Nitrogen inputs may improve soil biocrusts multifunctionality in dryland ecosystems

Running title: Nitrogen inputs and biocrusts

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Highlights

Biocrust pigments were influenced by both increased N inputs and plant legacy.

The N-driven changes resulted in trade-offs in biocrust functioning.

Increased N inputs improved biocrust multifunctionality.

Abstract

Soil biocrusts (communities of cyanobacteria, algae, mosses, lichens, and heterotrophs living at the soil surface) are fundamental components of dryland ecosystems worldwide. There is increasing concern over the potential for increasing nitrogen (N) inputs to affect biocrusts. This is of special concern in Mediterranean Basin drylands that face the threat of increased N inputs however, the effect on biocrusts remain poorly studied. We evaluated the potential effects of increased N inputs on biocrust structure and functioning in surrounding Mediterranean shrublands in the seventh year of a N-manipulation field experiment. We tracked the N-driven changes in biotope (changes in bare soil and in the non-legume and the legume occupation areas, and the percentage of radiation intercepted by plant canopies), evaluated biocrust functional traits (based on pigments) and measured biocrust functioning in terms of C and N cycling, soil fertility (macro and micronutrients) and biodiversity, and integrated these multiple soil functions simultaneously (i.e. soil multifunctionality).

Biocrust pigment concentration was significantly influenced by both plant legacy and N input. Biocrust pigments revealed a clear functional shift from: i) biocrusts dominated by photosynthetically inactive cyanobacteria that fix N_2 and are mostly committed to photoprotection at the expense of N-containing pigments under low N inputs; into ii) biocrusts more evenly composed of prokaryotes and eukaryotes, which are more photosynthetically active, but less committed to photoprotection and N_2 fixation under exposure to increased N inputs. The N-driven functional and structural changes in biocrusts resulted in trade-offs in biocrust functioning and processes (only N_2 fixation was affected) and an overall improvement in biocrust multifunctionality. By itself, biocrust pigment evenness accounted for ~50% of the observed variation in biocrust

multifunctionality. The biocrust pigment functional approach we adopted to study the effects of increased N inputs from patchy developed anthropogenic landscapes provides novel and critical knowledge of biocrusts community and functioning, which may be used as a tool in biodiversity conservation strategies, ecosystem functions and ecological modelling.

Keywords: biological soil crusts; biocrust functioning; biocrust pigments; increased N inputs; pigment functional traits; plant species legacy

1. Introduction

Drylands, which include dry sub-humid, semiarid, arid and hyper-arid areas, cover roughly 41% of the Earth's terrestrial surface (Maestre et al., 2012b). Besides hosting 38% of the global human population, drylands also host c.a. 20% of plant and 30% of bird biodiversity hotspots, thus making these terrestrial biomes especially vulnerable to global environmental changes (Maestre et al., 2012b). Indeed, faced with a global need to sequester more carbon (C), drylands may store up to 25% of the world's soil organic C (Ferrenberg et al., 2015), further prioritising soil conservation in these areas.

Globally, drylands are also at risk of excessive nitrogen (N) inputs, and this is especially true in N-limited drylands within the Mediterranean Basin (Galloway et al., 2004; Phoenix et al., 2006). A major source of increased N inputs are anthropogenic demands for food and energy. For example, global anthropogenic inputs were 16 Tg N yr^{-1} in 1860 and reached 210 Tg N yr^{-1} in 2005 (Galloway et al., 2008). Excessive N-inputs must therefore be seen as one of the most important drivers of biodiversity loss and ecosystem degradation (Bobbink et al., 2010; Sala et al., 2000; Steffen et al., 2015) affecting ecosystem structure and functioning (Dias et al., 2017).

Current opinion is conclusive in that excessive N inputs impact a variety of organisms, especially plants (Bobbink et al. 2010), and subsequently ecosystem functioning (Dias et al., 2017). However, there remain large gaps regarding the effects of excessive N inputs on other, lesser studied ecosystem components (Ochoa-Hueso et al., 2017). Biological soil crusts (biocrusts), which are comprised of communities of cyanobacteria, algae, lichens, mosses, and heterotrophic organisms, rank high among these understudied ecosystem components.

Biocrusts can cover up to 70% of the soil surface in drylands (Ferrenberg et al., 2015). They significantly influence ecosystem processes such as preventing soil erosion, nurturing ecosystems by fixing C and N from the atmosphere, altering soil albedo, regulating water relations, and supporting seed germination and optimum nutrient levels in vascular plants (Concostrina-Zubiri et al., 2017; 2014). However, their extensive coverage in the landscape, and the fact that they respond quite rapidly to changes in resource pulses, make them potentially vulnerable to increasing N inputs. Given the significant role biocrusts have in regulating ecosystem processes, an understanding of how they might respond to increased N inputs is essential for predicting ecological changes in drylands.

Increased N inputs have been shown to have significant effects on the structure and functioning of biocrusts, including shifts in species composition (Ochoa-Hueso et al., 2016; 2013), decrease of photosynthesis and N_2 fixation rates (Ochoa-Hueso et al., 2016; Sheridan, 1979), and reduction in concentrations of photosynthetic and photoprotective pigments. However, the reported N loads triggering these responses differ greatly. For example, Ochoa-Hueso et al. (2016) reported significant reductions in cyanobacterial abundance within Mediterranean Basin drylands, where N inputs ranged from 4.3 to 7.3 kg N ha^{-1} yr^{-1}. In turn, Wang et al. (2015) reported increasing cyanobacteria abundance

in biocrusts in the Tengger desert (China), where the area was exposed to seven years of 30 and 70 kg N ha^{-1} yr^{-1}. In fact, in the latter study, cyanobacteria only decreased upon adding 140 kg N ha^{-1} yr^{-1}. Disparities in the N load affecting biocrusts may have three causes: i) the most competitive biocrust groups are not adequately growing due to acute ecosystem limitations in water and other nutrients; ii) the biocrusts are at different successional stages (early, medium, and late successional) and/or have different community compositions (Belnap et al., 2008); successional stage has important consequences for determining the effects of increased N inputs (Liu et al., 2017); and iii) a significant part of the N added is lost without locally affecting the biocrusts. Therefore, more research is needed to understand the factors modulating biocrust responses to increased N inputs.

Taking into consideration that N deposition in the Mediterranean Basin is expected to increase to 15-20 kg N ha^{-1} yr^{-1} by 2050 (Galloway et al., 2004) biocrusts structure and functioning may be at risk. The increasing body of evidence involving biocrusts in ecosystem functioning suggests that the mechanisms involved in their response to increased N inputs should be considered for management and restoration of Mediterranean Basin drylands. Using a functional traits approach to study the effects of increased N inputs on biocrusts provides novel and critical knowledge of biocrusts community, and a tool to be used in biodiversity conservation strategies, ecosystem functioning or ecological modelling (Concostrina-Zubiri et al., 2014).

In the Mediterranean Basin, drylands of high conservation interest are typically patchily distributed within commodity production landscapes (e.g. ~20% of the Portuguese terrestrial area is conserved and patchily distributed – http://www.icnf.pt/portal/ap). Being situated in the matrix of agricultural, industrial or urban activities often results in heavy exposure to varying N forms. Previous studies have shown that because distinct

land uses emit different N forms (urban and industrial areas emit mostly NOx, while agricultural areas emit mostly NHy), they pose different threats to surrounding ecosystems: increased NHy inputs may increase the soil erosion and N leakage risks (Dias et al., 2014; 2017), whereas increased NOx inputs may increase the fire risk (Dias et al., 2014).

Our objective here was to assess the effects increasing N inputs from the surrounding environment, on biocrust structure and functioning in extant patches of Mediterranean shrubland. Whether plant legacy is an important factor determining biocrust formation is important to know, since most studies only examine the effect of biocrusts on the different life cycle stages of a plant, and thus not plant-specific life-history strategies (Deines et al., 2007; Gao et al., 2014; Langhans et al., 2009; Pendleton et al., 2003). We hypothesised that under Mediterranean climate conditions, where biological development is co-limited by several factors such as water, N and phosphorus availabilities, biocrusts developing under the influence of non-legume or legume plants could respond differently to increased N inputs. We choose two abundant shrub species that have been shown to respond similarly to increased N inputs (Dias et al., 2014): *Genista triacanthos*, a legume plant, forming round and dense shrubs; and *Cistus ladanifer,* a non-legume, forming more erect and less dense shrubs. We tracked the N-driven changes in biotope (changes in bare soil and in the non-legume and the legume occupation areas, and the percentage of radiation intercepted by plant canopies), evaluated biocrust functional traits (based on pigments) and measured biocrust functioning in terms of C and N cycling (C storage and N_2 fixation, respectively), soil fertility (macro and micronutrients) and biodiversity (pigment richness and evenness), and integrated these multiple soil functions simultaneously (i.e. soil multifunctionality) (Delgado-Baquerizo et al., 2016; Maestre et al., 2012a; 2012b).

2. MATERIALS AND METHODS

2.1. Study site

The study site (38°29'N - 9°1'W) is located in a Natura 2000 site (PTCON0010 Arrábida/Espichel) in Serra da Arrábida (Portugal), in the sub-humid thermomediterranean bioclimatic domain (Rivas-Martínez et al., 2004). Mean annual precipitation at the study site is 735 mm, mean maximum temperature is 30.1°C (August); highest maximum temperature is 43.5ºC (July); mean minimum temperature is 4.8°C (January); and lowest minimum temperature is -4.8ºC (January) (data recorded between1981 and 2010; Instituto Português do Mar e da Atmosfera – Setúbal meteorological station), However, based on the aridity index, the climate changed from sub-humid to semi-arid between 2000 and 2010 (Autoridade Nacional Florestal). The skeletal soil (topsoil is c.a. 15 cm) is classified as calcic rhodo-chromic luvisols and calcareous chromic cambisols (Specht et al., 1988), being mainly composed of silt (50%), while sand and clay contents are 32% and 18%, respectively (silt-sand-loam texture). The experiment is located on a southeast-facing slope (5%) at 130 m altitude, which is protected from public access and has not been managed in recent decades. Mediterranean maquis vegetation dominates the site, comprising dense vegetation: mainly shrubs with some small trees, annuals and geophytes (Eunis class F5.2 – Mediterranean maquis). The standing community developed after a fire event in the summer of 2003, four years before the first N addition of this experiment. *Cistus ladanifer* L., a Cistaceae, is the dominant plant species (Dias et al., 2014; 2011a). Other abundant plant species include *Genista triacanthos* Brot. (Fabaceae), *Ulex densus* Welw. ex Webb (Fabaceae), *Erica scoparia* L. (Ericaceae) and *Calluna vulgaris* (L.) Hull (Ericaceae). Herbaceous species comprise approximately 10% of the total plant cover (Dias et al., 2011a).

2.2. Experimental design and N additions

Since the beginning of the experiment, the estimated background N deposition is <4 kg N ha^{-1} yr^{-1}, and in 2013 it was 2.8 kg ha^{-1} yr^{-1} (1.6 kg NO_x + 1.2 kg NH_y) according to the model used by the European Monitoring and Evaluation Programme (grid location: x = 53 and y = 4 - http://www.emep.int/mscw/index_mscw.html). The N doses were designed to simulate 'worst case' scenarios of increased N inputs, but were lower than the values reported for Mediterranean-type areas with excessive N inputs (Fenn et al., 2003). The N forms applied mimicked the most likely scenarios of increased N inputs from developed patchy anthropogenic landscapes within the Mediterranean Basin: agricultural sources (NHy); or agricultural and urban/industrial sources (NHy and NOx). Three N addition treatments were tested: 40 kg NH_4^+-N ha^{-1} yr^{-1}, as NH_4Cl and $(NH_4)_2SO_4$ (1:1) (designated **40A**); 20 kg NH_4^+-N and 20 kg NO_3^--N ha^{-1} yr^{-1}, as NH_4NO_3 (designated **40AN**); and 40 kg NH_4^+-N and 40 kg NO_3^--N ha^{-1} yr^{-1}, as NH_4NO_3 (designated **80AN**). 40A and 40AN therefore provided the same N dose, whereas 40A and 80AN provided the same NH_4^+ dose. N was added as dry N salts sprinkled homogenously over the soil surface, by hand. The total N was split into three equal applications, performed in the spring, summer and mid-autumn/winter, beginning in January 2007. Control plots received no added N, being subjected only to background N deposition (low N input). Each treatment was replicated three times (3 plots of 400 m^2 each). The experimental plots were randomly distributed in three rows across the slope. The control plots were placed in the uppermost row to prevent N 'contamination' due to runoff from the N-plots. All measurements, analyses and sample collection were performed in the central 100 m^2 square, in order to limit boundary effects and dilution processes (Fig. S1).

2.3. Assessing changes in biotope and biocrust sampling

The perennial shrubs *Cistus ladanifer* L. (non-legume) and *Genista triacanthos* Brot. (legume) were selected for this study due to their abundance under low N inputs (25-45% and 20-35% respectively), distinct traits (*C. ladanifer* is a summer semi-deciduous while *G. triacanthos* is a legume), and similar responses to the applied N treatments (Dias et al., 2014). The non-legume and the legume occupation area, and that of bare soil were assessed within one 5x5 m square per experimental plot (within the internal 100 m^2) in June 2007 and 2013. The legume and the non-legume occupation areas (%) were calculated from the recorded total projected crown areas.

Changes in bare soil and in the non-legume and the legume occupation areas between 2007 (the first spring of N additions) and 2013 (the seventh spring of N additions) were calculated as positive (an increase) or negative (a decrease) as follows (Dias et al., 2014):

$$\text{Changes in bare soil or occupation area (\%)} = \frac{(\text{area 2013} - \text{area 2007})}{(\text{area 2013} + \text{area 2007})/2} \text{ x } 100$$

Solar radiation was measured in October 2012 (seventh autumn of N additions), on the same day that the biocrusts were sampled. Radiation was measured between 10:00 and 11:00 using a radiometer (LICOR, Inc, model LI-185B). Ten paired radiation readings were performed in each experimental plot: ambient radiation (above plant canopies) followed by ground radiation (below plant canopies, at ground level). The radiation intercepted by plant canopies is the percentage of total ambient radiation that reaches the ground/biocrusts, and was calculated as follows:

$$\text{Radiation intercepeted by plant canopies (\%)} = 100 - \frac{(\text{Ground radiation x } 100)}{(\text{Ambient radiation})}$$

Biocrust sampling consisted of collecting one soil sample (0-1 cm depth) underneath five *C. ladanifer* plants and five *G. triacanthos* plants, at 20 cm from the main stem, in all

experimental plots (Fig. S1). Bulk samples (equal mixtures of the five soil samples collected underneath each plant species per experimental plot) and stored at 4ºC until arrival at the laboratory. Upon arrival at the laboratory, each sample was subdivided into 3 sub-samples for determining: i) biocrust pigment composition and abundance, with the sub-sample being frozen at -20ºC until analyses; ii) biocrust N_2 fixation, with the sub-sample being kept at 4ºC until analysis; and iii) biocrust C storage and fertility, with the sub-sample being oven-dried at 60ºC until constant weight.

2.4. Biocrust pigment composition and abundance

As richness and evenness are main components of biodiversity (Wilsey and Potvin, 2000), we used the number of pigments as a proxy for biocrust richness, and the relative abundance of pigments as a proxy for biocrust evenness. By focusing on biocrust pigments, we adopted a functional approach (Concostrina-Zubiri et al., 2014).

Biocrust pigments were determined by HPLC, using acetone-extraction (Ochoa-Hueso and Manrique, 2011). Chlorophylls (*a* and *b*), β-carotene, neoxanthin, lutein, and VAZ cycle pigments (violaxathin, anteraxanthin, and zeaxanthin) were separated by HPLC (Waters, U.S.A.), according to Martínez-Ferri et al. (2000): 1 g of soil was thawed, ground with 0.5 mL of deionised water; then another 4 mL of HPLC-acetone was added and the grinding was repeated. The samples were then transferred to test tubes and the final volume was brought to 10 mL (final concentration of 95% acetone). The head-space of each tube was filled with helium, sealed with Parafilm, and kept at 8°C overnight. Following 24 h, samples were filtered through GF/F filter paper and condensed to a 3 mL volume using helium. Soil pigments were separated by HPLC according to the method of Val et al. (1994) with slight modifications (Martinez-Ferri et al., 2000). After passing through a 0.45 μm nylon filter, 25 μL of the extract was injected into a C18 column (ACE

5 C18- AR, ACE, Scotland). The mobile phase rate was 1.2 mL min^{-1} and the elution time lasted 30 min. Solvents for HPLC analysis were degassed before use by bubbling helium. The Waters HPLC system was equipped with a Waters 996 photodiode array detector (Waters, USA). Peak identification and quantification were performed using pure commercial standards (VKI, Hørsholm, Denmark) of chlorophylls *a* and *b*, β-carotene, echinenone, lutein, neoxanthin, violaxanthin and zeaxanthin and were used. Because scytonemin was not commercially available, it was estimated from its peak area at 436 nm (Bowker et al., 2002). Pigment concentrations were expressed as μg C in the pigment per g^{-1} of dry soil.

Based on pigment abundance, we determined indicators based on: i) classes – total chlorophylls (Chl *a*+*b*), xantophylls (VAZ), N-containing pigments (+N pig), pigments without N (-N pig), photoprotective (pro), photosynthetic (syn), exclusive to prokaryotes (prok), exclusive to eukaryotes (euk) and occurring in both superdomains (both); and ii) on ratios – chlorophyll *a* to *b* (Chl *a*/Chl *b*), xantophylls to chlorophylls (VAZ/Chl *a*+*b*), β-carotene to chlorophylls (Car/Chl *a*+*b*), lutein to chlorophylls (Lut/Chl *a*+*b*) and N-containing to pigments without N (+N pig/-N pig), photoprotective to photosynthetic (prot/syn), and prokaryotic exclusive to eukaryotic exclusive and both (prok/others).

2.5. Biocrust functioning

We used two different approaches to assess biocrust functioning: i) assessed separately biocrust C storage and N_2 fixation rates as proxies of C and N cycling; concentrations of macro and micronutrients as proxies of fertility; and pigment richness and evenness as proxies of biodiversity; and ii) multifunctionality based on the average approach (Maestre et al., 2012a). Average multifunctionality, is being increasingly used (Delgado-Baquerizo et al., 2016). It consists in calculating the average of the previously standardized multiple

functions measured, thus providing a straightforward and easily interpretable measure of multifunctionality (Byrnes et al., 2014). To obtain the average multifunctionality index (from herein multifunctionality) for each biocrust (from underneath the non-legume and the legume species and receiving the different N treatments), we first standardized each of our six variables to a 0–1 scale by dividing each value by the maximum value for that particular variable. The standardized variables were then averaged to obtain the multifunctionality values.

Biocrusts were dried at 60°C until constant weight, and the following were determined: i) C storage, by measuring C concentrations; and iii) macro- (N, potassium, calcium and magnesium) and micro-nutrient (copper, iron, manganese and zinc) fertility. C and N concentrations were determined using an elemental analyser (Carlo Erba model 1108EA, Milan, IT), and the remaining nutrients were determined by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES – Spectro Ciros CCD, Spectro, Germany). Biocrust N_2 fixation was estimated using the acetylene reduction assay, according to Ochoa-Hueso and Manrique (2013). Pigment richness and evenness were calculated based on the pigment composition and abundance, obtained by HPLC.

2.6. Statistics

We correlated the N input treatments with key environmental conditions per plot, these being the percentage of radiation intercepted by plant canopies, and the changes in bare soil and in the non-legume and the legume occupation areas (*C. ladanifer* and *G. triacanthos*) over a 7-year period. This was done using a principle component analysis (PCA), in CANOCO 5. Response data were centered and standardized.

To determine whether N input treatments and plant species legacy (non-legume or legume) influence biocrust pigment composition and abundance, we performed a

redundancy analysis (RDA) using CANOCO 5. The forward selection procedure in the CANOCO software, using a permutation test with 9999 permutations, was used to identify the strongest categories that could help explain soil-pigment variation. Variance partitioning (testing conditional effects using 9999 permutations) was then performed to determine the unique and combined contribution of each significant variable group in explaining soil pigment variation: an abiotic, N input treatment group *vs.* a biotic, plant species legacy (Borcard et al., 1992). Covariates that were linearly dependent were ignored in these analyses (in this case the non-legume and low N input treatment – Control), but included in the final ordination scheme (Lepš et al., 2003). In all cases we used the adjusted R^2 variation to account for sample size and the number of exploratory variables included (Peres-Neto et al., 2006). Response data were centered.

The effect of the N input treatments on plant and biocrust parameters was tested separately using a two-way ANOVA, with N input treatment and plant species as fixed factors. Bonferroni post-hoc multiple comparisons tested for differences ($P < 0.05$) in plant and biocrust parameters between N input treatments. In all cases, preliminary analyses were performed to ensure that there was no violation of statistical assumptions (including the Levene's test of homogeneity of variances). SPSS (version 23.0, IBM, Inc.) was used for these analyses.

We tested the association between N input treatments and functional indicators derived from biocrust pigments, by performing another RDA (in CANOCO 5) followed by the forward selection procedure. For this RDA, data were centred and standardized due to differences in measuring units.

Finally, we tested if our biocrust data could support a correlation between biodiversity and multifunctionality as shown by Maestre et al. (2012b) using dryland plant species. For that, the relationship between biocrust pigment evenness and multifunctionality was

modelled using a quadratic regression, using SPSS (version 23.0, IBM, Inc.). Because pigment evenness was one of the components used to calculate multifunctionality, we repeated the quadratic regression without pigment evenness.

3. RESULTS

3.1. Biotope changes after 7 years of increased N inputs

Although initially the occupation areas of the non-legume (*C. ladanifer*) and the legume (*G. triacanthos*) plants differed within the experimental plots, after seven years of increased N inputs the changes in their occupation areas followed the same pattern (Fig. S2). Under low N inputs (control plots) and up to 40AN the occupation area of both plant species expanded after the fire disturbance previous to the experiment set up. In turn, exposure to 80AN markedly decreased the occupation area of both species. Exposure to 40A tended to decrease the two plant species' occupation area, but not significantly.

Addition of 40 kg NH_4^+ ha^{-1} yr^{-1}, alone or together with nitrate (40A and 80AN) increased the area of bare soil (Dias et al., 2017), and decreased the percentage of radiation intercepted by plant canopies by 15-30% (Fig. 1). Therefore, biocrusts exposed to 40A and 80AN were less protected from ambient radiation (1372±44 μE m^{-2} s^{-1}) and wind and rain erosion than those under low N inputs (control and 40AN).

3.2. Biocrust pigment composition and abundance

Nine pigments were detected and quantified in the biocrusts (Table 1). From these, only three (chlorophylls *a* and *b*, and scytonemin) contained N. Except for the chlorophylls, all the pigments were photoprotective, with scytonemin and echinenone being exclusively produced by prokaryotes (cyanobacteria).

Under low N inputs (control), composition and abundance of biocrust pigments were similar under the legacy of both plant species (Table S1). However, increasing the N inputs reduced the total abundance of biocrust pigments below those of the control treatment, however the reduction of the biocrust pigments underneath the legume exposed to 40AN was not significant (Table S1). No pigment disappeared and no pigment appeared in the biocrusts in response to increased N inputs. Therefore, N input treatments had no effect on pigment richness (Table S2).

In relation to low N inputs (control), most pigments showed no difference in their abundance upon increased N inputs (e.g. echinenone), some increased (e.g. chlorophyll *a*), while scytonemin decreased (Table S1). Scytonemin was the dominant biocrust pigment under low N inputs (accounting for >80% of the pigments). However, with increased N inputs scytonemin's abundance dropped more than 8-fold and chlorophylls become the most abundant pigments, except in biocrusts underneath the legume exposed to 40AN, where scytonemin was only reduced by 30%. As a result of both scytonemin decreasing and other pigments increasing, the relative proportion of the pigments within the biocrusts became much more even following increased N inputs (Table S2).

Biocrust pigments were significantly influenced by both the plant legacy (non-legume or legume) and N input treatment (Fig. 2). In total, these two groups of variables explained 83.7% of the observed variation in biocrust pigments. However, N input treatment, the abiotic factor, had a much stronger influence on pigments' abundance than the plant species legacy, the biotic factor. Specifically, N input treatment explained 84.4% of the variation ($F = 39.0$, $P < 0.001$), and plant species legacy 3.4% ($F = 5.2$, $P = 0.026$). Nevertheless, both groups were important to determine pigments composition and abundance in these Mediterranean shrublands biocrusts (tested fraction $a+b+c$: $F = 30.6$, $P < 0.001$). Furthermore, the negative value of the shared fraction confirms that both plant

species legacy and N input treatment had marked yet opposite effects on biocrust pigment abundance (Peres-Neto et al., 2006). This means, for example, that exposure to 40AN underneath the legume had a smaller effect than underneath the non-legume.

Biocrust pigments were grouped into classes according to the following criteria: (i) pigment contains N or not; ii) pigment is photosynthetic or photoprotective; and iii) pigment can be found in prokaryotic or eukaryotic organisms or in both. Both N input treatment and plant species legacy affected the abundance of each class of pigments in the biocrusts (Fig. 3). Under low N inputs, ~90% of the pigments contained N, were photoprotective and exclusively found in prokaryotic organisms. Following increased N inputs, the proportion of: i) N-containing pigments dropped from >90% to 80% of the pigments; ii) photoprotective pigments dropped from 90% to 30-50% of the pigments; iii) and of pigments exclusively found in prokaryotic organisms dropped from 85% to 20-30% of the pigments. Again, the legume legacy buffered the effects of exposure to 40AN, with pigment partitioning (and proportion) showing an intermediate response between the low N input and 40A and 80AN treatments.

Several indicators based on biocrust pigments (Table S1) were significantly influenced (at the 5% level) by N input treatments (Fig. 4). In the PCA Axis 1, which explained most of the variance, was mostly associated with pigments evenness and ratios between pigments with N and without N (+N/-N), photo**pro**tective and photo**synt**hetic (pro/syn), and those exclusive to prokaryotes and the others (prok/others). Although there was a strong positive association for most indicators with low N input, some pigments were positively associated with increased N inputs, particularly total chlorophylls (Chl_a+Chl_b) and total xanthophylles (VAZ). Interestingly, biocrust diversity, as indicated by pigment richness and evenness, increased with increased N inputs.

3.3. Biocrust functioning

Despite seven years of increased N inputs, biocrust macronutrient concentration did not change (Table S2 and Fig. 5). However, with increased N inputs we did observe changes in biocrust C storage, N_2 fixation, micronutrients fertility and pigments evenness. Biocrust C storage, micronutrients fertility and pigments evenness were promoted when biocrusts were exposed to increased N inputs, while N_2 fixation was affected when biocrusts were exposed to 40A and 80AN. Furthermore, the relative positive effect of increased N inputs on biocrust pigments evenness and micronutrients fertility was greater than the relative negative effect it had on N_2 fixation. Consequently, when the six proxies for biocrust functioning were averaged, the N input treatments did not affect biocrust multifunctionality (Fig. 6). Instead, increasing N inputs (irrespective of dose and form) promoted multifunctionality in relation to low N inputs (control), with exposure to 40AN promoting the highest multifunctionality. Biocrust multifunctionality was highly dependent on pigments evenness as the best fitting model, quadratic regression including biocrust pigment evenness as a predictor, accounted for ~50% of the variation in multifunctionality. Even when we removed pigment evenness from multifunctionality calculations, the quadratic regression was still highly significant ($F = 5.572$; $P = 0.011$) and the coefficients were similar (Multifunctionality without pigments evenness = $- 2.9\ \text{evenness}^2 + 3.4\ \text{evenness} - 0.2$; $R^2 = 0.333$). Maximum values of multifunctionality were therefore observed when biocrust pigment evenness was ~0.7, whereas decreased biocrust multifunctionality was expected with lower and higher pigment evenness, as observed for the low N input biocrusts.

4. DISCUSSION

Our study shows that seven years of increased N inputs to a low N input shrubland resulted in trade-offs in biocrust functioning; cyanobacteria lowered their N_2 fixation rates while biocrust communities improved other functions. As a result, our data suggest that increased N inputs may promote biocrusts multifunctionality, which may help counterbalance the negative effects of climate change and desertification in drylands (Maestre et al., 2012b). Furthermore, biocrust pigment evenness, as sole predictor, accounted for ~50% of the variation in biocrust multifunctionality. The use of the biocrust pigment functional approach to study the effects of increased N inputs provides insights into biocrust community and functioning, which may be used as a tool in biodiversity conservation strategies, ecosystem functioning and ecological modelling.

4.1. Increased N inputs changed the biotope

Increasing N inputs can positively stimulate plant productivity, resulting in shading out of certain species (Bobbink et al., 2010; Ceulemans et al., 2017; Hautier et al., 2009; Vojtech et al., 2007), as observed for the 40AN treatment (Fig. 1). But depending on the form and dose, N may also have negative effects on plant productivity (Dias et al., 2015). For example, some Mediterranean maquis vegetation tend to be more sensitive to NH_4^+ than to NO_3^- (Cruz et al., 2008; Dias et al., 2015; 2011b). NH_4^+ might have played an important role in changing the plant-soil cover (Figs S2 and 1), biocrust pigments (Table S1 and Figs 2 and 3) and functioning (Table S2 and Fig. 5). In fact, the higher NH_4^+ inputs (40A and 80AN) did not contribute to increase soil shading. Instead, it may have contributed to enhance the risk of biocrust photodamage due to excessive radiation, and of biocrust drought due to higher temperatures. These changes in the biotope appear to have reduced biological activity and increased mortality (Housman et al., 2006) as shown

by the reduction in N_2 fixation (Table S2 and Fig. 5) and the decline in biocrust total pigments (Table S1 and Fig. 3).

4.2. Increased N inputs changed biocrust pigments

The legume (*G. triacanthos*) 'buffered' more the effects of increased N inputs on biocrust pigments (Table S1 and Figs 2 and 3) than the non-legume (*C. ladanifer*). This may be related to the rounder shape and denser canopy of the legume, which may lead to higher interception of N when compared to the non-legume canopy. The biocrusts underneath the legume may also have been adapted to higher N inputs, and thus been less susceptible to the increased N inputs. Despite being significant, the influence of the plant species legacy on biocrust pigments was much weaker than that of the N input treatments.

The effects of the N input treatments explained most of the observed biocrust pigments variability (Table S1 and Figs 2 and 3). Similarly to other studies (Belnap et al., 2008; Ochoa-Hueso et al., 2016), the dominance of scytonemin (a small sunscreen molecule synthesized by cyanobacteria in response to UV exposure – Balskus et al., 2011; Table S1) points to its dominance in these early stage biocrusts under low N inputs (control). The sharp decrease in scytomenin concentration in response to increased N inputs, in spite of the higher UV exposure under higher NH_4^+ input (40A and 80AN – Fig. 1), suggests a drastic N-driven mortality of cyanobacteria, as reported for other studies of increased N inputs (Ochoa-Hueso et al., 2016; Wang et al., 2015). However, because the biocrusts exposed to the higher NH_4^+ input (40A and 80AN) most likely dried faster, cyanobacteria may have escaped the increased UV radiation by downward vertical migration in microbial mats (Kruschel and Castenholz, 1998; Nadeau et al., 1999). Not exclusively, since cyanobacteria synthesize scytonemin mainly or solely when metabolically inactive (Fleming and Castenholz, 2008), they would still have been abundant in our biocrusts

under increased N inputs, but were still metabolically active and using the applied inorganic N instead of fixing N_2 (Table S2 and Fig. 5). Indeed, Fleming and Castenholz (2008) showed that the cyanobacteria *Nostoc* synthesized 3-7 times more scytonemin when fixing N_2 than when using NO_3^- or NH_4^+, and *vice versa* when inorganic N became depleted and *Nostoc* switched to diazotrophic metabolism. Although the mechanism is unclear, it seems that the greater the N limitation, the more scytonemin is synthesized. Further, the fact that echinenone (another photoprotective pigment synthetized exclusively by cyanobacteria – Table 1) did not disappear from the biocrusts exposed to increased N inputs (Table S1), and the relatively constant abundance of cyanobacterial OTU's (pyrosequencing – unpublished) further indicate that cyanobacteria did not suffer such a drastic mortality as suggested by the decrease in scytonemin.

The N-driven increase in the concentration of pigments occurring in both superdomains, and especially those exclusive to eukaryotes (Tables 1 and S1 and Fig. 3-e, f), suggests that increased N inputs favoured green microalgae. This potential promotion of green microalgae by increased N inputs is in line with previous reports (Ochoa-Hueso et al., 2016; Poikolainen et al., 1998) and further supports green algae as indicators of ecosystems being exposed to increased N inputs.

4.3. Pigment functional traits as ecological indicators of increased N inputs

All tested biocrust indicators (Table S1) were significantly influenced by long-term increased N inputs (Fig. 4). However, the biocrust pigments studied here revealed a clear functional community shift from: i) biocrusts dominated by inactive cyanobacteria that fix N_2 and are mostly committed to photoprotection at the expense of N-containing molecules; into ii) biocrusts more evenly composed of prokaryotes and eukaryotes, which are more actively photosynthesising, but less committed to photoprotection and fix less

N_2 (Figs 3 and 5). As a result, the indicators we propose based on functional traits (pigments evenness and the ratios between pigments with N and without, between photoprotective and photosynthetic and those exclusively found in prokaryotes and the others), displayed higher resolution and sensitivity to increased N inputs (Fig. 4), reinforcing the use of functional traits as accurate and robust indicators of the effects of increased N inputs.

4.4. Implications of increased N inputs on biocrust functioning

Unlike most published studies, we saw little negative effect of increased N inputs on nitrogenase activity (i.e. N_2 fixation rates – Table S3 and Fig. 5), despite the long-term exposure of the biocrusts to relatively high N levels. Two factors may contribute to explain the persistence of N_2 fixation in our biocrusts: i) larger fractions of phosphorus are being turned into bio-available phosphate (Ulm et al., 2017), which may stimulate N_2 fixation inputs (Reed et al., 2011) and counter-balance the negative effects of increased N inputs; and ii) the population of N_2 fixing bacteria (including cyanobacteria) may still be present in the plots exposed to increased N inputs (Dias et al., 2017). Further, increased N inputs mimicking combined inputs from agricultural and urban/industrial areas (40AN and 80AN) improved biocrust C storage (Table S2 and Fig. 5) as shown in previous studies (Maestre et al., 2016). In the same N-manipulation experiment, it was also shown that exposure to the high N input (80AN) increased soil organic matter (Dias et al., 2014), most likely reflecting a decrease in decomposition (Dias et al., 2013). This is of particular importance for Mediterranean Basin soils due to their naturally low organic matter concentrations and hence higher risk of soil erosion and desertification (Jones et al., 2012). Unlike previous studies (Ochoa-Hueso et al. 2013a), our data suggest that low-productivity Mediterranean systems might be able to store extra C in soil as part of

organic matter (living and/or dead) under increased N inputs, as soil C storage and productivity are N-limited in these ecosystems.

Contrary to our expectations, macronutrients availability did not increase despite adding N along seven years. Instead, increased N inputs enhanced micronutrients fertility 1-4-fold (Table S2 and Fig. 5), which was mainly driven by a >10-fold increase in manganese (Mn) concentration (data not shown). Even though the mechanism is unknown, high Mn concentrations have been shown to decrease scytonemin's synthesis in *Collema* (Bowker et al., 2008), which further supports that the observed decrease in scytonemin (Table S1) was not attributable to cyanobacteria's mortality. Because carotene synthesis is Mn dependent (Blaya and García, 2003), the enhanced Mn concentration may reflect accumulation mechanisms by both photosynthetic microorganisms, which, particularly cyanobacteria, which depend on Mn for their photosynthetic activity (Salomon and Keren, 2011). Indeed, as positively charged nutrients bind to negatively charged clay particles and the cyanobacterial sheath materials, cyanobacteria may accumulate nutrients by forming connections with soil particles (Belnap and Gardner, 1993).

Nine pigments characteristic of photosynthetic microorganisms were detected in the biocrusts, highlighting the biocrust role as a highly valuable and often neglected natural capital reservoir (Dominati et al., 2010). High biodiversity can stabilize ecosystems through functional complementarities, and thus buffer environmental change impacts (Hooper et al., 2012; MacDougall et al., 2013). In our study, biocrust pigment richness was not affected by increased N inputs, whereas evenness increased (Table S2 and Fig. 5), highlighting that different and independent ecological processes determine the response of richness and evenness to edaphic drivers (Dias et al., 2011a; Ma, 2005). The N limitation under low N inputs constitutes a disturbance factor that naturally drives communities into becoming uneven (Naeem, 2009; Wilsey and Potvin, 2000) as

previously shown for the plant community (Dias et al., 2014; 2011a). According to the revised Grime's humped-back model (Grime, 1973; Michalet et al., 2006), alleviating the N limitation by adding readily available N forms (NH_4^+ and/or NO_3^-) enabled the co-existence of diazotrophic, NO_3^--preferring and NH_4^+-tolerant species as previously observed in plant communities (Dias et al., 2014; 2011a). Although anthropogenic environmental changes are predicted to reduce regional coexistence (regional dominance and low beta diversity) (Hillebrand et al., 2008), our data suggest that increased N inputs may actually promote coexistence and make these more even biocrust communities more resistant to environmental changes (Wittebolle et al., 2009).

Collectively, the N-driven trade-offs in biocrust functioning (Fig. 5) resulted in improved biocrust multifunctionality (Fig. 6-a). Although 40AN was the treatment that promoted biocrusts multifunctionality the most, N-driven increases in biocrust multifunctionality in response to 40A and 80AN are especially important in biotopes exposed to high NH_4^+ inputs (40A and 80AN) due to the smaller vascular plant cover (Fig. 1). Our results therefore suggest that increased N inputs may promote biocrusts multifunctionality, which may help counterbalance the negative effects of climate change and desertification in drylands (Maestre et al., 2012b).

The fact that biocrust pigment evenness accounted for ~50% of the observed variation in biocrust multifunctionality (Fig. 6-b), suports the hypothesis that the correlation shown by Maestre et al. (2012b), between biodiversity and multifunctionality in drylands may be a general pattern in nature, reflecting a cause-and-effect link. The biocrust pigment functional approach we adopted to study the effects of increased N inputs from patchy developed anthropogenic landscapes provided novel and critical knowledge regarding biocrust community and functioning, which may be used as a tool in biodiversity conservation strategies, ecosystem functioning and ecological modelling.

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6. REFERENCES

Balskus, E.P., Case, R.J., Walsh, C.T., 2011. The biosynthesis of cyanobacterial sunscreen scytonemin in intertidal microbial mat communities. Fems Microbiology Ecology 77, 322-332.

Belnap, J., Gardner, J.S., 1993. Soil microstructure in soils of the Colorado Plateau - the role of the cyanobacterium *Microcoleus vaginatus*. Great Basin Naturalist 53, 40-47.

Belnap, J., Phillips, S.L., Flint, S., Money, J., Caldwell, M., 2008. Global change and biological soil crusts: effects of ultraviolet augmentation under altered precipitation regimes and nitrogen additions. Global Change Biology 14, 670-686.

Blaya, S.M., García, G.N., 2003. Química agrícola, 2nd Edition ed. Ediciones Mundi-Prensa, Madrid.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of

nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20, 30-59.

Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. Ecology 73, 1045-1055.

Bowker, M.A., Koch, G.W., Belnap, J., Johnson, N.C., 2008. Nutrient availability affects pigment production but not growth in lichens of biological soil crusts. Soil Biology & Biochemistry 40, 2819-2826.

Bowker, M.A., Reed, S.C., Belnap, J., Phillips, S.L., 2002. Temporal variation in community composition, pigmentation, and F-v/F-m of desert cyanobacterial soil crusts. Microbial Ecology 43, 13-25.

Byrnes, J.E.K., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., Cardinale, B.J., Hooper, D.U., Dee, L.E., Duffy, J.E., 2014. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. Methods in Ecology and Evolution 5, 111-124.

Ceulemans, T., Hulsmans, E., Berwaers, S., Van Acker, K., Honnay, O., 2017. The role of above-ground competition and nitrogen vs. phosphorus enrichment in seedling survival of common European plant species of semi-natural grasslands. Plos One 12.

Concostrina-Zubiri, L., Molla, I., Velizarova, E., Branquinho, C., 2017. Grazing or Not Grazing: Implications for Ecosystem Services Provided by Biocrusts in Mediterranean Cork Oak Woodlands. Land Degradation & Development 28, 1345-1353.

Concostrina-Zubiri, L., Pescador, D.S., Martinez, I., Escudero, A., 2014. Climate and small scale factors determine functional diversity shifts of biological soil crusts in Iberian drylands. Biodiversity and Conservation 23, 1757-1770.

Cruz, C., Bio, A.M.F., Jullioti, A., Tavares, A., Dias, T., Martins-Loucao, M.A., 2008. Heterogeneity of soil surface ammonium concentration and other characteristics, related

to plant specific variability in a Mediterranean-type ecosystem. Environmental Pollution 154, 414-423.

Deines, L., Rosentreter, R., Eldridge, D.J., Serpe, M.D., 2007. Germination and seedling establishment of two annual grasses on lichen-dominated biological soil crusts. Plant and Soil 295, 23-35.

Delgado-Baquerizo, M., Maestre, F.T., Eldridge, D.J., Bowker, M.A., Ochoa, V., Gozalo, B., Berdugo, M., Val, J., Singh, B.K., 2016. Biocrust-forming mosses mitigate the negative impacts of increasing aridity on ecosystem multifunctionality in drylands. New Phytologist 209, 1540-1552.

Dias, T., Clemente, A., Martins-Loucao, M.A., Sheppard, L., Bobbink, R., Cruz, C., 2014. Ammonium as a driving force of plant diversity and ecosystem functioning: observations based on 5 years' manipulation of N dose and form in a mediterranean ecosystem. PloS one 9, e92517-e92517.

Dias, T., Crous, C.J., Liberati, D., Munzi, S., Gouveia, C., Ulm, F., Afonso, A.C., Ochoa-Hueso, R., Manrique, E., Sheppard, L., Martins-Loucao, M.A., Bernardes da Silva, A., Cruz, C., 2017. Alleviating Nitrogen limitation in Mediterranean maquis vegetation leads to ecological degradation. Land Degradation & Development 28, 2482-2492.

Dias, T., Malveiro, S., Martins-Loucao, M.A., Sheppard, L.J., Cruz, C., 2011a. Linking N-driven biodiversity changes with soil N availability in a Mediterranean ecosystem. Plant and Soil 341, 125-136.

Dias, T., Martins-Loução, M.A., Sheppard, L., Cruz, C., 2015. Plant tolerance of ammonium varies between co-existing Mediterranean species. Plant and Soil 395, 243-252.

Dias, T., Neto, D., Martins-Loucao, M.A., Sheppard, L., Cruz, C., 2011b. Patterns of nitrate reductase activity vary according to the plant functional group in a Mediterranean maquis. Plant and Soil 347, 363-376.

Dias, T., Oakley, S., Alarcon-Gutierrez, E., Ziarelli, F., Trindade, H., Martins-Loucao, M.A., Sheppard, L., Ostle, N., Cruz, C., 2013. N-driven changes in a plant community affect leaf-litter traits and may delay organic matter decomposition in a Mediterranean maquis. Soil Biology & Biochemistry 58, 163-171.

Dominati, E., Patterson, M., Mackay, A., 2010. A framework for classifying and quantifying the natural capital and ecosystem services of soils. Ecological Economics 69, 1858-1868.

Fenn, M.E., Baron, J.S., Allen, E.B., Rueth, H.M., Nydick, K.R., Geiser, L., Bowman, W.D., Sickman, J.O., Meixner, T., Johnson, D.W., Neitlich, P., 2003. Ecological effects of nitrogen deposition in the western United States. Bioscience 53, 404-420.

Ferrenberg, S., Reed, S.C., Belnap, J., 2015. Climate change and physical disturbance cause similar community shifts in biological soil crusts. Proceedings of the National Academy of Sciences of the United States of America 112, 12116-12121.

Fleming, E.D., Castenholz, R.W., 2008. Effects of nitrogen source on the synthesis of the UV-screening compound, scytonemin, in the cyanobacterium Nostoc punctiforme PCC 73102. Fems Microbiology Ecology 63, 301-308.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vorosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153-226.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320, 889-892.

Gao, S.Q., Pan, X., Cui, Q.G., Hu, Y.K., Ye, X.H., Dong, M., 2014. Plant Interactions with Changes in Coverage of Biological Soil Crusts and Water Regime in Mu Us Sandland, China. Plos One 9.

Grime, J.P., 1973. Competitive exclusion in herbaceous vegetation. Nature 242, 344-347.

Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324, 636-638.

Hillebrand, H., Bennett, D.M., Cadotte, M.W., 2008. Consequences of dominance: A review of evenness effects on local and regional ecosystem processes. Ecology 89, 1510-1520.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486, 105-108.

Housman, D.C., Powers, H.H., Collins, A.D., Belnap, J., 2006. Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. Journal of Arid Environments 66, 620-634.

Jones, A., Panagos, P., Barcelo, S., Bouraqui, F., Bosco, C., Dewitte, O., Gardi, C., Erhard, M., Hervás, J., Hiederer, R., Jeffery, S., Lükewille, A., Marmo, L., Montanarella, L., Olazábal, C., Petersen, J.-E., Penizek, V., Strassburger, T., Tóth, G., Van Den Eeckhaut, M., Van Liedekerke, M., Verheijen, F., Viestova, E., Yigini, Y., 2012. The state of soil in Europe, in: Agency, E.E., Centre, J.R. (Eds.).

Kruschel, C., Castenholz, R.W., 1998. The effect of solar UV and visible irradiance on the vertical movements of cyanobacteria in microbial mats of hypersaline waters. Fems Microbiology Ecology 27, 53-72.

Langhans, T.M., Storm, C., Schwabe, A., 2009. Biological soil crusts and their microenvironment: Impact on emergence, survival and establishment of seedlings. Flora 204, 157-168.

Lepš, J, Šmilauer, P., 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press, UK.

Liu, Y.R., Delgado-Baquerizo, M., Trivedi, P., He, J.Z., Wang, J.T., Singh, B.K., 2017. Identity of biocrust species and microbial communities drive the response of soil multifunctionality to simulated global change. Soil Biology & Biochemistry 107, 208-217.

Ma, M., 2005. Species richness vs evenness: independent relationship and different responses to edaphic factors. Oikos 111, 192-198.

MacDougall, A.S., McCann, K.S., Gellner, G., Turkington, R., 2013. Diversity loss with persistent human disturbance increases vulnerability to ecosystem collapse. Nature 494, 86-89.

Maestre, F.T., Castillo-Monroy, A.P., Bowker, M.A., Ochoa-Hueso, R., 2012a. Species richness effects on ecosystem multifunctionality depend on evenness, composition and spatial pattern. Journal of Ecology 100, 317-330.

Maestre, F.T., Eldridge, D.J., Soliveres, S., Kefi, S., Delgado-Baquerizo, M., Bowker, M.A., Garcia-Palacios, P., Gaitan, J., Gallardo, A., Lazaro, R., Berdugo, M., 2016. Structure and Functioning of Dryland Ecosystems in a Changing World. Annual Review of Ecology, Evolution, and Systematics, Vol 47 47, 215-237.

Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., Garcia-Gomez, M., Bowker, M.A., Soliveres, S., Escolar, C., Garcia-Palacios, P., Berdugo, M., Valencia, E., Gozalo, B., Gallardo, A., Aguilera, L., Arredondo, T., Blones, J., Boeken, B., Bran, D., Conceicao, A.A., Cabrera, O., Chaieb, M., Derak, M., Eldridge, D.J., Espinosa, C.I., Florentino, A., Gaitan, J., Gatica, M.G., Ghiloufi, W., Gomez-Gonzalez, S., Gutierrez, J.R., Hernandez, R.M., Huang, X.W., Huber-Sannwald, E., Jankju, M., Miriti, M., Monerris, J., Mau, R.L., Morici, E., Naseri, K., Ospina, A., Polo, V., Prina, A., Pucheta, E., Ramirez-Collantes, D.A., Romao, R., Tighe, M., Torres-Diaz, C., Val, J., Veiga, J.P., Wang, D.L., Zaady, E., 2012b. Plant Species Richness and Ecosystem Multifunctionality in Global Drylands. Science 335, 214-218.

Martinez-Ferri, E., Balaguer, L., Valladares, F., Chico, J.M., Manrique, E., 2000. Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. Tree Physiology 20, 131-138.

Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I., Valiente-Banuet, A., Callaway, R.M., 2006. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? Ecology Letters 9, 767-773.

Nadeau, T.L., Howard-Williams, C., Castenholz, R.W., 1999. Effects of solar UV and visible irradiance on photosynthesis and vertical migration of *Oscillatoria* sp (cyanobacteria) in an Antarctic microbial mat. Aquatic Microbial Ecology 20, 231-243.

Naeem, S., 2009. ECOLOGY Gini in the bottle. Nature 458, 579-580.

Ochoa-Hueso, R., Delgado-Baquerizo, M., Gallardo, A., Bowker, M.A., Maestre, F.T., 2016. Climatic conditions, soil fertility and atmospheric nitrogen deposition largely determine the structure and functioning of microbial communities in biocrust-dominated Mediterranean drylands. Plant and Soil 399, 271-282.

Ochoa-Hueso, R., Maestre, F.T., de los Rios, A., Valea, S., Theobald, M.R., Vivanco, M.G., Manrique, E., Bowker, M.A., 2013. Nitrogen deposition alters nitrogen cycling and reduces soil carbon content in low-productivity semiarid Mediterranean ecosystems. Environmental Pollution 179, 185-193.

Ochoa-Hueso, R., Manrique, E., 2011. Effects of nitrogen deposition and soil fertility on cover and physiology of *Cladonia foliacea* (Huds.) Willd., a lichen of biological soil crusts from Mediterranean Spain. Environmental Pollution 159, 449-457.

Ochoa-Hueso, R., Manrique, E., 2013. Effects of nitrogen deposition on growth and physiology of *Pleurochaete squarrosa* (Brid.) Lindb., a terricolous moss from Mediterranean ecosystems. Water Air and Soil Pollution 224.

Ochoa-Hueso, R., Munzi, S., Alonso, R., Arróniz-Crespo, M., Avila, A., Bermejo, V., Bobbink, R., Branquinho, C., Concostrina-Zubiri, L., Cruz, C., de Carvalho, R.C., De Marco, A., Dias, T., Elustondo, D., Elvira, S., Estébanez, B., Fusaro, L., Gerosa, G., Izquieta-Rojano, S., Lo Cascio, M.a., Marzuoli, R., Matos, P., Mereu, S., Merino, J., Morillas, L., Nunes, A., Paoletti, E., Paoli, L., Pinho, P., Rogers, I.B., Santos, A., Sicard, P., Stevens, C.J., Theobald, M.R., 2017. Ecological impacts of atmospheric pollution and interactions with climate change in terrestrial ecosystems of the Mediterranean Basin: current research and future directions. Environmental Pollution 227, 194.

Pendleton, R.L., Pendleton, B.K., Howard, G.L., Warren, S.D., 2003. Growth and nutrient content of herbaceous seedlings associated with biological soil crusts. Arid Land Research and Management 17, 271-281.

Peres-Neto, P.R., Legendre, P., Dray, S., Borcard, D., 2006. Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology 87, 2614-2625.

Phoenix, G.K., Hicks, W.K., Cinderby, S., Kuylenstierna, J.C.I., Stock, W.D., Dentener, F.J., Giller, K.E., Austin, A.T., Lefroy, R.D.B., Gimeno, B.S., Ashmore, M.R., Ineson,

P., 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Global Change Biology 12, 470-476.

Poikolainen, J., Lippo, H., Hongisto, M., Kubin, E., Mikkola, K., Lindgren, M., 1998. On the abundance of epiphytic green algae in relation to the nitrogen concentrations of biomonitors and nitrogen deposition in Finland. Environmental Pollution 102, 85-92.

Reed, S.C., Cleveland, C.C., Townsend, A.R., 2011. Functional Ecology of Free-Living Nitrogen Fixation: A Contemporary Perspective. Annual Review of Ecology, Evolution, and Systematics, Vol 42 42, 489-512.

Rivas-Martínez, S., Penas, A., Díaz, T.E., 2004. Bioclimatic map of Europe-Thermoclimatic belts.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Biodiversity - Global biodiversity scenarios for the year 2100. Science 287, 1770-1774.

Salomon, E., Keren, N., 2011. Manganese Limitation Induces Changes in the Activity and in the Organization of Photosynthetic Complexes in the Cyanobacterium Synechocystis sp Strain PCC 6803. Plant Physiology 155, 571-579.

Sheridan, R.P., 1979. Effects of airborne-particulates on nitrogen-fixation in legumes and algae. Phytopathology 69, 1011-1018.

Specht, R.L., Rundel, P.W., Westman, W.E., Catling, P.C., Majer, J.D., Greenslade, P., 1988. Mediterranean type ecosystems. A data source book. Kluwer Academic Publishers, Dordrecht.

Steffen, W., Richardson, K., Rockstrom, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., de Vries, W., de Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sorlin, S., 2015. Planetary boundaries: Guiding human development on a changing planet. Science 347.

Takaichi, S., 2011. Carotenoids in Algae: Distributions, Biosyntheses and Functions. Marine Drugs 9, 1101-1118.

Takaichi, S., 2013. Distributions, biosyntheses and functions of carotenoids in algae. Agro Food Industry Hi-Tech 24, 55-58.

Ulm, F., Gouveia, C., Dias, T., Cruz, C., 2017. N fertilization in a Mediterranean ecosystem alters N and P turnover in soil, roots and the ectomycorrhizal community. Soil Biology & Biochemistry 113, 60-70.

Val, J., Monge, E., Baker, N.R., 1994. An improved HPLC method for rapid analysis of the xanthophyll cycle pigments. Journal of Chromatographic Science 32, 286-289.

Vojtech, E., Turnbull, L.A., Hector, A., 2007. Differences in light interception in grass monocultures predict short-term competitive outcomes under productive conditions. Plos One 2.

Wang, J., Bao, J., Su, J., Li, X., Chen, G., Ma, X., 2015. Impact of inorganic nitrogen additions on microbes in biological soil crusts. Soil Biology & Biochemistry 88, 303-313.

Wilsey, B.J., Potvin, C., 2000. Biodiversity and ecosystem functioning: Importance of species evenness in an old field. Ecology 81, 887-892.

Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P., Verstraete, W., Boon, N., 2009. Initial community evenness favours functionality under selective stress. Nature 458, 623-626.

Tables

Table 1 – Main characteristics of the detected biocrust pigments.

Pigment	Molecular composition	N containing pigment	Function	Superdomain*
Echinenone	$C_{40}H_{54}O$	No	Photoprotective	Prokaryotes
Scytonemin	$C_{36}H_{20}N_2O_4$	Yes	Photoprotective	Prokaryotes
Chl *a*	$C_{55}H_{72}O_5N_4Mg$	Yes	Photosynthetic	Both
Chl *b*	$C_{55}H_{70}O_6N_4Mg$	Yes	Photosynthetic	Both
β-carotene	$C_{40}H_{56}$	No	Photoprotective	Both
Zeaxanthin	$C_{40}H_{56}O_2$	No	Photoprotective	Both
Lutein	$C_{40}H_{56}O_2$	No	Photoprotective	Eukaryotes
Neoxanthin	$C_{40}H_{56}O_4$	No	Photoprotective	Eukaryotes
Violaxanthin	$C_{40}H_{56}O_4$	No	Photoprotective	Eukaryotes

*Pigments' occurrence across superdomains was done according to Takaichi (2011; 2013).

Figures

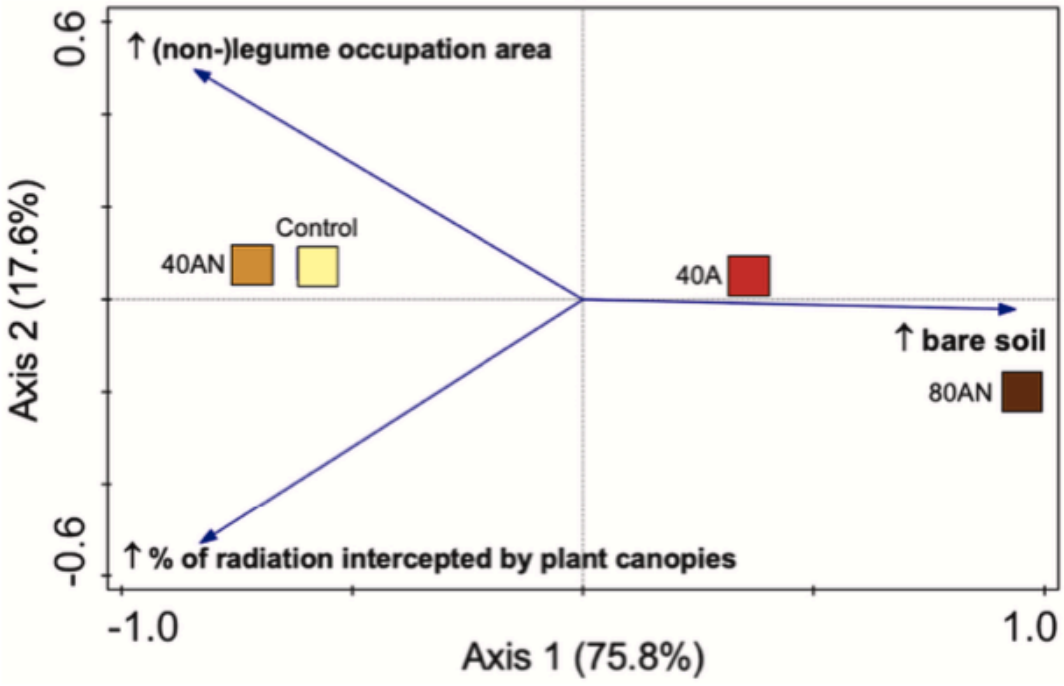

Figure 1 – Principal component analysis (PCA) depicting changes in biotope associated with each N input treatment in 12 Mediterranean shrubland plots. Response variables per plot were the percentage of radiation intercepted by plant canopies and the changes in the non-legume and legume (shrub) occupation areas and in bare soil. Symbols represent the explanatory variables (i.e. the N input treatments). Total variation is 36, and N input treatments explain 48.4% of the variation (adjusted).

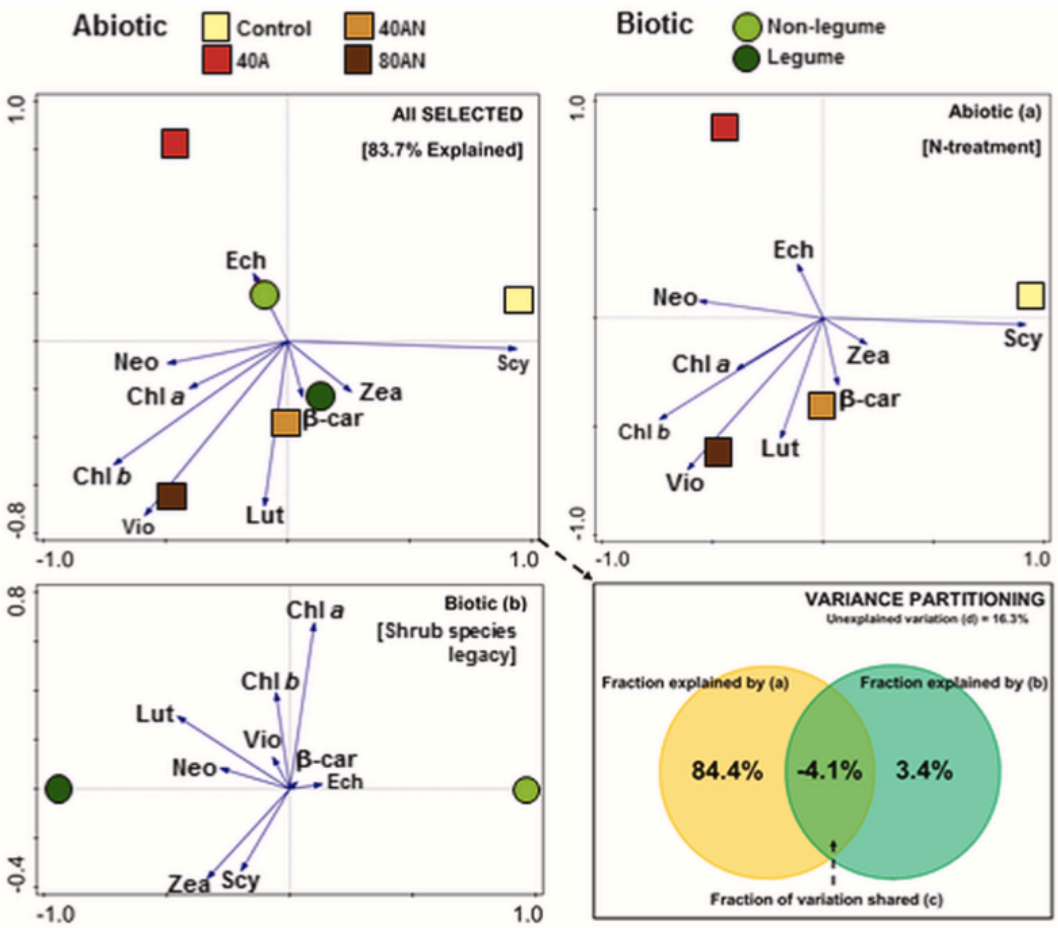

Figure 2 – Redundancy analysis (RDA) with variance partitioning, showing that both the N input treatments over 7 years and sampling underneath the non-legume (*C. ladanifer*) or the legume (*G. triacanthos*) plants significantly changed biocrust pigment composition and abundance (Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; β-car – β-carotene; Ech – echinenone; Lut – lutein; Neo – neoxanthin; Scy – scytonemin; Vio – violaxanthin; and Zea – zeaxanthin) in 12 Mediterranean shrubland plots. Total adjusted variation was 83.7%. Tested fraction *a*+*b*+*c*: F = 30.6, P < 0.001; Tested fraction *a*: F = 39.0, P < 0.001; Tested fraction *b*: F = 5.2, P = 0.026. Note the negative value of shared fraction *c*, confirming the opposite effect that groups *a* and *b* had on soil pigment abundance and distribution (Peres-Neto et al. 2006). Response data were centered.

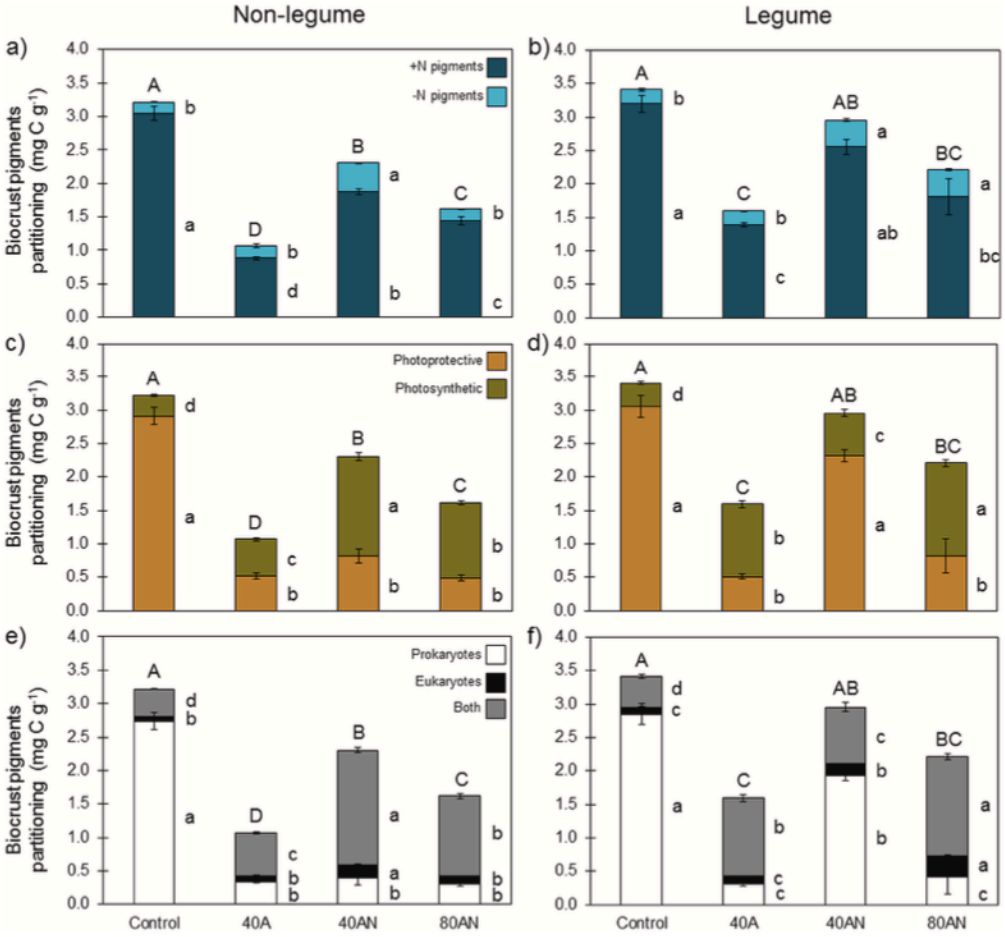

Figure 3 – Effects of both the N input treatments and plant species legacy (the non-legume *C. ladanifer* or the legume *G. triacanthos*) on pigments functional partitioning according to: a and b) pigments with or without N; c and d) photosynthetic or photoprotective pigments; and e and f) pigments can be found in prokaryotic or eukaryotic organisms or in both. Different letters show significant effects at the 5% level. Bars are the mean ± 1SE (n = 3).

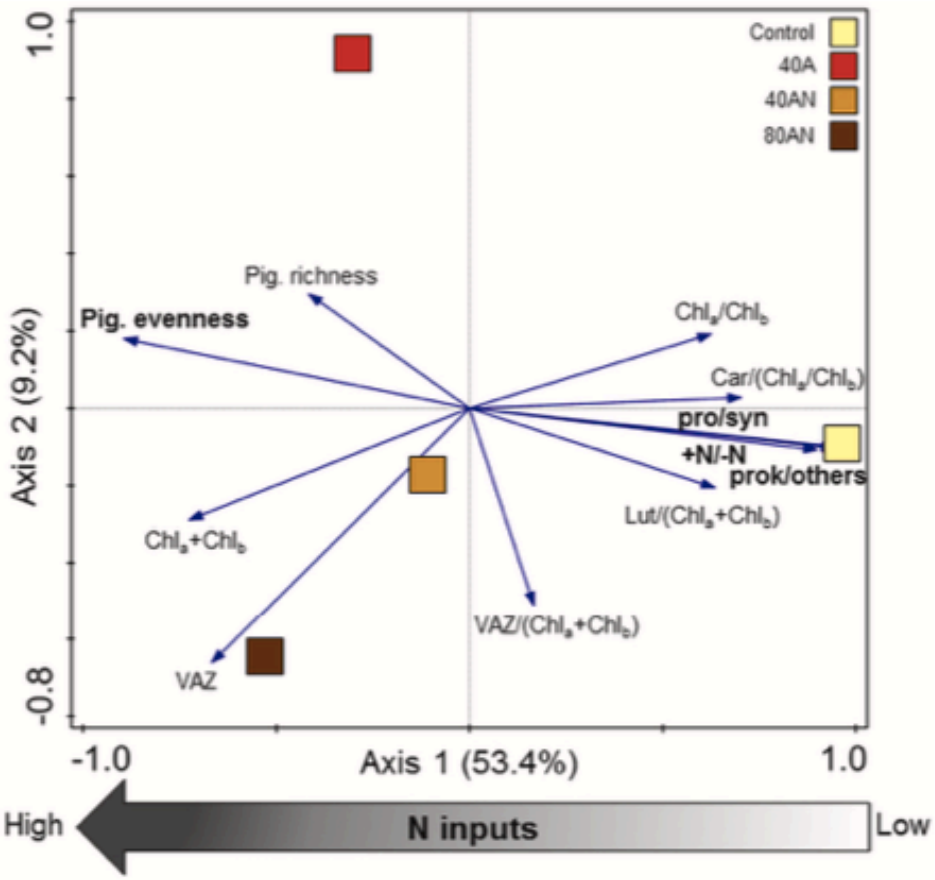

Figure 4 – Redundancy analysis (RDA) showing how the N input treatments significantly influenced biocrust ecological indicators (Table S1) in 12 Mediterranean shrubland plots. Total variation was 264, and explanatory variables accounted for 61.7% of the variation (adjusted variation). Forward-selection of variables rendered Control: pseudo-F = 22.0, P < 0.001; 40A: pseudo-F = 4.3, P = 0.003; 80AN: pseudo-F = 4.9, P < 0.001; and 40AN: unnecessary to calculate due to linear combinations (Lepš and Šmilauer 2003).

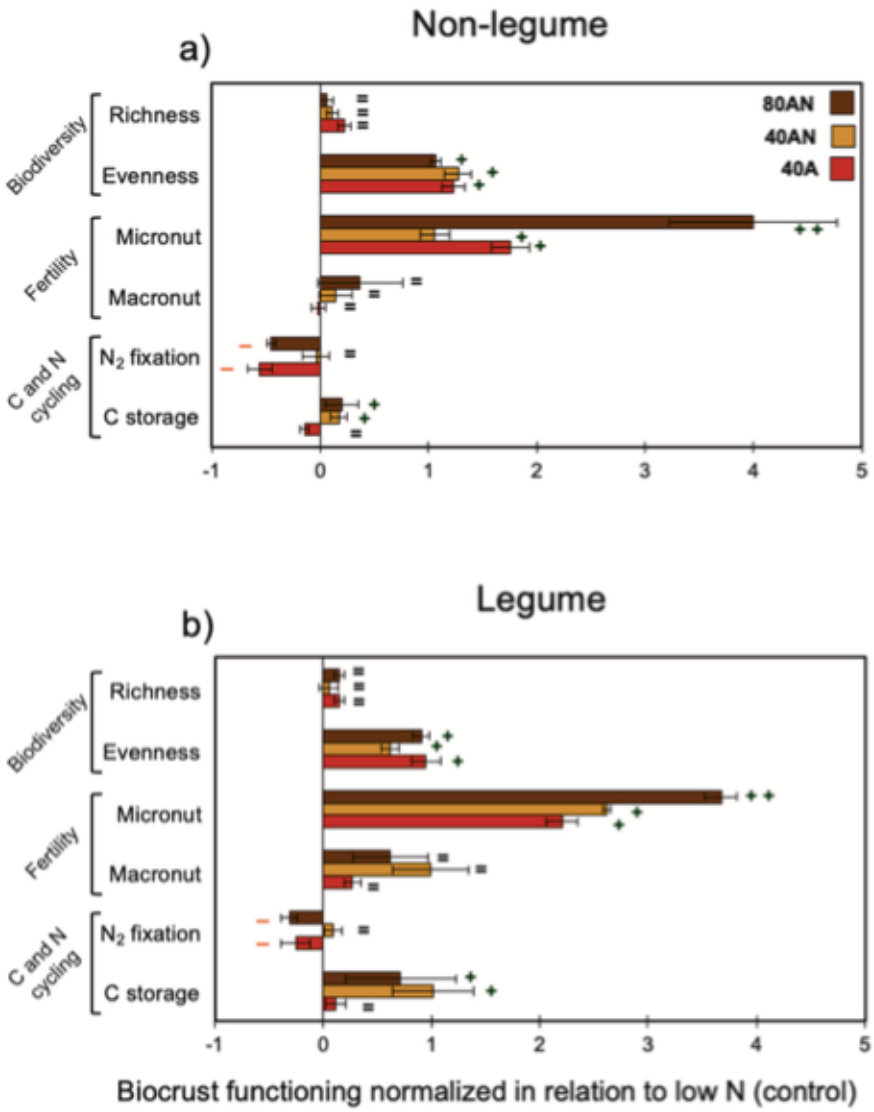

Figure 5 – Effects of both the level of the N input treatments and plant species legacy (the non-legume *C. ladanifer* or the legume *G. triacanthos*) on biocrust functioning relative to the treatment with low N input (control). Biocrust functioning included C and N cycling (C storage and N_2 fixation), fertility (concentration of macro and micronutrients) and biodiversity (pigment richness and evenness). Biocrust functioning were first normalized to its value under low N input (control). '-', '+' and '++' show significant negative and positive effects in relation to low N at the 5% level. Bars are the mean ± 1SE (n = 3).

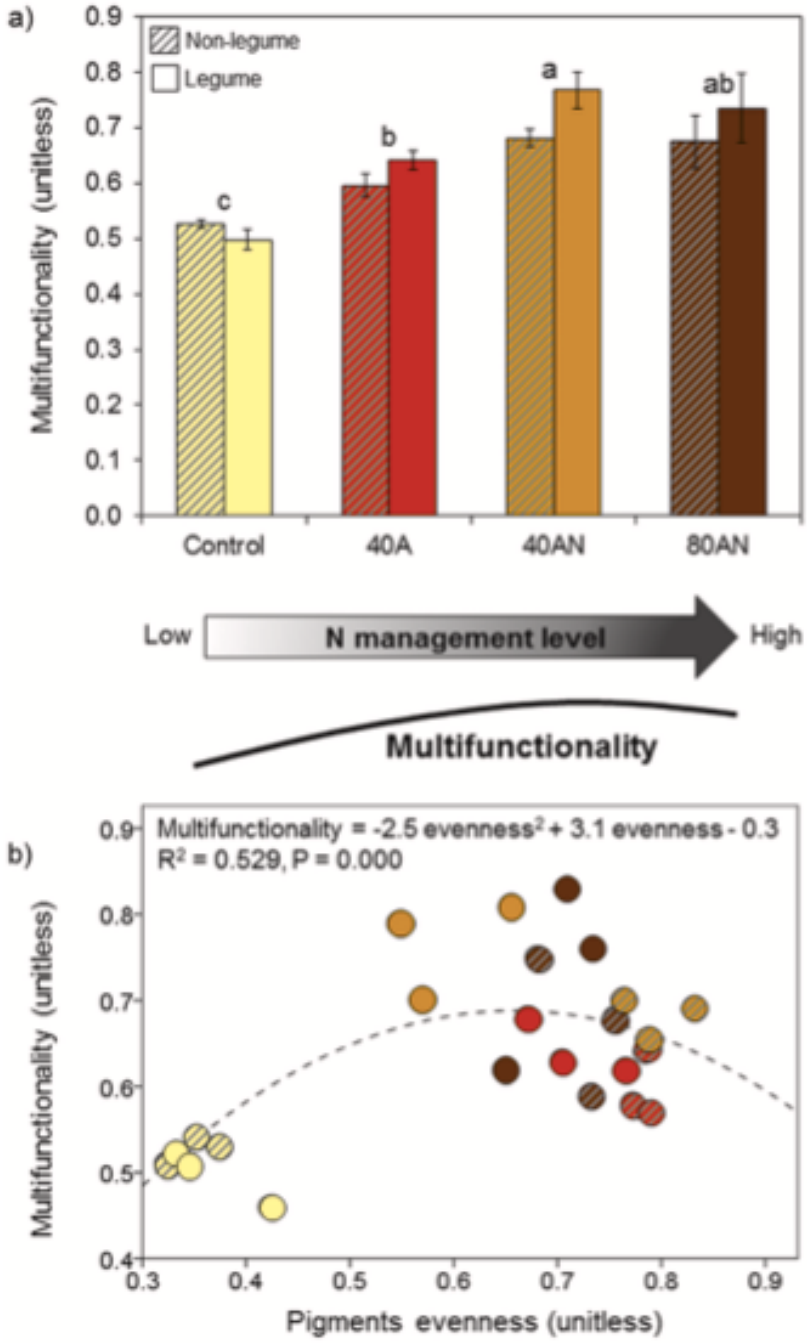

Figure 6 – Effects of both the level of the N input treatments and plant species legacy (the non-legume *C. ladanifer* or the legume *G. triacanthos*) on biocrust multifunctionality (a). Different letters show significant effects at the 5% level. Bars are the mean ± 1SE (n = 3). Quadratic regression analysis showing how biocrust multifunctionality is related with pigments evenness in 12 Mediterranean shrubland plots (pigments evenness explains 48.4% of the adjusted variation – b).