N inputs on the N cycling in drylands (Delgado-Baquerizo et al., 2014).

Despite the well-known influence of biocrusts on the regulation of soil nutrient cycles and microbial communities, little is known about how identities of biocrust-forming species (e.g. lichen species), and their associating microbial communities, modulate the impact of global change drivers on nutrient cycling. A recent study suggested that, similar to vascular plants, biocrust-forming lichens have species-specific effects on soil nutrient cycling and microbial abundance (Delgado-Baquerizo et al., 2015). However, the role of different biocrust-forming species in controlling the response of nutrient cycling (here N transformation processes) to global environmental change remains unresolved. Improving our understanding on the role of different species of biocrust-forming lichens in controlling multiple global change impacts on the N cycle is crucial for accurately forecasting their impacts on dryland ecosystems, the largest biome on Earth. Yet no previous study has evaluated how individual species of biocrust-forming lichens modulate the responses of key N cycling processes to interactive global environmental disturbances such as N inputs and altered rainfall frequencies, which are threatening the proper functioning of drylands worldwide (Reynolds et al., 2007; Solomon, 2007; Phoenix et al., 2012; Concilio et al., 2013).

Herein, we conducted a microcosm study to evaluate the potential role of biocrust-forming lichen species (*Diploschistes thunbergianus*, *Psora crystallifera* and *Xanthoparmelia reptans*) in

controlling responses of soil N cycling (i.e. nitrate availability and N_2O flux) to simultaneous changes in rainfall frequency and N additions. We explicitly considered both direct and indirect effects mediated via the diversity and abundance of microbial communities related to N availability and losses using structural equation modeling. We hypothesized that: i) the species identity of biocrust-forming lichens will drive the response of N cycle processes (i.e. nitrification and denitrification) to disturbances from added N and altered rainfall frequencies; ii) different species of biocrust-forming lichens will differentially modulate the resistance of the N cycle to N addition alongside different watering frequencies; and iii) changes in microbial diversity linked to different lichen species will influence the response of N availability to the interactive impacts from the disturbance factors.

2. Materials and methods

2.1. Experimental design

Samples for the microcosm study were collected from Nyngan (31°34', 147°12'E), New South Wales, Australia. The climate in this region is semiarid, with a mean annual rainfall and temperature of 431 mm and 18.7 °C, respectively (1920–2014). Open areas between plant-patches contained well-developed biocrust communities dominated by the lichens studied: *D. thunbergianus*, *P. crystallifera* and *X. reptans*. Sampling was carried out in May 2014 within a 50 m × 50 m area under each of the most abundant biocrust-forming lichens. All lichens randomly distributed within the same flat ground (Fig. S1). We randomly collected 30 intact soil cores (5 cm diameter and 5 cm height PVC tubes), as well as their respective lichen thalli, for each of the species studied (exclusively covered by *D. thunbergianus*, *P. crystallifera* and *X. reptans* in each case) and another 30 cores of bare ground areas. A total of 120 soil cores were collected (30 for each of the three biocrusts studied and bare ground areas; Fig. S1). All these soil cores were collected from open areas between plants avoiding areas under plant canopies. After sampling, soil cores were transported to the laboratory and air-dried at room temperature for three weeks before starting the microcosm experiment. Previous studies have found that air drying dose not appreciably alter variables such as C and N we studied (Zornoza et al., 2006; Delgado-Baquerizo et al., 2014). The soil is classified as alfisol with a content of 54% sand, 13% silt and 32% clay. Some basic chemical characteristics of the soils (0–4 cm depth, removing the above biocrusts) were obtained before our incubation experiment and are listed in Table 1.

We established a full factorial microcosm experimental design with three factors: biocrust-forming species (three lichen species and bare ground areas), N amendment (0 and 20 kg N ha⁻¹ year⁻¹) and changes in watering frequency (i.e. high frequency, 3.61 mm each 3 days; moderate frequency, 7.22 mm each 6 days; and low frequency, 14.44 mm each 12 days; Fig. S2). Five replicated cores per combination of treatments, (120 pots in total) were incubated for 72 days. It is important to note that the amount of water added to the different pots at the end of the experiment is exactly the same; with the only change being watering frequency. The amount of water added was adjusted to mimic the exact amount of water (via rainfall) these soils received during the spring season of the previous year under field conditions. N amendment was conducted at the beginning of the experiment by adding NH_4NO_3 with the first watering (0.78 mg per soil core, an amount that is comparable to many previous studies, Ramirez et al., 2010; Ochoa-Hueso et al., 2013). These N additions and watering treatments were selected to evaluate the potential role of species identity of biocrust-forming lichens in driving the response of the N cycle to future global change impacts. Thus, our treatments are within the limits

Table 1

Main chemical and biological properties of bare soil and soils under three species of biocrust-forming lichens at the beginning of incubation experiment.

		Bare soil	<i>Diploschistes thunbergianus</i>	<i>Psora crystallifera</i>	<i>Xanthoparmelia reptans</i>
pH	(H ₂ O)	5.73 ± 0.07a	5.86 ± 0.07a	5.83 ± 0.04a	5.89 ± 0.05a
TC	(%)	1.13 ± 0.01a	1.20 ± 0.07a	1.40 ± 0.14a	1.91 ± 0.11b
TN	(%)	0.09 ± 0.001a	0.09 ± 0.005a	0.11 ± 0.006a	0.16 ± 0.011b
DOC	(mg kg ⁻¹)	117.7 ± 8.71a	142.4 ± 24.3a	160.6 ± 21.7a	144.7 ± 3.5a
MBC	(mg kg ⁻¹)	143.7 ± 21a	188.3 ± 24a	169.1 ± 15a	243.0 ± 68a
NH ₄ ⁺ –N	(mg kg ⁻¹)	2.52 ± 0.56a	8.77 ± 2.10b	2.17 ± 0.64a	6.61 ± 1.80 ab
NO ₃ ⁻ –N	(mg kg ⁻¹)	1.14 ± 0.051a	3.86 ± 1.55a	6.62 ± 1.92 ab	10.60 ± 2.05b
DON	(mg kg ⁻¹)	4.81 ± 1.08a	2.97 ± 0.78a	2.66 ± 1.02a	1.76 ± 1.37a
MBN	(mg kg ⁻¹)	0.36 ± 0.35a	13.7 ± 2.1b	10.6 ± 1.5b	8.87 ± 1.01b
PNR	(mg NO ₂ ⁻ –N kg ⁻¹ soil h ⁻¹)	0.06 ± 0.01ab	0.03 ± 0.0a	0.05 ± 0.02ab	0.11 ± 0.01b
AOA abundance	(<i>amoA</i> gene copies g ⁻¹ soil)	7.58E + 07a	8.31E + 07a	9.48E + 07 ± a	1.36E + 08a
AOB abundance	(<i>amoA</i> gene copies g ⁻¹ soil)	2.91E + 04a	5.62E + 04 ab	8.91E + 04 ab	1.23E + 05b
<i>nosZ</i> abundance	(<i>nosZ</i> gene copies g ⁻¹ soil)	7.25E + 05a	8.43E + 05a	1.54E + 06a	1.49E + 06a

TC and TN, total carbon and total nitrogen, respectively; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PNR, potential nitrification rate; AOA, ammonia oxidizing archaea; AOB, ammonia oxidizing bacteria; *nosZ*, denitrifiers consisting of *nosZ* gene. Data are means ± SE (n = 5). Different lowercase letters indicate significant differences between the examined variables.

predicted by environmental N inputs and climate change models for dryland regions and are largely used in long and short-term experiments in the current literature (Phoenix et al., 2006; Feng and Fu, 2013; Ochoa-Hueso et al., 2013). A climate-controlled glass-house was used to precisely manipulate light, temperature, humidity, and day–length cycles. The monthly day and night temperature and humidity conditions were controlled based on 60 year's average data from the Meteorological Bureau Station (<http://www.bom.gov.au>). The temperature ranged from 26 °C in the day time to 15 °C during night time. The cores were rotated regularly within the glass-house to avoid any potential position effects. In total 120 soil samples (0–4 cm depth) were collected at the end of the incubation period for chemical and molecular analysis, carefully removing the above biocrusts. Biocrusts are known to have a strong influence on soils up to 4 cm in depth, which is why we restricted our sampling to this depth (usually top 0–4 cm of soil; Delgado-Baquerizo et al., 2013c; Bowker et al., 2014).

2.2. Soil characteristics analysis and N related variables measurements

Soil pH was measured using a fresh soil to water ratio of 1: 2.5 with a Delta pH-meter (Mettler-Toledo Instruments Co., Columbus, OH, USA). Soil moisture content was measured by oven-drying the samples at 105 °C for 24 h. Total carbon (TC) and total nitrogen (TN) in the soils were measured on a LECO macro-CN analyzer (LECO, St. Joseph, MI, USA). Potential nitrification rate (PNR) was assessed using the chlorate inhibition soil-slurry method as previously described (Hu et al., 2015). Ammonium, nitrate, dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in the soils were extracted with 0.5 M K₂SO₄ in a ratio of 1:5 by shaking at 200 rpm for 1 h and filtered using 0.45-μm Millipore filter paper (Jones and Willett, 2006). In parallel, the N present in microbial biomass (MBN) was determined using the fumigation-extraction method (Brookes et al., 1985). Full details of the N-related measurements is provided in the method description of the Supplementary Materials.

Soil N₂O flux was monitored using the entire soil column including soil and biocrusts. Each core was placed in a glass jar (12 cm depth, 7.5 cm diameter, Ball, USA), and then sealed with a gas-tight lid, which had a rubber stopper in the middle. Fifteen ml gas samples were taken from the headspace at 0, 30 and 60 min after sealing, using a 25 ml gas-tight syringe. The gas sample was immediately injected into a pre-evacuated 10-ml glass vial (Agilent Technologies, USA) sealed with a butyl rubber stopper and aluminium seal (Sigma–Aldrich, USA). Then, N₂O was measured

with an electron capture detector in an Agilent-7890a gas chromatograph equipped with a flame ionization detector (FID) and an electron capture detector (ECD) (Agilent Technologies, Wilmington, DE, USA). A linear model was then applied to estimate the N₂O flux rate inside the jar headspace.

2.3. Quantification and diversity of *amoA* and *nosZ* genes

Genomic DNA was extracted from 0.25 g of soil using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions with slight modifications; a FastPrep bead beating system (Bio-101, Vista, CA, USA) at a speed of 5.5 m s⁻¹ for 60 s was used at the initial cell-lysis step. The quantity and quality of extracted DNA was checked using a NanoDrop® ND-2000c UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

The abundances of *amoA* for ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) and *nosZ* genes were quantified on a CFX-96 thermocycler (Biorad, USA). These ammonia-oxidizers are responsible for the first step involved the conversion of ammonia to nitrite (Purkhold et al., 2000). Moreover, *nosZ* gene is considered to be one of the most important functional genes associated with denitrification (Henry et al., 2006). Primer pairs of CrenamoA23f/CrenamoA616r (Tourna et al., 2008), *amoA*-1F/*amoA*-2R (Rotthauwe et al., 1997) and *nosZ*2F/*nosZ*r (Henry et al., 2006) were used for quantifying *amoA* for AOA, AOB, and *nosZ* genes, respectively. Microbial species diversity (e.g. Shannon index) of *amoA* (for AOA) and *nosZ* gene were characterized using terminal-restriction length polymorphism (T-RFLP) analysis, using the fluorescently-labelled primers FAM-CrenamoA23f/CrenamoA616r and FAM-*nosZ*1211F/*nosZ*1719R (Enwall et al., 2005), respectively. Here, the AOB community was not analyzed because of low AOB abundance according to our results from qPCR. Thus, the PCR products did not satisfy the requirements for T-RFLP.

2.4. Statistical analysis

First, we used a three-way ANOVA to evaluate the overall effect of biocrust species, watering frequencies and N amendment on N related variables (i.e. nitrate, N₂O flux rate and microbial gene abundance and diversity) included in this study. A two-way ANOVA was used to check the effects of N amendment and watering frequency on the examined variables under different species of biocrust-forming lichens. The *amoA* and *nosZ* gene copy numbers were log-transformed prior to statistical analysis to meet normality assumptions. Multiple comparisons of group means between

treatments were performed with a Student-Newman-Keuls test after a one-way ANOVA on nitrate, MBN and N₂O flux, *amoA* and *nosZ* gene. All statistical analyses were performed using R.3.1 software (<http://www.r-project.org>).

We then calculated the resistance of the N variables evaluated (ammonia, nitrate, dissolved inorganic nitrogen (DIN), DON, MBN, potential nitrification rate and available N to the altered water frequencies and added N using the Orwin & Wardle (Orwin and Wardle, 2004) resistance index (RS), according to the following equation:

$$RS = 1 - \frac{2|D_0|}{(C_0 + |D_0|)}$$

where D_0 is the difference between the control (C_0 ; value of each N variables in the treatments without N addition) and the N amended soil. This index has the advantage of being standardized by the control, being bounded between -1 (less resistance) and $+1$ (maximal resistance); it remains bounded even when extreme values are encountered (Orwin and Wardle, 2004). We used two-way ANOVA to evaluate differences in the resistance of N variables to N amendment among biocrusts species and watering frequencies (both factors being fixed in these analyses). We further explored the relationship between watering frequency and the resistance of N transformation to added N using Spearman's correlation analysis.

Finally, we used structural equation models (SEMs) to evaluate the direct and indirect (through the microbial community) effects of watering frequency and N amendment on N turnover (i.e. nitrate synthesis and N₂O flux) in the soils under the three lichen species. The N treatments were set as categorical exogenous variables with two levels: 0 and 1 (Grace, 2006). Before conducting SEMs, gene abundance values were log-transformed to improve linearity. The microbial community was introduced as a composite variable into the model after obtaining a satisfactory model fit. This composite variable consists of the abundance of *amoA* or *nosZ* gene and their diversity (i.e. Shannon index, Fierer and Jackson, 2006). The use of composite variables does not alter the underlying assumptions of SEM, but collapses effects of multiple conceptually-related variables into a single composite effect, aiding interpretation of model results (Shipley, 2002). When these data manipulations were completed, we parameterized our model using our dataset and tested its overall goodness of fit. There is no single universally accepted test of overall goodness of fit for SE models. Thus, we used the Chi-square test (χ^2 ; the model has a good fit when $0 \leq \chi^2 \leq 2$ and $0.05 < P \leq 1.00$) and the root mean square error of approximation (RMSEA; the model has a good fit when RMSEA $0 \leq RMSEA \leq 0.05$ and $0.10 < P \leq 1.00$) (Schermelleh-Engel et al., 2003). Additionally, because some variables were not normal, we confirmed the fit of the model using the Bollen-Stine bootstrap test (the model has a good fit when $0.10 < \text{bootstrap } P \leq 1.00$). The different goodness-of-fit metrics indicate that our *a priori* model was satisfactorily fitted to our data, and thus no post hoc alterations were made.

To aid with the final interpretation of our SEMs, we also calculated the standardized total effects of the global change drivers and microbial community features characteristics (e.g. abundance, structure, diversity) on NO₃[−] and N₂O. The net influence that a given variable has upon another is calculated by summing all direct and indirect pathways between these two variables. If the model fits the data well, the total effect should approximate the bivariate correlation coefficient for pair of variables (Shipley, 2002; Grace, 2006).

3. Results

3.1. Species identity of biocrust-forming lichens modulates the response of N cycle to added N and altered watering frequency

Our results indicate that species identity of biocrust-forming lichens modulates the response of soil nitrate availability and N₂O flux to N addition and shifted watering frequencies (Fig. 1; Table S1). This modulator effect was evidenced by the various interactions among biocrust species $\times$ N amendment $\times$ watering frequency found for both nitrate content and N₂O flux rates. Specifically, soil nitrate content decreased with elevated watering frequency under *P. crystallifera* (Fig. 1, N treatment) and *X. reptans* (without N addition). Soils under *D. thunbergianus* had the highest average N₂O fluxes ($P < 0.05$); especially under medium and high watering frequencies without N amendment compared to *P. crystallifera* and *X. reptans* covering soils (Fig. 1). Moreover, we found that soils under *D. thunbergianus* showed a sharp decrease in the N₂O flux in response to N addition under a high watering frequency, a response that was not observed in the other lichen species (Fig. 1; Table S2).

Fig. 1. Contents of NO₃[−]-N and N₂O efflux rates in response to varied watering frequency and nitrogen (N) amendment in the bare soil (BS) and soils with three biocrust-forming lichen species *Diploschistes thunbergianus* (DT), *Psora crystallifera* (PC) and *Xanthoparmelia reptans* (XR). For N₂O, a positive value means that a production of N₂O occurs, whereas a negative flux denotes a sink of N₂O. Letter L, M and H mean low (added water each 12 days), moderate (added water each 6 days) and high watering frequency (added water each 3 days), respectively. Different capital letters indicate significant differences between the soils under altered watering frequencies without N addition ($P < 0.05$). Different lowercase letters indicate significant differences between the soils under altered watering frequencies with N amendment ($P < 0.05$). Data are means $\pm$ SE ($n = 5$).

3.2. Species identity of biocrust-forming lichens modulates the response of nitrifiers and denitrifier microbes to added N and altered watering frequency

Biocrusts and watering frequency rather than N amendment significantly influenced AOA abundance in our experiment (Fig. 2; Table S1). Intriguingly, the response of the AOA community to altered watering frequencies and N addition varied under different species of biocrust-forming lichens (Fig. 2; Table S2). In particular, we found the highest soil AOA abundance under *X. reptans*, which was not affected by altered watering frequency or N addition ($P > 0.05$; Table S2). However, AOA diversity was the highest under low watering frequency without N amendment, for all type of biocrusts (Fig. S3; Table S1). We also found that different species of biocrust-forming lichen modulated the response of *nosZ* diversity and abundance to altered watering frequencies as for the observed interaction between biocrust species $\times$ watering frequency ($P < 0.05$; Fig. 2; Fig. S3; Table S1). In particular, we found lower abundance of the *nosZ* gene under *D. thunbergianus* compared with *P. crystallifera* and *X. reptans*, though no difference of *nosZ* gene diversity was observed between the different biocrusts ($P > 0.05$; Fig. 2; Fig. S3; Table S1). Different species of biocrust-forming lichens altered the response of the

nitrifying and denitrifying microbial communities to changed watering frequencies, as observed with both AOA and *nosZ* gene community structures in the conducted non-metric multidimensional scaling analysis (Fig. S4; PerMANOVA, $P < 0.05$).

3.3. Species identity of biocrust-forming lichens modulates the resistance of N cycling processes to N addition

Biocrust species had differential effects on the resistance of N-related variables to N addition ($P < 0.05$; Fig. 3). Overall, the soil under *X. reptans* showed the highest and lowest resistance to N addition across different watering frequencies for nitrate availability and N_2O fluxes, respectively. Individually, watering frequency had a negative impact on the resistance of ammonia, N_2O fluxes and available N under the *D. thunbergianus* ($P < 0.05$; Fig. 3; Table 2). Interestingly, increased watering frequency tended to reduce the resistance of N_2O flux in all cases.

3.4. Species identity of biocrust-forming lichens modulates indirect effects of watering frequency and N amendment on nitrate availability and N_2O flux via microbial community

Our SEMs indicate that each species of biocrust-forming lichens differentially modulate the effect of N amendment and watering frequency on nitrate content and N_2O flux (Fig. 4; Table 3). For example, while the effect of N addition and watering frequency on nitrate were indirectly driven by microbial community under *X. reptans* ($P < 0.01$; Fig. 4), however, in the case of the *P. crystallifera*, we found that N amendment directly affected nitrate availability regardless of the microbial community ($P < 0.05$; Fig. 4; Table 3). We did not observed any direct or indirect significant effect of the environmental disturbances on nitrate availability under *D. thunbergianus* ($P > 0.05$), although they had a significant,

Fig. 2. Abundances of ammonia-oxidizing archaea (AOA) *amoA* and *nosZ* genes in response to altered watering frequency and N addition in the bare soil and the soils under different biocrust-forming lichen species *Diploschistes thunbergianus* (DT), *Psora crystallifera* (PC) and *Xanthoparmelia reptans* (XR). Letter L, M and H mean low (added water each 12 days), moderate (added water each 6 days) and high watering frequency (added water each 3 days), respectively. Different capital letters indicate significant differences between the soils under altered watering frequencies without N addition ($P < 0.05$). Different lowercase letters indicate significant differences between the soils under altered watering frequencies with N amendment ($P < 0.05$). Data are means $\pm$ SE ($n = 5$).

Fig. 3. Changes in the resistance indexes of $NO_3^- - N$ and N_2O flux in response to N addition in the bare soil (BS) and the soils under different biocrust-forming lichen species *Diploschistes thunbergianus* (DT), *Psora crystallifera* (PC), and *Xanthoparmelia reptans* (XR). Letter L, M and H mean low (added water each 12 days), moderate (added water each 6 days) and high watering frequency (added water each 3 days), respectively. Different letters indicate significant differences between different simulated rainfall frequencies ($P < 0.05$). Data are means $\pm$ SE ($n = 5$).

Table 2

Correlation coefficients (Spearman's ρ) between the resistance of N variables and simulated rainfall frequency (added water every 3, 6, and 12 days, respectively, with the same annual precipitation). P values below 0.05 are in bold.

	DIN	AVBN	N ₂ O	NH ₄ ⁺ -N	NO ₃ ⁻ -N	DON	MBN	PNR
<i>D. thunbergianus</i>	-0.25(0.377)	-0.60(0.017)	-0.84(<0.001)	-0.79(<0.001)	0.28(0.305)	0.095(0.737)	0.09(0.738)	0.15(0.591)
<i>P. crystallifera</i>	-0.04(0.894)	0.57(0.028)	-0.55(0.034)	-0.23(0.416)	0.47(0.075)	0.59(0.021)	0.55(0.034)	0.13(0.638)
<i>X. reptans</i>	-0.51(0.050)	-0.18(0.947)	-0.59(0.021)	-0.13(0.638)	-0.04(0.893)	0.21(0.455)	0.94(0.737)	0.019(0.947)

DIN, dissolved inorganic nitrogen; AVBN, available nitrogen; DON, dissolved organic nitrogen; MBN, microbial biomass nitrogen; PNR, potential nitrification rate.

Fig. 4. Structural equation models showing the effects of watering frequency and N amendment on soil microbial communities (AOA and *nosZ* gene), and NO₃⁻-N availability and N₂O flux rates under *Diploschistes thunbergianus* (DT), *Psora crystallifera* (PC), and *Xanthoparmelia reptans* (XR). Microbial community includes gene abundance and diversity based on Shannon index. Bold and dashed arrows indicate significant and non-significant pathways respectively; green and red arrows denote positive and negative relationships, respectively. Numbers adjacent to arrows are path coefficients, and the width of arrows is proportional to the strength of path coefficients. As in other linear models, R^2 denotes the proportion of variance explained and appears under every response variable in the model. The hypothetical model created was satisfactorily fitted to our data, as suggested by non-significant χ^2 values ($\chi^2 = 0$; $P = 1$; $d.o.f = 1$ in all cases), non-parametric Bootstrap $P = 0.999$ and by values of RMSEA = 0.000 with a $P = 1$. Significance levels are as follows: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contrasting impact on the AOA community. Regarding the emission of gasses, we found positive direct and total impacts for both N addition and watering frequency on the N₂O flux under *D. thunbergianus* ($P < 0.001$; Fig. 4; Table 3), which was negatively impacted by N addition under *X. reptans* ($P < 0.01$; Fig. 4; Table 3). However, we did not observe any indirect effect of the three species of biocrust-forming lichens on N₂O flux rate through microbial communities.

4. Discussion

We evaluate the potential role of species identity of biocrust-forming lichens in controlling the response of the N cycle (nitrification and denitrification) to shifted rainfall frequency and N addition. Our study provides evidence that the species identity of biocrust-forming lichens drives the response of N cycling processes to the disturbance factors. For instance, N addition did not have any effect on nitrate availability under *X. reptans* which had showed the

highest resistance to this disturbance, regardless of watering frequency. However, N addition produced an overall increase in nitrate availability under *P. crystallifera* with the lowest resistance to this impact under high and low rainfall frequencies. The importance of biocrusts in enhancing the resistance of the N cycle in response to global changes was previously reported (Reed et al., 2012; Delgado-Baquerizo et al., 2013b, 2013c), however, this is the first study providing empirical evidence that species of biocrust-forming lichens differentially modulated the resistance of the N transformation processes to simultaneous global change drivers. Differences in the response of N cycle resistance to global change drivers under different biocrust-forming lichen species can be explained by several complementary mechanisms, which include soil properties, biocrust traits and microbial communities under each lichen species.

Regarding soil properties, soils under *X. reptans* had the highest nitrate availability, as well as DOC and total N contents in the control (Table 1), which may have diluted the impact derived from

Table 3

Standardized total effects (direct plus indirect effects) derived from the structural equation modeling, including the effects of N amendment, watering frequency, microbial biomass N (MBN), microbial abundance and diversity on N cycle (NO_3^- availability and N_2O flux) for the different species of biocrust-forming lichens studied here. DT = *Diploschistes thunbergianus*; PC = *Psora crystallifera* (PC); XR = *Xanthoparmelia reptans*. Microbial abundance and diversity represent total effects of AOA and *nosZ* genes on NO_3^- and N_2O , respectively.

Biocrusts		N amendment	Watering frequency	MBN	Microbial abundance	Microbial diversity
DT	NO_3^-	-0.120	0.200	-0.100	-0.180	0.220
	N_2O	0.394	0.741	-0.086	-0.266	-0.122
PC	NO_3^-	-0.342	-0.513	0.063	0.036	-0.076
	N_2O	-0.140	0.140	-0.090	0.060	-0.570
XR	NO_3^-	-0.055	-0.252	-0.220	0.054	0.530
	N_2O	-0.320	0.220	0.350	-0.090	-0.050

N inputs (Gruber and Galloway, 2008), leading to a relatively stable nitrate availability compared to the control (no N addition). In addition, the soil under *X. reptans* also had the highest microbial activity and MBN in this study (e.g. PNR in Table 1; Fig. S5). A higher soil nitrate content under *X. reptans* than under *D. thunbergianus* and *P. crystallifera* may incorporate N to microbial biomass, absorbing any impact from added N in the soils (Treseder, 2008). This may be especially true for drylands where N is one of the most important limiting factors for both plant and microbial growth (Hooper and Johnson, 1999; Austin, 2011; Schlesinger and Bernhardt, 2013). Indeed, the importance of the microbial community controlling the impact of N amendment on nitrate availability is especially noticeable for *X. reptans*. It should also be noted that such dose of N inputs may have negative effects on N cycling under some biocrusts. For example, the MBN content under the three biocrusts tended to decrease in responses to N addition (Fig. S5; Table S1), especially that under the *D. thunbergianus* ($P < 0.05$; Table S2), which could be associated with its relatively low microbial abundances that are crucial for N cycling.

Albeit soils under *X. reptans* had the highest resistance for nitrate availability in this study, strikingly, they also had lower resistance for average N_2O fluxes compared to the soil under other biocrust species. This interesting result may be the consequence of different biocrust traits linked to different species of lichens studied here. For example, *D. thunbergianus* thalli are firmly attached to the soil (Fig. S1), which may produce local anaerobic conditions that promote processes involved in N_2O production (Henry et al., 2006; Jones et al., 2013). The sharp increase and decrease of N_2O fluxes and nitrate, respectively, in response to N amendment and under high rainfall frequency support this idea. Contrary to this, *X. reptans* is loosely attached to the soil which may favor aerobic processes such as nitrification, providing the highest resistance for nitrate availability. The thallus of *P. crystallifera* is also highly attached to the soil, albeit *P. crystallifera* usually forms smaller patches than *D. thunbergianus* and thus may promote more similar conditions to *X. reptans* than to *D. thunbergianus* (Fig. S1). In addition, the stoichiometry of plants could have a strong effect on the belowground soil properties (Bell et al., 2014). The nutrient content in thalli thus may have played a role when controlling the resistance of belowground N cycling to the N addition and altered rainfall frequencies.

For example, although we did not measure N in lichen tissue in this study, Delgado-Baquerizo et al. (2015) reported that species such as *Diploschistes diacapsis* (similar to *D. thunbergianus* here) had the lowest N availability in thalli tissue and soil among the six examined species from the Iberian peninsula, whereas *Psora decipiens* (similar to *P. crystallifera*) contained the highest N content. Soil microsites with high N availability for plants have been reported to reduce N_2O fluxes (Zaman et al., 2009; Liu et al., 2013), likely by promoting AOA and AOB communities, which could explain the lowest N_2O flux rates under *P. crystallifera* and *X. reptans* microsites (Table 1). Our results are also supported by previous study suggesting the positive role of *Xanthoparmelia* spp (similar *X. reptans* here) in N fixation (Seneviratne and Indrasena, 2006).

Beside soil properties and lichen traits, we also found that the microbial communities under particular lichen species (i.e. *X. reptans*) may drive the species-specific response of the N cycle to altered rainfall frequencies and added N. This observation is supported by the SEMs which suggest that under *X. reptans* thalli, AOA mediated the effect of N addition and changes in rainfall frequency on nitrate availability, which was not observed for any other lichen species included in this study. The importance of AOA in nitrification is largely supported by laboratory studies (e.g. Verhamme et al., 2011), but our results further indicate that this relationship may change among different dryland microsites. In addition to this, we found strong, significant and direct effects of microbial community involvement in the N_2O flux for the soil under *P. crystallifera*. Nitrification and denitrification are specialized processes performed by very specific group of microorganisms (Schimel et al., 2005; Singh et al., 2014). In this respect, two factors may explain the lack of direct effects from microbes on N_2O flux under *D. thunbergianus* and nitrate availability under *D. thunbergianus* and *P. crystallifera*. First, we want to highlight that for logistical reasons we did not measure the diversity and abundance of all microbial functional groups such as *nirK*, *nirS* and *norB* which also play important roles in controlling both nitrification and denitrification in soils (Jones et al., 2013). Second, we argue that at least for AOA, the high gene abundance (vs. bare soil) in low nutrient environments (e.g. *D. thunbergianus* and *P. crystallifera*) may not have limited the production of nitrate that may offset N loss through denitrification; thus this direct effect may have been

undetected for these biocrusts. In addition, our argument is also supported by the correlation between AOA abundance and nitrate as well as the relationship between *nosZ* gene abundance and N₂O flux (Table S3). Building from previous reports on the importance of interactions between rainfall frequency and N inputs on nutrient cycling in terrestrial ecosystems (Steudler et al., 1989; Mosier et al., 1991; Zheng et al., 2007; Morillas et al., 2015), we provide empirical evidence that the responses of N cycling to global change treatments is driven by the species identity of biocrust-forming lichens as a consequence of their different soil properties, lichen traits and microbial communities.

The observed direct and indirect (via microbes) control of species identity of biocrust-forming lichens on N processes in response to N amendment and shift in rainfall patterns, suggests that any change in biocrust composition derived from global change may have important consequences for nutrient availability in dryland ecosystems (Escobar et al., 2012; Reed et al., 2012), affecting both C and N cycling (Zelikova et al., 2012; Maestre et al., 2013). For instance, Escobar et al. (2012) found that climate change reduced the relative abundance of *Squamaria lentigera* and *D. diacapsis* in a dryland ecosystem from Spain. The negative effect of climate change on the abundance of other important biocrusts such as mosses has also been reported (Reed et al., 2012). Changes in the relative abundance of particular biocrust species may alter the resistance of the N cycle to global environmental change (Delgado-Baquerizo et al., 2015). In our study, any reduction in *X. reptans* and *D. thunbergianus*, with the highest nitrate content and N₂O flux resistance to N addition at different rainfall frequencies respectively, may constrain the availability of nitrate in these systems. Similarly, a reduction of *P. crystallifera* may alter the resistance of N cycling under particular scenarios (low and high rainfall frequencies) and in response to N addition.

5. Conclusions

In conclusion, our results provide novel and empirical evidence that the species identity of biocrust-forming lichens modulate the response of N cycling processes to altered rainfall frequency and N addition. In particular, *X. reptans* plays a more important role than *P. crystallifera* and *D. thunbergianus* in sustaining N availability and reducing N losses from drylands. Our study also emphasizes the importance of the microbial community for N cycling in response to environmental N inputs and changes in rainfall frequency. The significance of our results is constrained by microcosm nature of the experimental design and needs to be validated in field conditions. Nonetheless, these findings are critical for predicting the possible consequences of climate change and anthropogenic disturbances on N cycling in dryland ecosystems. This study also advances our understanding of the potential role of species identity of biocrust-forming lichens in ecosystem functions, which may be obscured when these communities are considered as one entity in drylands.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2016.01.021>.

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