The effect of lichen-dominated biological soil crusts on growth and physiological characteristics of three plant species in a temperate desert of northwest China

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ABSTRACT

Biocrusts (biological soil crusts) cover open spaces between vascular plants in most arid and semi-arid areas. Information on effects of biocrusts on seedling growth is controversial, and there is little information on their effects on plant growth and physiology. We examined impacts of biocrusts on growth and physiological characteristics of three habitat-typical plants, *Erodium oxyrhynchum*, *Alyssum linifolium* and *Hyalea pulchella*, growing in the Gurbantunggut Desert, northwest China. The influence of biocrusts on plant biomass, leaf area, leaf relative water content, photosynthesis, maximum quantum efficiency of PSII (F_v/F_m), chlorophyll, osmotic solutes (soluble sugars, protein, proline) and antioxidant enzymes (superoxide dismutase, catalase, peroxidase) was investigated on sites with or without biocrust cover. Biomass, leaf area, leaf water content, photosynthesis, F_v/F_m and chlorophyll content in crusted soils were higher than in uncrusted soils during early growth and lower later in the growth period. Soluble sugars, proline and antioxidant enzyme activity were always higher in crusted than in uncrusted soils, while soluble protein content was always lower. These findings indicate that biocrusts have different effects on these three ephemeral species during growth in this desert, primarily *via* effects on soil moisture, and possibly on soil nutrients. The influence of biocrusts changes during plant development: in early plant growth, biocrusts had either positive or no effect on growth and physiological parameters. However, biocrusts tended to negatively influence plants during later growth. Our results provide insights to explain why previous studies have found different effects of biocrusts on vascular plant growth.

INTRODUCTION

Biological soil crusts (hereafter 'biocrusts') typically contain several organisms, *e.g.* mosses, lichens, algae, cyanobacteria and heterotrophic microorganisms, which live in the uppermost millimetres of the soil surface (Belnap 2002). As a particularly important component of arid regions, the ecological functions of biocrusts and their potential effects on desertification are attracting more attention, and recently, numerous studies have addressed aspects of the influence of biocrusts on vascular plants (Belnap 2002; Belnap & Lange 2003; Li *et al.* 2005; Escudero *et al.* 2007; Kidron 2014). Some studies found that this influence is related to modifications of the soil substrate by biocrusts. For example, they play an important role in stabilising the soil surface (Eldridge & Leys 2003; Zhang *et al.* 2006) and in providing carbon (C) and nitrogen (N) (Ettershank *et al.* 1978; Lange *et al.* 1992) to the ecosystem. They also play a role in pedogenesis (Fischer *et al.* 2010) and hydrological processes and thus in water redistribution (Kidron & Yair 1997; Kidron 1999; Zhang *et al.* 2009); this activity may be especially important in regions where water is the main limiting factor for plant growth (Noy-Meir 1973).

Water availability and N supply are important determinants of plant species composition, diversity and productivity in arid and semi-arid ecosystems (Zhang & Zak 1998). Despite the importance of biocrusts in controlling water and nutrients, experimental evidence of their effects on vascular plant species is scarce and contradictory. Relationships between biocrusts and vascular plant species are extremely complex, with positive, negative and neutral responses in components of both communities at the species level, and involve plant traits related to seed morphology and dispersal and the functional type of biocrust (Bowker *et al.* 2010). This controversy is a consequence of the fact that plant–biocrust interactions are likely to be highly species-specific, similar to the situation in plant–plant interactions (Callaway 2007).

Therefore, researchers have suggested that more studies are needed in order to completely understand interactions between biocrusts and vascular plants (Belnap & Lange 2003; Godínez-Alvarez *et al.* 2012), especially in areas with different background vegetation, soil and climate conditions. Moreover, despite increasing numbers of international studies that document the important roles of biocrusts and their relationship with vascular plants (Belnap & Lange 2003), such studies

mainly focus on plant emergence, survival and seedling establishment (Li *et al.* 2005; Escudero *et al.* 2007; Godínez-Alvarez *et al.* 2012). However, there are few studies on growth and physiological characteristics of ephemeral plant species, particularly during their growth period, which are required to evaluate plant suitability for growth on biocrusts.

The Gurbantunggut Desert, the largest fixed and semi-fixed desert in China, is characterised by lichen-dominated biocrusts, which serve an essential role together with adjacent ephemeral plants in sand fixation (Zhang *et al.* 2007). Ephemeral plants can emerge during snowmelt and complete their life cycle within 2 or 3 months before the onset of the dry season. Ephemerals, as the shortest stature vascular plants, make a major contribution to sand dune stability. Biocrusts embedded among these plants can alter soil characteristics, such as water and nutrient levels, which are important for the growth of ephemeral plants. For example, recent findings have documented that biocrusts enhance evaporation from underlying soil in a dune field and thus negatively affect annual plant growth (Kidron 2014). Therefore it is possible that the performance of ephemerals in Gurbantunggut Desert might differ in biocrusted or uncrusted soils. However, it remains unclear how biocrusts affect the growth and development of ephemeral plants in this desert.

In the present research, we attempted to experimentally investigate whether and how biocrusts affect desert vascular plant growth and physiological characteristics during growth in the Gurbantunggut Desert of northwest China. Due to the abundant nutrient supply in biocrusts and their ability to influence soil moisture availability (Belnap 2002; Kidron 2014), we hypothesised that biocrusts would stimulate growth and physiological performance of ephemerals in the early growth period when snowmelt occurs and soil moisture is not limited, and have different effects on plant growth in the later growth period after the onset of the dry season.

MATERIAL AND METHODS

Description of study area

The study area is located in the southern part of the Gurbantunggut Desert (44°87′ N, 87°82′ E), Xinjiang Uygur Autonomous Region, China. It is the largest fixed and semi-fixed desert in China, with an area of 4.88×10^4 km^2. The mean annual temperature is 7.3 °C, annual precipitation ranges from 70 to 150 mm, while mean potential annual evaporation is 2607 mm. Precipitation is unevenly distributed among seasons, with half of the annual precipitation falling between April and July. In winter, approximately 20 cm of snow covers the surface of the desert. The temporal pattern of temperatures and precipitation creates favourable conditions for growth and development of ephemeral plants in spring. In the study area, ephemeral plants grow vigorously in spring and early summer after snowmelt and rain, resulting in up to 30% vegetation cover. Ephemerals, *e.g.* Geraniaceae, Brassicaceae and Asteraceae, mainly appear on inter-dunes and middle to lower slopes of dunes. The areas between vascular plants are colonised by well-developed biocrusts, dominated by lichens such as *Collema tenax*, *Psora decipiens*, *Xanthoria elegans*, *Acarospora strigata* and *Lecanora argopholis* (Zhang *et al.* 2007).

Field experiment design

In October 2011, 6 months prior to the start of the experiment, a site with well-developed biocrusts was selected in the lowlands of the desert. The site, 60 m × 100 m, was fenced to protect the ground from disturbance by people or animals prior to commencement of the study. In early April 2012, before the time at which seed dispersal begins, 40 2 m × 2-m plots were randomly established within the enclosure. The experimental design was planned to ensure that there were enough plots to account for between-plot variability and to avoid spatial interdependencies between the plots (pseudo-replication). Twenty plots were selected randomly from among 40 2 m × 2-m plots as disturbed plots from which biocrusts were removed (biocrust-removed). The remaining plots served as control plots in which the biocrusts remained intact (biocrust-intact). Thus there were 20 plots with an intact lichen/cyanobacteria crust and 20 plots without a lichen/cyanobacteria crust. To accomplish biocrust removal, we removed the top 0–8 cm of soil when the soil was slightly moist and less vulnerable to break up (Serpe *et al.* 2008). After removal of biocrust, no visible components of biocrusts remained in the biocrust-removed site.

Collection of samples

Plant samples were collected five times during the growing season at 10- to 15-day intervals. When snow melted at the end of March, ephemeral plants gradually began to appear. Our first harvest was on 7 April 2014 and the last one on 25 May 2014, when plants had reached peak vegetative biomass and begun to set seed. On 25 May 2014, the plants had reached peak vegetative biomass and a very small proportion had seeded. We collected plant samples, avoiding individuals setting seed, from both biocrust-intact and biocrust-removed plots.

Normally, seedling emergence of ephemerals in this desert occurs at the beginning of April and finishs at the end of May. According to the whole growing period of ephemeral plants, we considered 7–30 April 2014 as 'early growth period', and 30 April to 25 May 2014 as 'late growth period'. For each species and plot, 80 plants were harvested at each sampling date in April and 60 at each sampling date in May. After harvest, the samples were kept in plastic containers at 4 °C and transported to the laboratory for analysis of biomass, leaf water content and chemical characteristics. All measurements were conducted in three ephemeral plant species: *E. oxyrrhynchum*, *A. linifolium* and *H. pulchella*.

At the time of plant collection, soil samples from crusted and uncrusted plots were also collected. Since ephemeral plant roots are mainly concentrated within the top 15 cm of soil, the soil was sampled from this layer using a soil knife and separated into fractions collected at depths of 0–5, 5–10 and 10–15 cm. These samples were used to analyse soil moisture and chemical characteristics, as described below.

Analysis of soil moisture, organic matter and nutrients

Soil moisture was determined gravimetrically, with oven drying at 105 °C for 24 h. Air-dried soil samples were used to measure

physical and chemical characteristics. Soil particle size was assessed with the pipette method; soil bulk density was determined by a inserting a metallic corer (0.05-m depth and diameter) into the soil. Organic matter was measured with the dichromate oxidation method of Walkey and Black (Weaver *et al.* 1994). Soil total C was measured with the K_2CrO_7 method (Walkley 1947), and total N with the Kjeldahl procedure using a UDK140 automatic steam distillation unit (Automatic Titroline 96, Italy; Weaver *et al.* 1994). Available N was determined with the alkali hydrolysation–diffusion method, available phosphorus (available P) and potassium (available K) with the Bray method and leaching–flame luminosity method, respectively (Chen *et al.* 2007).

Plant biomass, leaf area and water content measurements

For biomass determination, each plant was oven-dried at 70 °C to constant weight. Individual biomass represents the sum of the weight of roots, stem and leaves. The leaves were also scanned (Uniscan B800, Tsinghua Unisplendour, Beijing, China) and leaf areas estimated from digital images using CI-400 CIAS (CID, Camas, WA, USA) software.

Leaf water status was evaluated as relative water content (RWC, %) calculated based as:

$$RWC(\%) = (FW - DW)/(TW - DW) \times 100$$

where FW is leaf fresh weight (FW), TW full turgor weight after soaking leaves in water for 24 h, and DW dry weight after drying for 48 h at 80 °C (Schonfeld *et al.* 1988).

Determination of chlorophyll, soluble sugars, soluble protein and proline

Chlorophyll was extracted from samples taken from the centre of fresh leaves, using 95% (v/v) ethanol. Absorption of the filtered extract was measured at 665 nm, 649 nm and 470 nm, and chlorophyll content calculated according to Lichtenthaler (1987).

The concentration of soluble sugars was estimated in extracts obtained from fresh leaves with the anthrone method, using glucose as standard (Yemm & Willis 1954). Fresh leaf samples (0.5 g) were homogenised in 2 ml 80% (v/v) alcohol in a pestle and mortar and washed three times with 3 ml 80% alcohol. The homogenates were kept at room temperature for 30 min then centrifuged at 4000 *g* for 20 min. The supernatant was stored at 4 °C, and 0.5 ml supernatant mixed with 3 ml anthrone and incubated at 95 °C for 10 min. Absorbance at 620 nm was then recorded.

Free proline was extracted in aqueous sulphosalicylic acid and measured using ninhydrin according to Bates *et al.* (1973). Approximately 0.5 g fresh leaf sample was homogenised with 10 ml 3% (w/v) aqueous sulphosalicylic acid and the homogenate centrifuged at 3000 *g* for 20 min. The supernatant was boiled for 1 h with acid-ninhydrin and the reaction terminated in a waterbath at a room temperature (25 °C) for 10 min. Absorbance at 520 nm was determined using L-proline as standard.

Soluble protein was determined using Coomassie brilliant blue G-250. A 0.5-g leaf tissue sample was homogenised in 15 ml phosphonate buffer and the supernatant centrifuged and used to determine protein content following Sedmak & Grossberg (1977) with bovine serum albumin as standard.

Concentrations of chlorophyll, soluble sugars, soluble protein and proline were calculated on a fresh weight basis ($mg \cdot g^{-1}$ FW).

Antioxidant enzyme assay

Superoxide dismutase (SOD) activity was determined following the method of Kuk *et al.* (2003). Activity was expressed in enzyme units$\cdot mg^{-1}$ protein. One unit of SOD is defined as the amount of enzyme that inhibits the reduction of nitro blue terazolium chloride by 50%.

Peroxidase activity (POD) was determined with the guaiacol oxidation method (Nakno & Asada 1981). The reaction mixture consisted of 50 ml 0.2 M phosphate buffer (pH 6.0), 19 μl guaiacol and 28 μl 30% H_2O_2. The reaction started after adding 0.5 ml supernatant to 1.5 ml reaction mixture, and changes at 470 nm were recorded for 3 min. Enzyme activity was expressed as an increase in absorbance per min $\cdot mg^{-1}$ protein.

Catalase (CAT) activity was estimated by monitoring the disappearance of H_2O_2 and recording decline in absorbance at 240 nm, following Ai *et al.* (2008), in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.8), 100 mM 30% H_2O_2, and crude enzyme extract.

Gas exchange measurements

For each plot and species, net photosynthesis (P_N), transpiration (E) and stomatal conductance (g_s) were measured in the field using a Li-6400 open gas exchange system (Li-Cor, Lincoln, NE, USA). These measurements were conducted during the growing season at 10- to 15-day intervals. All measurements were made in fully expanded leaves between 07:30 and 10:30 h to avoid potential stomatal closure at midday. One or two leaves were positioned across a 20 mm × 30 mm leaf chamber taking precautions to avoid self-shading. All measurements were conducted at 30 °C with a saturating PPFD of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, provided from a red blue 6400-02B light source. Saturating PPFD was determined based on light response curves. After measurements, the leaves were scanned and estimated leaf areas used to calculate P_N and E per unit leaf area. Water use efficiency (WUE) was calculated as P_N/E (Condon *et al.* 2002).

Chlorophyll fluorescence

The chlorophyll fluorescence parameter F_v/F_m was measured on the youngest fully expanded leaf using a pulse-modulated chlorophyll fluorometer (PAM2100; Walz, Effeltrich, Germany). Measurements were made during the growing season at 10- to 15-day intervals. The leaves were pre-darkened for at least 30 min prior to measurements. For each seedling, maximum photochemical efficiency of PSII (F_v/F_m) was estimated based on two measurements. F_v/F_m was calculated as:

Table 1. Effects of biocrusts (B), plant species (S), growth periods (P) and the interaction among them on growth and physiological characteristics using factorial ANOVA.

	source of variance						
	F_B	F_S	F_P	$F_{B \times S}$	$F_{B \times P}$	$F_{S \times P}$	$F_{B \times S \times P}$
df	1	2	4	2	4	8	8
Biomass	30.78**	1389.48**	2406.83**	12.82**	40.14**	539.48**	19.34**
ALA	25.16**	731.89**	94.91**	245.58**	13.49**	37.48**	91.54**
RWC	42.54*	33.06**	14.81**	2.23	3.06*	2.94**	0.79
P_N	4.58	14.88**	385.65**	2.62	19.26**	2.77*	2.43*
g_s	0.76	247.04**	238.82**	1.06	17.46**	3.57	1.7
E	0.91	163.96**	118.69**	1.51	13.95**	5.15**	1.52
WUE	1.43	2660.39**	171.44**	2.18	32.23**	3.26**	1.48
F_v/F_m	0.21	161.35	113.56**	0.61	16.95**	2.06	1.18
Chl	0.19	58.54**	292.73**	0.54	13.41**	7.42**	2.29*
Soluble sugars	30.42**	20.54**	49.52**	1.64	3.64*	4.05**	1.18
Soluble protein	33.47**	124.50**	326.56**	0.19	5.09**	4.43**	0.78
Proline	35.81**	72.71**	615.52**	1.59	2.54	0.83	0.54
POD	0.31	156.29**	14.89**	0.18	0.91	0.19	0.48
SOD	16.29**	673.09**	8.17*	1.11	0.73	2.15	1.13
CAT	31.08**	376.15**	19.31**	5.36**	0.57	2.84*	0.24

Treatment effects are: F_B = comparing crusted and uncrusted soils; F_S = comparing three different plant species; F_P = comparing five different growth periods; $F_{B \times S}$ = comparisons of two sites and three species; $F_{B \times P}$ = comparisons of two sites and five growth periods; $F_{S \times P}$ = comparisons of three species and five growth periods; $F_{B \times S \times P}$ = comparisons of two sites, three species and five growth period.
*$P < 0.05$, **$P < 0.01$.

$$F_v/F_m = (F_m - F_o)/F_m$$

where F_o is initial fluorescence (all reaction centres open) and F_m is maximum fluorescence (all reaction centres closed; Krause & Weis 1991).

Statistical treatment of the data

General linear model procedures were performed to evaluate the effects of treatment, species, sampling date and their interactions on plant biomass, water content and chemical and physiological characteristics. t-Tests were used to determine differences between crusted and uncrusted treatments, such as soil physiochemical properties and plant characteristics, on each sampling date. The plant samples were collected randomly from six or seven plots and then mixed each time, thus providing three replicates from the 20 plots for each treatment. These statistical tests were performed with SPSS 13.0 (SPSS, Chicago, IL, USA). Data were tested for normality with the Kolmogorov–Smirnov test and significance of
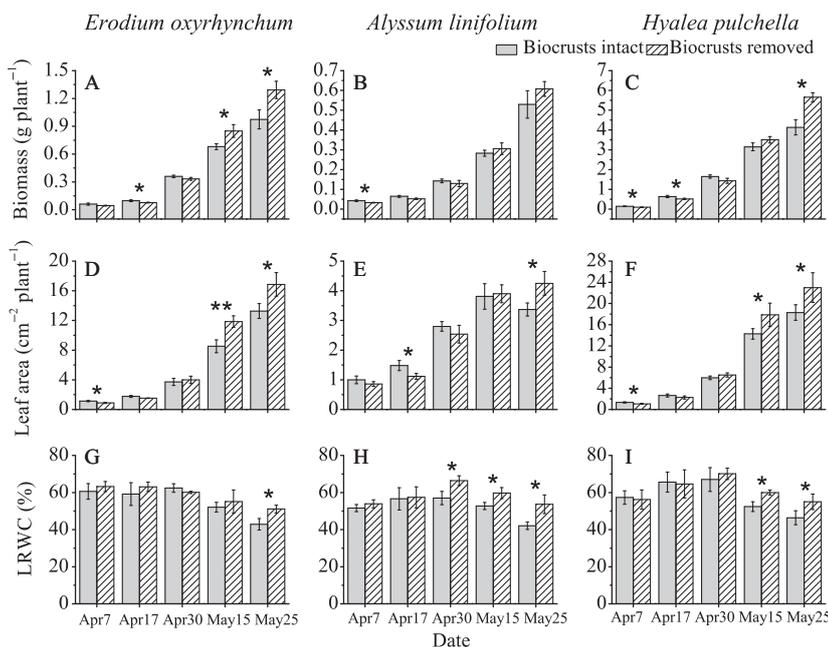
Fig. 1. Biomass, leaf area (LA) and leaf relative water content (RWC) of *Erodium oxyrhynchum* (A, D, G), *Alyssum linifolium* (B, E, H) and *Hyalea pulchella* (C, F, I) in crusted soils and uncrusted soils of five growing periods (Mean ± SD; n = 3). Asterisks indicated significant difference between crusted soils and uncrusted soils on each growing period: *$P < 0.05$ or **$P < 0.01$.
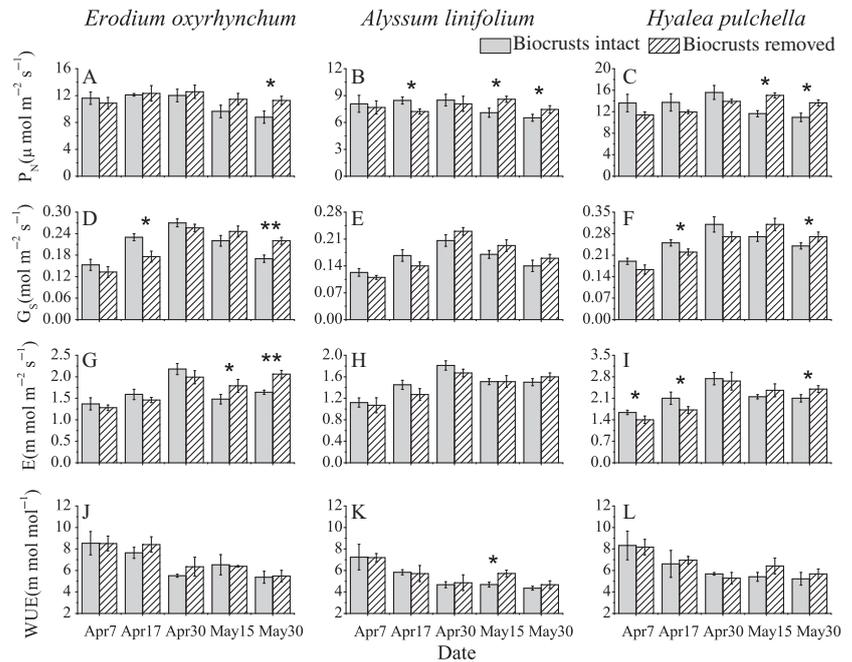
Fig. 2. Net photosynthetic rate (P_N), stomatal conductance (Gs), transpiration rate (E) and water use efficiency (WUE) of *Erodium oxyrhynchum* (A, D, G, J), *Alyssum linifolium* (B, E, H, K) and *Hyalea pulchella* (C, F, I, L) in crusted soils and uncrusted soils of five growing periods (Mean ± SD; n = 3). Figure annotations are the same as in Fig. 1.

differences between treatment means was evaluated at $P < 0.05$.

In addition to these tests, we conducted a redundancy analysis (RDA) to examine the extent to which the variation in water and nutrient content between crusted and uncrusted plots explained differences in plant biomass and P_N. A partial Monte Carlo permutation (499 permutations) was applied for significance analysis. RDAs were conducted with axes 1 and 2 scores as dependent variables and soil nutrient and water content as independent variables to determine which variable had the largest effect on biomass and P_N. The RDA was conducted on samples collected on 17 April and 15 May. We chose these days as representative of the early and late portion of the growth period. Based on root distribution at the time of sampling, soil properties in the 0–5 cm layer were used as independent variables on 7 April and average properties of the 0–5 cm and 5–10 cm layers as independent variables on 15 May.

RESULTS

Biomass, leaf area and leaf RWC

Biomass, leaf area and leaf RWC differed significantly between crusted and uncrusted soils (Table 1). Biocrust presence tended to have a positive effect on biomass and leaf area early in the growth period, but a negative effect late in the season (Fig. 1A–F). There were also differences among species biomass in relation to the crust. Removal of the crust did not have a significant effect on final biomass (25 May) of *A. linifolium*, but promoted biomass accumulation in *E. oxyrhynchum* and *H. pulchella*. Biocrust presence also had an effect on RWC, particularly towards the end of the growing season. For all three species, removal of the biocrust was associated with an increase in RWC for samples harvested on 15 May and/or 25 May (Fig. 1G–I). In addition, significant interactions occurred between crust treatment and species and crust treatment and

growth period (Table 1; $P < 0.01$), indicating that the effect of the biocrust differed among species or throughout the growth period.

Gas exchange

At the end of the growing season, biocrust removal tended to increase P_N, g_S, E and WUE, particularly in *E. oxyrhynchum* and *H. pulchella* (Fig. 2). The major differences in gas exchange parameters were observed among species (Table 1; $P < 0.01$). In both biocrust-covered and biocrust-removed plots, P_N, g_S, E and WUE of *H. pulchella* were higher than those of *E. oxyrhynchum* and *A. linifolium* (Fig. 2A–L). In contrast to biomass and leaf area, biocrust removal did not affect P_N, g_S, E and WUE (Table 1). However, there were interactions between biocrust treatment and growing period.

Chlorophyll content and F_v/F_m

In both crusted and uncrusted soils, F_v/F_m values of all three species tended to increase from 7 to 30 April, while remaining constant or slightly decreasing thereafter (Fig. 3A–C). Biocrust presence did not have an overall effect on chlorophyll content, but there was a significant interaction between biocrust treatment and sampling date (Table 1). In April, chlorophyll content for plants growing in crusted plots was similar or higher than that of plants growing in uncrusted soil. In contrast, in May, plants in the crusted plots had lower chlorophyll content than those in uncrusted ones. An exception was *A. linifolium* on 25 May, which had similar chlorophyll content under both treatments. Chlorophyll content also varied among species: *H. pulchella* had higher chlorophyll content than *E. oxyrhynchum* and *A. linifolium* (Fig. 3D–F). Differences in chlorophyll content were also somewhat related to differences in F_v/F_m. For example, *H. pulchella* had higher chlorophyll content and F_v/F_m values than *A. lynifolium* (Fig. 3).
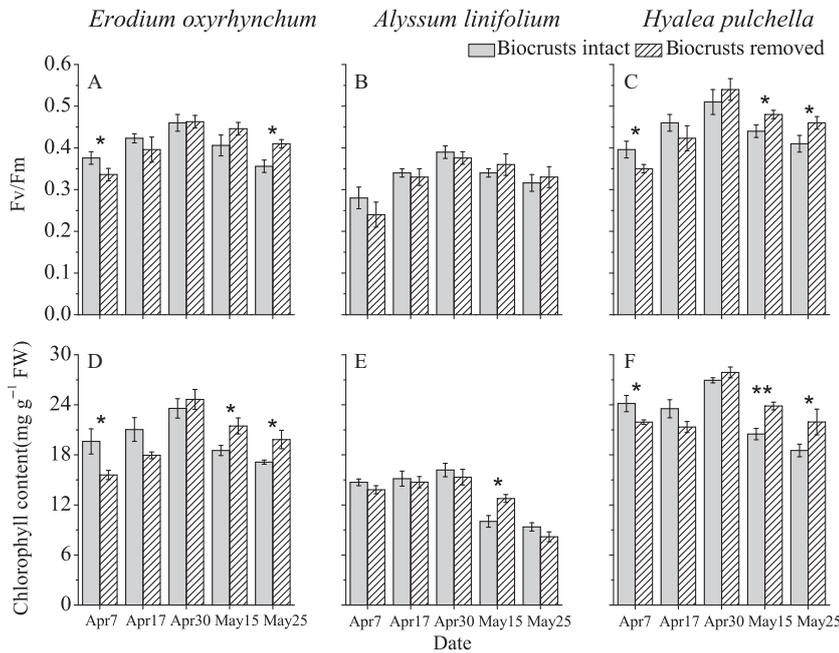
Fig. 3. The maximum quantum efficiency of PSIIphotochemistry (F_v/F_m) and chlorophyll content of *Erodium oxyrhynchum* (A, D), *Alyssum linifolium* (B, E) and *Hyalea pulchella* (C, F) in crusted soils and uncrusted soils of five growing periods (Mean ± SD; n = 3). Figure annotations are the same as in Fig. 1.

## Accumulation of soluble sugars, protein and proline

In all three species and throughout the growing season, plants in crusted plots had higher soluble sugars and proline than those in uncrusted plots (Fig. 4A–F). In contrast, the opposite trend was observed for soluble protein (Fig. 4G–I). Accumulation of soluble sugars, proline and soluble protein differed between the biocrust treatments (Table 1; $P < 0.01$). Other factors affecting levels of sugars, proline, and protein were species and sampling date (Table 1; $P < 0.01$). The highest concentrations of soluble sugars, proline, and soluble protein were found in *A. linifolium* followed by *E. oxyrhynchum* and *H. pulchella* (Fig. 4A–I). Similarly, the concentration of soluble sugars and proline was highest on 15 May, and of soluble protein on 15 and 25 May (Fig. 4A–F).

Antioxidant enzyme activity

Activity of POD was not significantly affected by biocrust presence and no significant interactions were observed among the three factors considered (Table 1). In contrast, species and sampling date affected POD activity: POD activity was higher in *H. pulchella* and *E. oxyrhynchum* than in *A. linifolium*. Differences in POD activity were also observed among sampling dates, but no clear trend was apparent throughout the growth period (Fig. 5A–C). In contrast to POD, biocrust presence had a significant effect on activity of SOD and CAT (Table 1); these enzymes tended to be lower in uncrusted than crusted soils (Fig. 5D–I). Similar to results for POD, SOD and CAT activity was higher in *H. pulchella* and *E. oxyrhynchum* than in *A. linifolium*. In addition, SOD
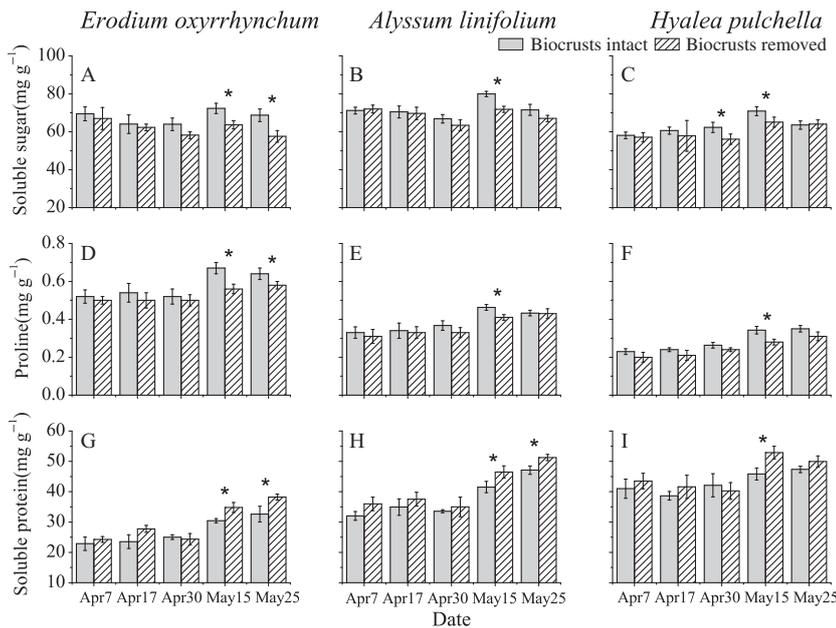
Fig. 4. The contents of soluble sugar, proline and soluble protein of *Erodium oxyrhynchum* (A, D, G), *Alyssum linifolium* (B, E, H) and *Hyalea pulchella* (C, F, I) in crusted soils and uncrusted soils of five growing period (Mean ± SD; n = 3). Figure annotations are the same as in Fig. 1.
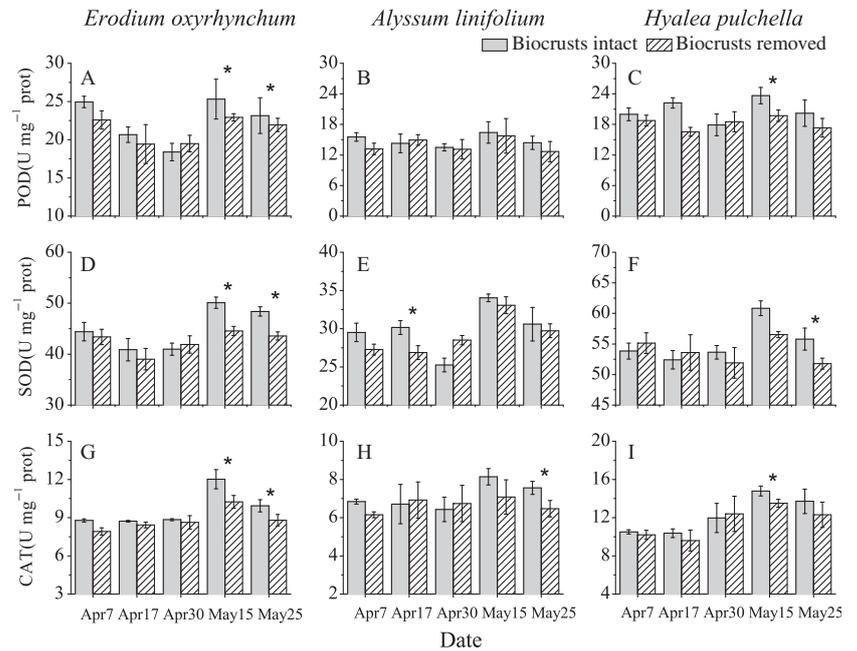
Fig. 5. Activities of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) of *Erodium oxyrhynchum* (A, D, G), *Alyssum linifolium* (B, E, H) and *Hyalea pulchella* (C, F, I) in crusted soils and uncrusted soils of five growing periods (Mean ± SD; n = 3). Figure annotations are the same as in Fig. 1.

and CAT activity was higher for all three species in May than in April (Fig. 5D–I).

Effects of soil conditions on biomass and P_N

There were no significant differences in soil characteristics among sampling dates, but differences were detected between crusted and uncrusted soils. Measurements from samples collected on 7 April are representative of these differences (Table 2). In the 0–5 cm soil layer, organic matter, total N, available N and available P were higher in crusted than uncrusted soils. Similarly, in the 5–10 cm layer, total N, available N and available P were higher in crusted than uncrusted soils, while no differences were detected in organic matter and available K. The deepest layer studied, 10–15 cm, did not show differences in organic matter or nutrient levels, except for available P, which was higher in crusted than uncrusted soil. During early and mid-April, soil moisture in crusted soils was not different from that in uncrusted soil. However, some differences were

observed between 30 April and 25 May, when soil moisture tended to be lower in crusted than uncrusted soils (Fig. 6).

The effects of soil chemical characteristics and water content on biomass and P_N were also examined with RDA. The cosine of the angle between the species line and a particular soil characteristic line is indicative of the effect of soil characteristics on a dependent variable (biomass or P_N; Fig. 7). Soil water and nutrient variables explained more than 90% of total variability in plant growth (RDA; $P < 0.05$). Soil available P and K had the smallest angle with the three species line (Fig. 7A), indicating that these soil properties were strongly related to biomass in the early growth period. In contrast, soil water content was the most important factor promoting biomass in the later part of the growing season (Fig. 7B). Soil organic matter and available P were the dominant factors for P_N variation of *A. linifolium* and *H. pulchella* in the early growth period (Fig. 7C), while soil water was most important for P_N of all three species in the later season (Fig. 7D).

Table 2. Main soil characteristics in crusted and uncrusted sites (n = 3, mean ± SD).

	0–5 cm			5–10 cm			10–15 cm		
	Crusted soils	Uncrusted soils	*t*-Value	Crusted soils	Uncrusted soils	*t*-Value	Crusted soils	Uncrusted soils	*t*-Value
Silt (%)	18.26 ± 1.49	16.16 ± 1.34	2.16	16.69 ± 1.27	15.11 ± 0.22	1.33	16.16 ± 1.24	15.22 ± 0.31	1.22
Clay (%)	2.63 ± 0.49	2.42 ± 0.32	0.67	2.45 ± 0.45	2.52 ± 0.35	−0.52	2.64 ± 0.25	2.33 ± 0.47	0.96
Sand (%)	79.11 ± 1.02	81.41 ± 1.66	−1.76	80.85 ± 1.42	82.36 ± 0.37	−2.01	81.20 ± 1.06	82.44 ± 0.26	−2.07
Organic matter (g·kg^{-1})	3.69 ± 0.14	2.68 ± 0.42	3.89*	1.64 ± 0.22	1.37 ± 0.18	1.59	1.39 ± 0.11	1.12 ± 0.16	2.42
Total N (g·kg^{-1})	0.23 ± 0.04	0.14 ± 0.01	3.07*	0.13 ± 0.01	0.08 ± 0.01	7.23*	0.10 ± 0.02	0.07 ± 0.01	1.56
Available N (mg·kg^{-1})	9.56 ± 0.97	6.96 ± 1.11	4.04*	8.67 ± 0.78	6.01 ± 0.83	2.33*	6.15 ± 1.06	4.14 ± 1.05	3.76
Available P (mg·kg^{-1})	6.17 ± 0.58	4.51 ± 0.51	3.76*	4.88 ± 0.24	3.69 ± 0.36	4.72**	3.66 ± 0.32	2.89 ± 0.06	4.09*
Available K (mg·kg^{-1})	158.6 ± 7.1	145.6 ± 20.0	1.06	149.0 ± 6.5	139.3 ± 13.6	1.11	142.6 ± 10.4	127.6 ± 16.8	1.31

Significant differences between plots in initial concentrations of soil variables: *$P < 0.05$, **$P < 0.01$. Data are mean ± SD (n = 3).
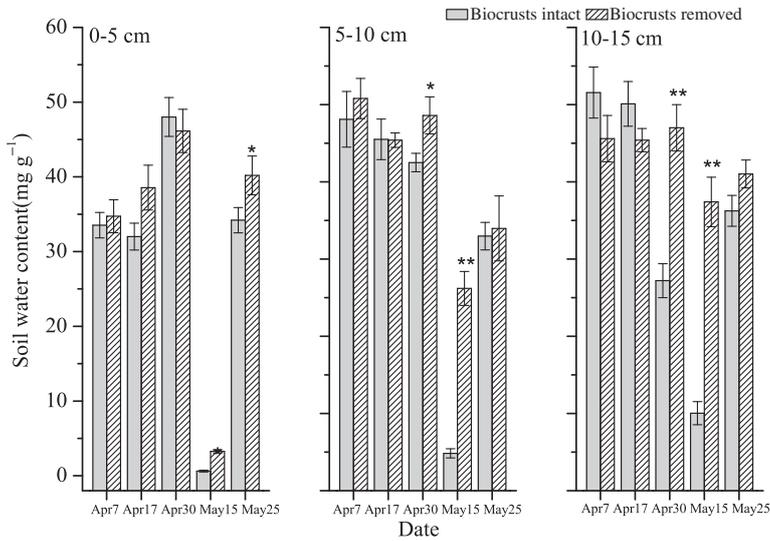
Fig. 6. Comparion of soil moisture in upper 0–5 cm, 5–10 cm and 10–15 cm of crusted soils and uncrusted soils during periodic sampling date. Bars represent standard deviation of mean (n = 3). Figure annotations are the same as in Fig. 1.

DISCUSSION

Effect of biocrusts on growth

Biocrusts had a positive or neutral effect on performance of all three ephemeral plant species in the early growth period, and then a negative or neutral effect in the later part of the growing season, consistent with our initial hypothesis. The positive effect of biocrusts on seedling growth in the early growth period might be the result of increases in nutrient content, particularly N. Biocrusts may contribute to the fine surface fraction and nutrient enrichment, given the ability of some species to fix and retain N (Mayland & McIntosh 1966; Belnap 2002; Hawkes 2003). Additionally, most studies have suggested that presence of biocrusts could enhance nutrient content of the underlying soil (Harper & Pendleton 1993; Jafari *et al.* 2004). Our investigation produced similar results in

the two different site conditions. Total N and available N and P in the 0–5 cm upper soil as well as 5–10 cm in the lower layer under biocrusts were significantly higher in crusted sites compared to uncrusted sites. In the early growth period of all three ephemeral plant species, RDA showed that soil available P and K were the main factors driving plant growth (Fig. 7). Moreover, soil water content in those layers did not differ significantly between crusted soils and uncrusted soils (Fig. 6). This is more likely to occur in early spring when melting snow and rain increase soil moisture content and growth is limited by nutrient availability. Thus, crusted soils may serve as a particularly potent micro-fertility belt. These results could well explain why the increase in soil nutrients below biocrusts has a positive effect on establishment and growth of vascular plants in the early growth period. Consistent with our results, simulations of semi-arid
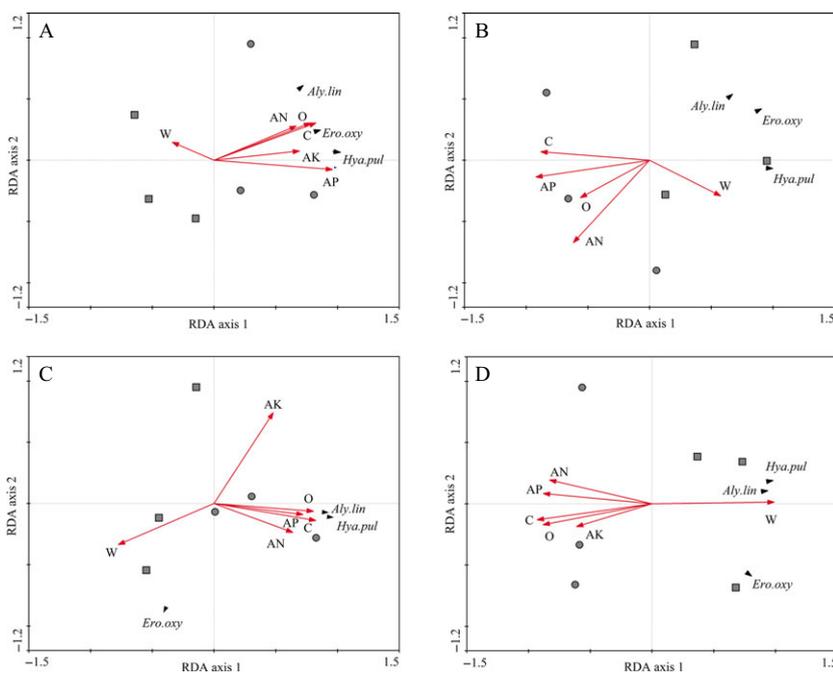
Fig. 7. Ordination diagrams of the reduced redundancy analysis (RDA) model containing the explanatory variables (O, organic matter; C, total carbon; AN, available N; AP, available P; AK, available K, and W, soil moisture) for biomass (A, B) and P_N (C, D) in the early (A, C) and later growing (B, D) period. For the sake of clarity, arrows have not been indicated for plant species vectors. Biocrusts-removed site (filled squares); biocrusts-intact site (filled circles).

systems have shown that productivity increases following N input if sufficient water is available (Asner *et al.* 2001).

Negative effects of biocrusts on plant growth in the later part of the growing season might be due to a lack of moisture in crusted soils. Our results showed that soil water content in crusted soils was significantly lower than in all three layers of uncrusted soils during the later part of the growth period (Fig. 6). It is well known that biological activity in desert ecosystems is primarily limited by water and secondly by N during seasons with high evaporation and low rainfall (McCrackin *et al.* 2008). Biologically crusted soils could reduce water throughput to vascular plant rooting zones, where polysaccharide root sheaths absorb water and swell during rainfall events (Kidron & Yair 1997; Eldridge *et al.* 2000). RDA also showed that water was crucial in determining plant growth during this period, relative to nutrients (Fig. 7). Similar results for herbaceous plants in relation to soil moisture and N have previously been found in this area (Zhou *et al.* 2011) and other arid ecosystems of China (Wu *et al.* 2008). Regardless of nutrient levels, water deficit dramatically reduced seedling growth and biomass production. In addition, the current data support the notion that biocrusts impede water infiltration (Coppola *et al.* 2011; Malam Issa *et al.* 2011), increase evaporation (Kidron & Tal 2012) and ultimately degrade soil water conditions, thus affecting plant growth. In contrast, analyses of biocrust effects on vascular plants have also shown that crusts frequently increase the survival and growth of plants (Li *et al.* 2005; Godínez-Alvarez *et al.* 2012). Such positive responses have been documented for several plant species growing in distinct deserts.

We also found that biocrusts affected not only growth of the ephemerals, but also plant community characteristics. Consistent with our results, seedling densities vary profoundly in response to soil surface property changes through the presence of biocrusts (Belnap & Lange 2003). According to our study, seedling densities were higher in crusted soils than in uncrusted soils, which indicates that biocrusts might have better ability to trap seeds. An increasing number of studies have shown that biocrusts affect crust entrapment ability for seeds and also influence seed emergence (Belnap & Lange 2003; Li *et al.* 2005; Godínez-Alvarez *et al.* 2012).

Effect of biocrusts on photosynthesis and chlorophyll fluorescence

Most of the physiological characteristics showed similar trends during growth of the three evaluated species. Few studies have reported physiological changes related by biocrusts during plant growth. The factors that affect plant growth might limit the physiological performance of the studied species. Correlations between growth and physiological responses could provide evidence for this relationship.

Water is the main factor limiting plant growth and physiological responses. Biocrusts play an important role in moderating soil water availability in xeric environments (Belnap & Lange 2003). They may also play an important role in moderating physiological characteristics of plants through affecting water status of the soil surface. In our study, all three tested plant species had higher leaf RWC in uncrusted in comparison to crusted soil, especially in the later growth period (Fig. 1,

Table 1; $P < 0.01$), which reflects the sensitivity of the species to soil water content.

Sensitivity of RWC to soil moisture in all three species reflects a relationship to photosynthesis (Lawlor & Cornic 2002). Soil water content in crusted soils was less than that in uncrusted soils, and the differences were significant in the later part of the growth period (Fig. 6). Biocrust-covered sites posed a water limitation on plants during the later growth period (Fig. 7), and both P_N and g_S of all three species in crusted soils were substantially lower than that in uncrusted soils (Fig. 2). The decrease in P_N in crusted soils with lower water availability was in part due to stomatal closure, which restricts water loss. This was reflected in synchronous changes of P_N and g_S during the entire growth period in both sites (Fig. 2).

Stomatal closure is a protective mechanism against water loss in relatively water-limited conditions (Flexas & Medrano 2002). In our study, plants with stomatal control survived well in crusted soils. Moreover, stomatal response to lower water availability in crusted soils and the lower E and WUE support the notion that plants conserve moisture through effective stomatal control (Flexas & Medrano 2002; Zhang *et al.* 2004). This capacity may be an important physiological mechanism ensuring growth of these species in crusted soils. Previous studies found that WUE can either improve under water limitation (Liu *et al.* 2005) or that species employ a prodigal water-use strategy (Clavel *et al.* 2005). In this study, the tested species employed the latter strategy, WUE declined with the decrease in soil water content at both sites, and values in crusted soils in the later part of the growth period were lower than in uncrusted soils (Fig. 2J–L). This phenomenon might be attributed to low biomass production under lower water conditions in crusted soils.

The intrinsic efficiency of PSII (F_v/F_m) and chlorophyll content of all three species were higher in crusted than in uncrusted soils in the early growth period, and the situation completely reversed in the later part of the growth period (Fig. 3A–F). Lima *et al.* (2002) reported high stability of PSII photochemical efficiency to water limitation in *Coffea robusta* and suggested that reductions in photosynthesis are mainly due to stomatal closure. In our study, lower F_v/F_m in the later part of the growing season in crusted soils might be the result of both a reduction in the photochemical processes and an increase in stomatal limitation (Silva *et al.* 2004). Decreases in chlorophyll content (Fig. 3D–F) in all three species in crusted soils might also cause a decrease of F_v/F_m (Fig. 3A–C), suggesting that chlorophyll breakdown was accompanied by a decrease of the maximum photochemical efficiency of PSII. In our study, there were significantly positive and linear relationships of P_N with chlorophyll content ($R^2 = 0.86$, $P < 0.05$) and F_v/F_m ($R^2 = 0.71$, $P < 0.05$) when pooling data for *E. oxyrrhynchum*, *A. linifolium* and *H. pulchella*. Overall, the negative effects of biocrusts on photosynthesis and chlorophyll fluorescence in the later growth period most likely led to lower biomass in comparison to uncrusted soils.

Effect of biocrusts on osmotic adjustment and antioxidant enzymes

In the present study, plants in crusted soils had significantly higher soluble sugar and proline content than in uncrusted soils in the later part of the season (Fig. 4), suggesting that

plants might suffer stress in biocrust plots; lack of soil moisture might be the most important factor. Soluble sugars, proline and soluble protein play an important role in osmotic adjustment and may protect plants against oxidative stress (Ben Ahmed *et al.* 2009). In our study, RWC was negatively correlated with soluble sugars and proline content, suggesting that these three ephemerals had high capacity for osmotic adjustment, especially when water was limited in the later growth period, and could maintain water absorption in crusted soils (Chaves *et al.* 2003).

Studies have shown that more than half of the enzymes of photosynthesis are soluble proteins (Evans 1989; Andrews *et al.* 1999). Therefore, increases in soluble proteins in the three species in uncrusted soils likely affected photosynthetic activity, and consequently plant growth. In our study, concentrations of soluble protein were correlated with biomass, similar to results in a study of two desert annuals, *Malcolmia africana* and *Bassia hyssopifolia* (Zhou *et al.* 2011).

Superoxide dismutase controls the first threshold of the water–water cycle of the antioxidant system (Zhu *et al.* 2009). It plays a key role in quenching active oxygen (Fu & Huang 2001), catalysing the dismutation of O_2^- into H_2O_2, which is eliminated by POD and CAT. Increased SOD activity is accompanied by increases in POD and CAT activity, indicating high demand for quenching H_2O_2. The higher activity of SOD, POD and CAT in the three desert species in biocrust-covered sites suggests that they were protected from damage caused by drought stress. An intimate relationship between enhanced or constitutive antioxidant enzyme activity in response to water deficiency has been observed in many species (Zhu *et al.* 2009; Shen *et al.* 2010). In the present study, variation in osmotic solute content (proline and soluble sugars) was similar to that of antioxidant enzyme activity (SOD, CAT and POD). Accumulation of proline and soluble sugars can activate antioxidant defence mechanisms (Ben Ahmed *et al.* 2009). Because proline and soluble sugars stabilise the structure and activity of

enzymes (Chaves *et al.* 2003), their higher accumulation in species growing in crusted soils may increase activity of antioxidant enzymes.

CONCLUSIONS

Our hypothesis that biocrusts might stimulate growth and physiological performance of ephemerals in the early growth period and affect plant characteristics in the late growth period through soil moisture availability was confirmed. In the early growth period of all three species there was no significant difference in soil moisture between the two soil cover types. Biocrusts dramatically increased biomass and average leaf area of all three species by increasing nutrient supply. Moreover, P_N, g_S, E, WUE, F_v/F_m and chlorophyll content also increased in crusted *versus* uncrusted soils. Thus, soil moisture availability was not the crucial factor limiting plant growth in this period. However, biocrusts increased evaporation from soil, and low soil moisture then negatively affected plant growth and physiological parameters in the later part of the growing season. Taken together, our results indicate that biocrusts have different effects on the three ephemeral species during the whole growth period in this desert. This result could contribute another explanation for divergent or even opposite effects of biocrusts on plant growth in different areas.

ACKNOWLEDGEMENTS

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