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Response of desert biological soil crusts to alterations in precipitation frequency

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Abstract Biological soil crusts, a community of cyanobacteria, lichens, and mosses that live on the soil surface, occur in deserts throughout the world. They are a critical component of desert ecosystems, as they are important contributors to soil fertility and stability. Future climate scenarios predict alteration of the timing and amount of precipitation in desert environments. Because biological soil crust organisms are only metabolically active when wet, and as soil surfaces dry quickly in deserts during late spring, summer, and early fall, the amount and timing of precipitation is likely to have significant impacts on the physiological functioning of these communities. Using the three dominant soil crust types found in the western United States, we applied three levels of precipitation frequency (50% below-average, average, and 50% above-average) while maintaining average precipitation amount (therefore changing both timing and size of applied events). We measured the impact of these treatments on photosynthetic performance (as indicated by dark-adapted quantum yield and chlorophyll *a* concentrations), nitrogenase activity, and the ability of these organisms to maintain concentrations of radiation-protective pigments (scytonemin, beta-carotene, echinenone, xanthophylls, and canthaxanthin). Increased precipitation frequency produced little response after 2.5 months exposure during spring (1 April–15 June) or summer (15 June–31 August). In contrast, most of the above variables had a large, negative response after exposure to increased precipitation frequency for 6 months spring–fall (1 April–31 October) treatment. The crusts dominated by the soil lichen *Collema*, being dark and protruding above the surface, dried the most rapidly, followed by the dark surface cyanobacterial crusts (*Nostoc-Scytonema-Microcoleus*), and then by the light cyanobacterial crusts (*Microcoleus*). This order reflected the magnitude of the observed response: crusts dominated by the lichen *Collema* showed the largest decline in quantum yield, chlorophyll *a*, and protective pigments; crusts dominated by *Nostoc-Scytonema-Microcoleus* showed an intermediate decline in these variables; and the crusts dominated by *Microcoleus* showed the least negative response. Most previous studies of crust response to radiation stress have been short-term laboratory studies, where organisms were watered and kept under moderate temperatures. Such conditions would give crust organisms access to ample carbon to respond to imposed stresses (e.g., production of UV-protective pigments, replacement of degraded chlorophyll). In contrast, our longer-term study showed that under field conditions of high air temperatures and frequent, small precipitation events, crust organisms appear unable to produce protective pigments in response to radiation stress, as they likely dried more quickly than when they received larger, less frequent events. Reduced activity time likely resulted in less carbon available to produce or repair chlorophyll *a* and/or protective pigments. Our findings may partially explain the global observation that soil lichen cover and richness declines as the frequency of summer rainfall increases.

Keywords Arid lands · Carbon · UV pigments · Climate change · Radiation stress

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Introduction

Biological soil crusts (BSCs), composed primarily of photosynthetic cyanobacteria, algae, lichens, and mosses, cover plant interspaces in relatively undisturbed areas and thus can constitute 70% or more of the living ground cover in these sparsely-vegetated regions (Belnap 1995). BSCs

play a key role in many ecosystem functions of semi-arid and arid ecosystems around the world (Belnap and Lange 2003), including soil fertility and soil stability. BSCs can be the dominant source of nitrogen (N) in deserts (Evans and Ehleringer 1993). As 5–70% of this fixed N can be released immediately, BSCs can be an important source of N for associated organisms that include vascular plants and other microbes (reviewed in Belnap et al. 2003). BSCs also fix substantial amounts of carbon (C; Evans and Lange 2003), increasing total surface soil C by up to 300% (reviewed in Belnap et al. 2003). This addition appears to benefit the often C-limited soil biota, especially in the interspaces between vascular plants (Belnap 2003a). BSCs secrete exopolymers that help prevent nutrient losses via leaching and concentrate plant-essential nutrients such as sodium (NaCl), potassium, magnesium, calcium, manganese, iron (Fe), nickel (Ni), copper (Cu), and zinc (Zn). They secrete powerful metal chelators that maintain metals in bio-available forms; peptide N and riboflavin which help keep phosphorus (P), Cu, Zn, Ni, and Fe plant-available; glycollate, which stimulates P uptake; and various other factors that stimulate growth such as B_{12} and auxin-like substances. BSCs are also important in trapping nutrient-rich dust (Verrecchia et al. 1995) and in reducing both wind and water erosion (Belnap 2003b; Warren 2003).

Biological activity in arid and semi-arid ecosystems is determined primarily by the size, frequency, and timing of precipitation pulses (Noy-Meir 1973). Because BSCs are metabolically active only when wet and their physiological functions are also highly responsive to temperature (Lange 2003; Lange et al. 1998; Tuba et al. 1996; Nash 1996), changes in precipitation characteristics are expected to have especially profound consequences for the physiological functioning of BSCs. Summer and early fall can be an especially stressful time for BSC organisms. Soil surface temperatures greater than 40°C are typical and are supraoptimal for photosynthetic activity (Lange 2003). Radiation is high, and times of soil wetness are infrequent and short. Rehydration of BSC organisms results in immediate C losses via cells bursting upon rewetting (Farrar 1973), respiration (Lange 2003), and membrane leakage (reviewed in Belnap et al. 2003). If hydration periods are too short, photosynthetic gains cannot compensate for these losses (Jeffries et al. 1993). Short hydration periods often occur in semi-arid lands, as most precipitation events are less than 3 mm (Sala and Laurenroth 1982, Loik et al., in press). Without sufficient C, BSC organisms likely lack the ability to perform basic maintenance and repair functions. Various crust species have characteristic responses to precipitation and temperature, resulting in different C gain among species under the same environmental conditions (reviewed in Lange 2003).

Three dominant crust types occur in southwest United States deserts. “Light” cyanobacterial BSCs are dominated by the cyanobacterium *Microcoleus vaginatus*. They occur in areas of very low rainfall (e.g., hyperarid and hot deserts) or in any desert where high disturbance levels prevent establishment of lichens and mosses. *Microcoleus* mostly lacks UV-protective pigments and is large and mobile. It generally resides below the soil surface, gliding upwards into the photosynthetic zone only when soils are wet and returning to depth as soils dry. “Dark” cyanobacterial BSCs are dominated by the cyanobacteria *Scytonema myochrous*, *Nostoc commune*, and *M. vaginatus*. Dark BSCs occur in hot and cool deserts where either precipitation or soil stability limits lichen development but where disturbance is low. *Scytonema* and *Nostoc* are small and relatively immobile species that reside on the soil surface and thus require heavy UV-protective pigmentation to prevent radiation damage.

The third type of BSC has a significant lichen and/or moss component, with the dominant lichen species most often either *Collema tenax* or *C. coccophorum*. Lichen-moss crusts occur in small patches in hot deserts and extensively in cool deserts (e.g., Colorado Plateau, northern Great Basin) on soils where disturbance is low to absent or where recovery times have been substantial. Because lichens protrude above the soil surface, they experience the most intense radiation exposure, while also drying the most rapidly among the three crust types. Organisms in dark crusts experience slightly less radiation, and as they are embedded in the soil, dry more slowly than the lichens. The organisms in light crusts experience the least radiation and also dry the most slowly among the crust types.

Because BSCs are such an essential part of desert ecosystems, there has been concern regarding the effect of future climate change on their species composition and physiological functioning. Future climate scenarios for the arid southwestern United States predict an increase in temperature and alteration of precipitation timing, intensity, and interannual variability (Schlesinger et al. 1990). However, how much or in what direction precipitation will change is a matter of debate (Weltzin and McPherson 2003). As BSCs are only metabolically active when wet, any alteration in precipitation patterns is likely to profoundly affect their physiological functioning (e.g., C and N fixation) and their response to stress (e.g., production of UV-protective pigments). In addition, species within a biological crust are expected to show a differential response to climate changes. This could lead to alterations in the BSC flora, which in turn would alter the influence of the BSC on a given ecosystem. Based on the above information, we designed an experiment that altered precipitation frequency without changing total precipitation amounts. We predicted that (1) BSCs experiencing more frequent but shorter hydration periods would experience C deficits; (2) that these C deficits would be reflected in reduced production of radiation-protective pigments; and (3) based on differential drying times and radiation exposure, *Collema* BSCs would be more impacted than dark BSCs, and dark BSCs more impacted than light BSCs.

Materials and methods

Crust samples were field-collected from a 20-ha area near Moab, Utah, during the last week of March 1999 (for the spring and spring-fall treatment) and during the second week of June (for the summer treatment). Four types of samples were collected: sterilized sand (controls) and light, dark, and *Collema* BSCs. Samples were exposed to treatments for either 2.5 months in the spring (1 April–15 June, hereafter referred to as "spring"), 2.5 months in the summer (15 June–31 August, hereafter referred to as "summer") or 6 months from 1 April–31 October (hereafter referred to as "spring-fall"). Each sample was collected in a tube 18 mm in diameter and 60 mm deep (to avoid any water pooling during the experiment). The ten replicates per treatment were placed on trays on top of 1.5-m-tall tables, above which a single layer of UV-transparent film (0.04-mm-thick Aclar Type 22A, Honeywell Specialty Films, Pottsville, Pa.) was suspended 40 cm above the experimental material and sloped slightly to the south so rain would run off. Shelter sides and ends remained open to maximize air movement. Photosynthetically-active radiation was measured under the shelter and was found to be 10% below ambient levels.

Our precipitation treatments were based on a 30-year record of daily precipitation amounts and timing. All samples received equal amounts of precipitation (the 30-year average). However, we applied three levels of precipitation frequency: 50% below average, average, and 50% above average of the 30-year record (Fig. 1). Therefore, our "average" treatment received the 30-year precipitation average in both amount and frequency. Because the total amount of precipitation was held constant per event while the frequency of events was varied, precipitation amounts were highest in the low frequency treatment (6–10 mm per watering event), intermediate in the average frequency treatment (3–6 mm per event) and lowest in the high frequency treatment (~2 mm per event). Though this method does not incorporate all the variability possible in this region, our individual rainfall events varied in both amount and the time between rainfall events in a way that simulated "average" natural variability. Samples were watered midday, regardless of cloud condition. This meant that many

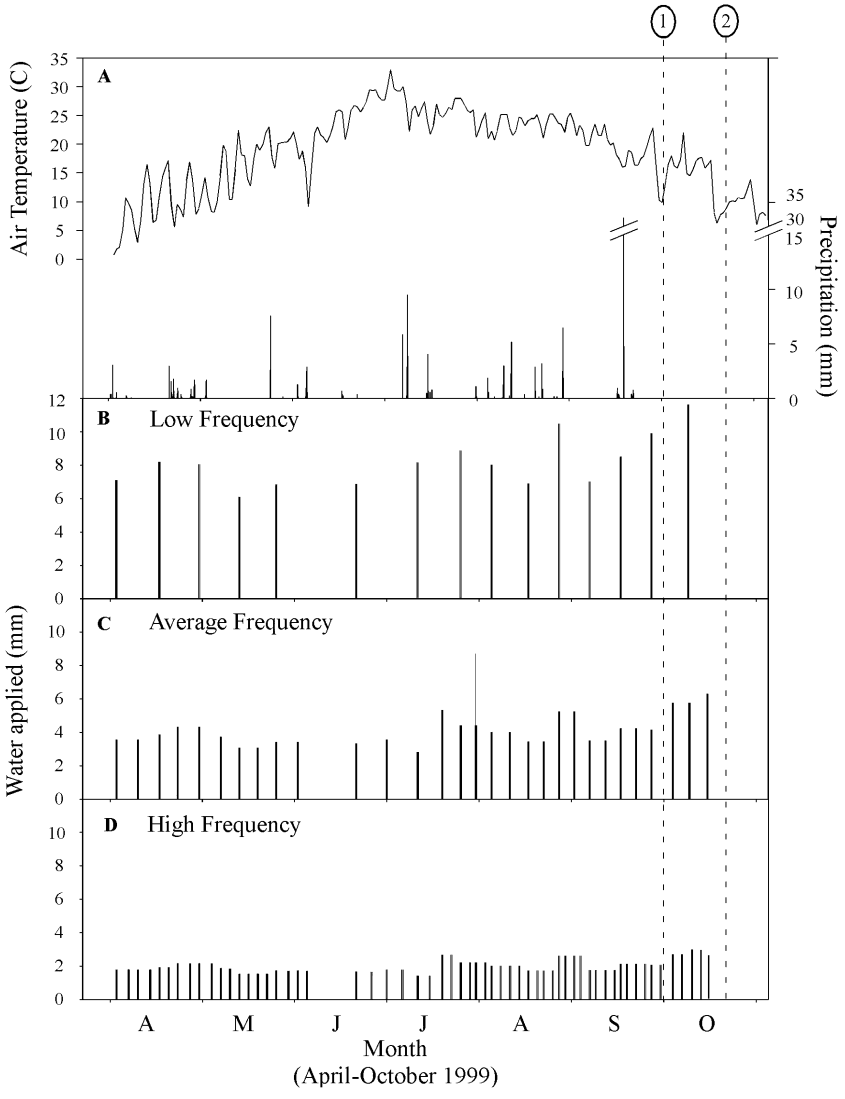

Fig. 1 **A** Natural precipitation and air temperatures at the study site; **B** timing and amount of low frequency precipitation treatment; **C** timing and amount of average precipitation frequency treatment; **D** timing and amount of high frequency precipitation frequency treatment. *Dotted lines* compare the times of sample collection for: *1* Bowker et al. 2002 study, post spring-fall; *2* this study, post spring-fall

Table 1 Values obtained for different response variables. Measurements were made at the end of the three seasonal treatments (post spring, post summer, and post spring-fall). Different letters denote significant differences ($P < 0.05$)

Crust	Season	Precip. frequency	Quantum yield Fv/Fm Mean ± SE	Chlorophyll *a* µg/g soil Mean ± SE	Scytonemin µg/g soil Mean ± SE	Xanthophylls µg/g soil Mean ± SE	Canthaxanthin µg/g soil Mean ± SE	Echinenone µg/g soil Mean ± SE	Beta-carotene µg/g soil Mean ± SE	N fixation rate nmol ethylene/m^2/h * 100 Mean ± SE
Light Crust	Spring	High	0.31±0.04	3.31±0.48	8.3±42	0.62±0.13	0.14±0.02	0.28±0.05	0.28±0.06	0.5±0.5
		Average	0.37±0.04	5.43±1.73	5.3±1.1	0.92±0.24	0.17±0.04	0.45±0.13	0.43±0.15	0.0±0.0
		Low	0.33±0.08	5.39±1.10	7.3±4.1	1.01±0.15	0.17±0.01	0.44±0.06	0.52±0.12	0.5±0.5
	Summer	High	0.41±0.05	7.57±0.86	21.8±3.7	0.59±0.20	0.17±0.02	0.54±0.06	0.58±0.10	4.1±1.7
		Average	0.39±0.06	6.30±1.62	24.9±6.5	0.25±0.15	0.13±0.03	0.43±0.11	0.57±0.20	7.5±3.5
		Low	0.44±0.06	6.22±1.33	16.3±4.3	0.61±0.26	0.12±0.02	0.45±0.12	0.56±0.17	2.8±1.4
	Spring thru fall	High	0.46±0.05 ab	4.16±0.65	5.7±0.8	0.31±0.11	0.10±0.03 ab	0.31±0.07	0.28±0.07 a	0.0±0.0
		Average	0.37±0.08 a	4.11±0.83	19.8±5.9	0.13±0.10	0.06±0.02 a	0.21±0.07	0.22±0.10 a	1.4±66.9
		Low	0.52±0.05 b	6.13±1.17	12.7±2.3	0.47±0.19	0.16±0.03 b	0.54±0.14	0.64±0.16 b	1.5±51.5
Dark Crust	Spring	High	0.18±0.05	14.98±1.45	124.9±23.3	3.05±0.54 ab	0.61±0.08	1.26±0.09	2.27±0.23	1.4±0.8
		Average	0.25±0.05	13.19±2.65	118.9±23.0	2.32±0.50 ab	0.62±0.12	1.11±0.20	1.79±0.35	11.3±5.5
		Low	0.26±0.06	14.88±1.88	116.1±14.8	4.16±0.52 b	0.78±0.09	1.39±0.17	2.04±0.31	16.4±14.3
	Summer	High	0.36±0.04	21.81±4.30	104.1±11.1	0.58±0.42	0.68±0.11	1.47±0.30	1.95±0.38	23.2±21.9
		Average	0.35±0.02	20.14±2.17	101.0±18.0	1.92±0.68	0.70±0.07	1.41±0.17	1.76±0.25	58.2±56.6
		Low	0.38±0.04	21.65±2.96	90.4±10.8	2.07±0.66	0.84±0.12	1.63±0.26	2.08±0.44	4.0±3.9
	Spring thru fall	High	0.35±0.03 a	10.26±1.97 a	149.0±8.8	0.00±0.00	0.45±0.08	0.61±0.14 a	0.78±0.27 a	136.2±82.4
		Average	0.34±0.03 a	10.79±1.41 a	140.3±18.7	0.71±0.37	0.38±0.07	0.74±0.11 a	1.05±0.21 a	43.7±34.4
		Low	0.44±0.03 b	19.43±1.67 b	190.1±20.8	0.89±0.42	0.62±0.06	1.39±0.13 b	2.21±0.31 b	0.0±0.0
Collerma	Spring	High	0.23±0.04	19.01±2.00	91.3±18.8	3.43±0.65	1.14±0.13	1.31±0.16	1.41±0.20	6.3±4.6
		Average	0.18±0.02	17.43±2.98	98.5±16.4	4.60±0.96	1.00±0.18	1.23±0.17	1.28±0.30	1.5±0.8
		Low	0.23±0.06	13.86±1.83	75.9±10.5	3.44±0.66	0.82±0.12	0.89±0.14	1.33±0.41	64.8±45.1
	Summer	High	0.27±0.05	38.69±4.40	213.0±36.8	3.05±1.41	1.96±0.20	2.36±0.28	2.15±0.44	6.2±2.6
		Average	0.28±0.03	39.29±5.19	195.4±33.5	4.08±1.26	1.93±0.26	2.56±0.36	1.99±0.27	11.8±6.5
		Low	0.34±0.03	34.69±2.38	148.2±26.6	2.98±0.99	1.76±0.15	2.06±0.15	1.50±0.21	28.3±22.3
	Spring thru fall	High	0.33±0.03 a	9.26±1.95 a	100.2±12.5	0.42±0.21	0.38±0.08 a	0.52±0.11 a	0.66±0.23 a	201.7±79.9
		Average	0.41±0.03 b	14.41±2.90 ab	122.2±14.3	1.27±0.56	0.59±0.12 ab	0.97±0.23 ab	1.04±0.24 ab	97.6±48.7
		Low	0.44±0.03 b	19.02±2.69 b	126.5±15.2	1.26±0.40	0.76±0.09 b	1.39±0.23 b	1.88±0.41 b	72.4±48.6

times crusts were watered under clear sky, unlike the conditions prevailing under natural rainfall. Thus, samples dried more quickly on average and, for a given amount of rainfall, experienced slightly shorter intervals under wet conditions. Air temperatures and natural rainfall during the experimental time were recorded (Fig. 1).

At the conclusion of the experimental treatments, the effects of increasing precipitation frequency (increasing from low to either average or high frequency) were quantified in the lab. Samples were preconditioned under dark and wet (1 mm precipitation equivalent) for 12 h (Lange et al. 1998). Dark-adapted quantum yield (hereafter referred to as "quantum yield") was assessed with a PAM-2000 pulse amplitude fluorometer (Walz, Germany), using the saturation pulse method (Bilger et al. 1995) at light levels of <25 μmol/m^2/s. Samples were maintained at 1 mm precipitation equivalent during measurements, with three measurements taken per sample. Wet samples were then placed in the light for 3 h. Nitrogenase activity was measured using the acetylene-reduction method (Belnap 2002). Samples were incubated for 4 h in the light at 26°C and analyzed with a Shimadzu FID gas chromatograph equipped with a 2.4-m, 8% NaCl on alumina column, using helium as the carrier gas (30 ml min^{-1}). Simultaneous calibrations with ethylene standards were done.

The top 2 mm of the samples were the analyzed for pigment concentrations, using quantitative and qualitative HPLC analysis on acetone-extracted samples (Karsten and Garcia-Pichel 1996). Concentrations for all pigments were quantified using peak areas integrated from photodiode array data at 436 nm and compared to commercially obtained standards. Because a scytonemin standard was not commercially available, scytonemin was quantified using its peak area at 436 nm and a modification of its extinction coefficient of 112.6 l g^{-1} cm^{-1} at 384 nm (Garcia-Pichel et al. 1992). An extinction coefficient of 60.8 l g^{-1} cm^{-1} for 436 nm was used. Data were analyzed using Millenium^{32}software (Waters, United States). The xanthophylls zeaxanthin, lutein, and myxoxanthophyll were grouped (hereafter referred to as xanthophylls) on the basis of similar function, absorbence spectra, retention times, and difficulty of distinguishing between lutein and zeaxanthin.

Data normality was tested using the Shapiro-Wilk test. Most data were normal, and if not, were transformed. The effects of precipitation frequency were determined using an Independent *t*-test or an ANOVA, with Tukey HSD employed to determine differences between means. However, values for N fixation could not be transformed to conform to normality assumptions. Therefore, these data were compared with either a Mann-Whitney U (for comparing two variables) or the Kruskal-Wallis H test (for comparing three variables). Kruskal-Wallis H tests were followed with Dunnett's T-3 tests to determine differences between means. A Wilcoxin sign-rank test was used to compare across all treatments, all crust types, and/or all seasons. Significant results are reported at P <0.10, 0.05, and 0.01. Results for the higher P value were also presented to evaluate the data under reduced risk of type II error in a more exploratory approach.

Results

As expected, sterile sand lacked nitrogenase activity, quantum yield, and pigments, regardless of season or precipitation treatment (data not shown). Among crust types and within each season, light BSCs generally had the least number of variables respond to the increased precipitation frequency treatment, dark BSCs had an intermediate number respond, and *Collema* had the greatest number respond (Tables 1, 2). When all response variables and seasons were combined, light and dark BSCs had a similar number of variables decline with the increased precipitation frequency treatment, whereas *Collema* again had more variables decline than either of the cyanobacterial BSCs. Percent change for most variables was quite large, with a few exceptions (Table 2).

Response to the precipitation frequencies within a given precipitation frequency was different in the different seasons. After the spring treatment, most response variables tended to increase. After the summer treatment, most variables showed a mixed decline. However, after the spring-fall treatment, almost all variables were significantly reduced (Table 1). The exception was the xanthophylls, which consistently declined after all seasons. When low frequency treatments were compared to average or high frequency treatments, most response variables declined (Tables 1, 3). When the data from all seasons are compared, quantum yield, beta-carotene, and myxoxanthophyll significantly declined with the increased precipitation frequency treatment in all three BSC types (Table 2). The other pigments, with the exception of scytonemin and chlorophyll *a*, tended to have lower values with the increased precipitation frequency treatment in 6–7 of the 9 crust/season combinations. All three BSC types had the most variables decline with the increased precipitation frequency treatment in the longer spring-fall trial relative to the shorter spring and summer treatments (Table 3). As with the comparisons within crust types, the percent changes observed were generally large.

Discussion

Differential response of crust types to increasing precipitation frequency

Late spring, summer, and early fall are times of high radiation and UV exposure for organisms on the soil surface. When light hits the soil surface, part of the light is reflected and the rest penetrates into the soil (Fig. 2). Therefore, both reflected and incoming light are present in the zone at and just above the soil surface. Organisms that occur in this zone are accordingly subjected to radiation levels higher than incident radiation. Once within the soil, light is subject to intense attenuation due to the high

Table 2 Percent difference in response variables when different harvest times were compared (post spring/post summer, post summer/post spring–fall, and post spring/post spring–fall; ~ indicates one value was zero, therefore percent change could not be calculated)

Time period	Crust type	Precip. frequency	Quantum yield % Change	Chlorophyll *a* % Change	Scytonemin % Change	Xanthophylls % Change	Canthaxanthin % Change	Echinenone % Change	Beta-carotene % Change	N fixation rate % Change	Wilcoxin *P*
% Change spring to summer	Light crust	High	32**	128**	162**	–5	22	93**	105**	652	0.03**
		Average	5	16	370***	–73**	–23	–5	32	~	0.33
		Low	32	15	122**	–40	–28	2	8	481	0.26
	Dark crust	High	99**	46	–17	–81	13	17	–14	1,607	0.89
		Average	40*	53***	–15	–17	14	26	–1	415	0.58
		Low	46	46	–22	–50*	8	17	2	–76	0.99
	Collema	High	21	104***	133**	–11	73**	81**	53	–2	0.05**
		Average	56**	125***	98*	–11	93***	108***	55	682	0.04**
		Low	48*	150***	95*	–14	115***	131***	13	–56	0.12
% Change spring to fall	Light crust	High	45*	26	–32	–51	–29	11	0	–100	0.67
		Average	–1	–24	273***	–85***	–65**	–53	–48	~	0.26
		Low	55**	14	74*	–54	–5	24	25	211*	0.12
	Dark crust	High	96**	–32**	19	–100***	–26	–52**	–66***	9,925	0.58
		Average	36	–18	18	–69**	–38	–34	–41	287	0.40
		Low	70**	31	64**	–79***	–20	0	8	–100	0.33
	Collema	High	45*	–51***	10	–88***	–67***	–60***	–53**	3,092**	0.48
		Average	128***	–17	24	–72***	–41**	–21	–19	6,361	0.58
		Low	93**	37	67**	–63**	–7	56**	41	12	0.12
% Change sommer to fall	Light crust	High	10	–45**	–74***	–48	–42	–42*	–51*	–100	0.03**
		Average	–5	–35	–21	–45	–55	–51	–61	–81	0.01**
		Low	18	–2	–22	–23	32	22	15	–46	0.67
	Dark crust	High	–2	–53*	43**	–100	–35	–59*	–60	487	0.48
		Average	–3	–46***	39	–63	–46***	–47**	–41**	–25	0.16
		Low	16	–10	110***	–57	–26	–14	6	–100	0.48
	Collema	High	30	–76***	–53**	–86	–81***	–78***	–69**	3,147*	0.09*
		Average	46***	–63***	–37	–69**	–70***	–62***	–48**	726	0.04**
		Low	31**	–45***	–15	–58	–57***	–32**	25	156	0.09*
Wilcoxin *P*	Spring to summer		0.01**	0.01**	0.31	0.01**	0.04**	0.02**	0.09*	0.37	
	Spring to fall		0.01**	0.52	0.01**	0.01**	0.01**	0.26	0.17	0.05*	
	Summer to fall		0.05**	0.01**	0.68	0.01**	0.01**	0.01**	0.09*	0.37	

* P <0.10, ** P <0.05, *** P <0.01

Table 3 Percent differences in response variables when different precipitation frequency treatments are compared (low/average frequency and low/high frequency)

Crust	Season	Precip. frequency Change	Quantum yield % Change	Chlorophyll *a* % Change	Scytonemin % Change	Myxoxanthophylls % Change	Canthaxanthin % Change	Echinenone % Change	Beta-carotene % Change	N fixation rate % Change	Wilcoxin *P*
Light crust	Spring	Low to ave.	12	1	–28	–9	–1	2	–16	–100	0.40
		Low to high	–6	–39	13	–39*	–17	–37*	–45	11	0.16
	Summer	Low to ave.	–12	1	53	–60	6	–4	2	167	0.58
		Low to high	–6	22	34	–3	40	20	4	44	0.09*
	Spring thru fall	Low to ave.	–28*	–33	55	–71	–64**	–61*	–65	–8	0.16
		Low to high	–12	–32	–55**	–35	–38	–43	–56	–100**	0.01**
Dark crust	Spring	Low to ave.	–4	–11	2	–44**	–21	–20	–13	–31	0.16
		Low to high	–31	1	8	–27	–22	–9	11	–92	0.78
	Summer	Low to ave.	–7	–7	12	–7	–16	–14	–15	1357	0.58
		Low to high	–6	1	15	–72	–18	–10	–6	481	0.99
	Spring thru fall	Low to ave.	–23***	–44***	–26	–20	–38**	–47***	–53**	~	0.05**
		Low to high	–20***	–47**	–22	–100*	–28	–56***	–65**	~	0.07*
Collema	Spring	Low to ave.	–21	26	30	34	22	38	–3	–98	0.16
		Low to high	0	37*	20	0	39*	46*	6	–90	0.20
	Summer	Low to ave.	–16*	13	32	37	10	24	32	–58	0.04**
		Low to high	–19	12	44	2	12	15	43	–78	0.07*
	Spring thru fall	Low to ave.	–6	–24	–3	1	–23	–31	–44	35	0.07*
		Low to high	–25***	–51**	–21	–66*	–51***	–63***	–65**	179	0.09*
Wilcoxin *P*		Low to ave.	0.03**	0.44	0.26	0.44	0.52	0.37	0.09*	0.86	
		Low to high	0.01**	0.68	0.59	0.02**	0.52	0.31	0.31	0.77	

* $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$

density of mineral and biogenic particles, with shorter wavelengths penetrating less deeply than longer wavelengths. Thus, organisms just above the soil surface (e.g., lichens such as *Collema*) receive the most radiation, while organisms at or just under the soil surface (e.g., cyanobacteria in dark BSCs) receive somewhat less radiation. Organisms deeper in the soil (e.g., *Microcoleus* in light BSCs) receive the least radiation. Drying times, and therefore activity times, of these organisms would be expected to follow radiation levels: *Collema* BSCs would have the least activity time, dark BSCs a bit more activity time, and light BSCs the most activity time.

Studies show exposure to both UV and radiation degrades internal cellular structures, including photosynthetic machinery and protective pigments (reviewed in Castenholz and Garcia-Pichel 2000). There are three general strategies crust organisms can use to avoid this damage: avoidance, repair of cellular damage, and/or production of radiation-protective pigments (Castenholz and Garcia-Pichel 2000). Avoidance requires a vertical migration from the soil surface (Garcia-Pichel and Pringault 2001) and so is only available to relatively large, mobile organisms such as the large filamentous cyanobacteria *Microcoleus*. The light BSCs used in this study were heavily dominated by *Microcoleus*. This species does not synthesize many pigments for radiation protection. Instead, it avoids high radiation exposure by residing below the soil surface and gliding to the surface only when soils are sufficiently moistened for photosynthesis (Garcia-Pichel and Pringault 2001). When surface soils begin to dry *Microcoleus* glides down out of the high radiation zone (Garcia-Pichel and Belnap 1996).

In contrast, organisms in dark and *Collema* BSCs rely on repair and protective pigments to cope with radiation stress. Dark crusts are a mixture of small, relatively non-motile cyanobacteria such as *Nostoc* and *Scytonema* that cannot seek refuge like *Microcoleus*. In order to obtain enough light for photosynthesis, these organisms occur on and just below the soil surface. However, their occurrence at the soil surface requires the manufacture of copious amounts of UV-protective pigments and polysaccharides for protection from radiation exposure. Similarly, the lichen *Collema* occurs above the soil surface and is completely immobile. The darkly-colored fungal tissue offers some protection to the photobiont embedded within it (Dodds 1989; Büdel et al. 1997).

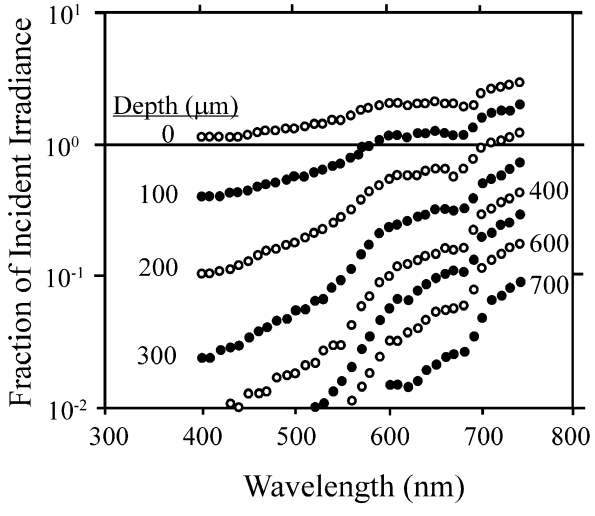

Fig. 2 Spectral attenuation: each spectrum shows the fraction of incident radiation in the soil at various depths (given in μm). *Filled and empty circles* are alternated to aid in reading the figure (adapted from Garcia-Pichel and Belnap 1996)

Repair of radiation damage consists of restoring PSII elements, repairing DNA damage, and/or replacing bleached chlorophylls and antenna pigments (Cameron 1960). Protection by pigments requires synthesizing compounds that either screen incoming radiation (Garcia-Pichel and Belnap 1996; Garcia-Pichel and Castenholz 1991) or quench intracellularly generated free radicals (Adams et al. 1993). Both of these processes require carbon and thus positive photosynthetic gain during most of the time when they are employed. Protective pigments are split into three groups: scytonemins, mycosporine-like amino acids (MAAs), and carotenoids. Scytonemin is dark orange and is found in the polysaccharide sheaths of terrestrial cyanobacteria. It is a passive protector, absorbing UV-A (320–400 nm) and UV-C (190–280 nm; Garcia-Pichel and Belnap 1996) before it enters the cell. Generally, MAAs are intercellular (Garcia-Pichel and Castenholz 1991), absorb at a wide range of wavelengths (Cameron 1960), and provide significant UV protection to DNA (Castenholz and Garcia-Pichel 2000). Carotenoids and xanthophylls (echinenone, canthaxanthin, beta-carotene) protect cells from lethal photooxidation via singlet oxygen (Karsten et al. 1998) and are concentrated mainly in thylakoid membranes, cell membranes and cell walls of cyanobacteria (Häder 1997). These compounds are considered a second-tier defense against photooxidative damage, as they act only after the radiation has entered the cell.

While we do not have complete information on how moisture affects photosynthesis in individual BSC species, the data we do have show that there is a wide variety of responses. The moisture levels at which organisms become photosynthetically active (the moisture compensation point) are highly variable. *Collema* has a very high moisture compensation point (0.22 mm water content), cyanobacterial BSCs from the Negev and the Colorado Plateau an intermediate moisture compensation point (0.1–0.2 mm water content), and other soil phycolichens a quite low moisture compensation point (0.04 mm water content; Lange 2003; Belnap et al., unpublished). Therefore, *Collema* requires 2–5 times more moisture for activation than these other species. On the other hand, the gelatinous *Collema* has a greater water-holding capacity than the other species. *Collema* and dark BSCs show low C fixation at <20% and >60% water content. The net compensation point, where sufficient C fixation occurs to compensate for respiratory losses that begin immediately with wetting, is reached in 30–60 min for *Collema* at optimal water content (Lange et al. 1998), whereas cyanobacterial BSCs can take just over 10 min at optimal

water content to reach their net compensation point (Jeffries et al. 1993; Belnap et al., unpublished).

Combining the differences in radiation exposure, activity times, physiological responses and behavior among the three BSC types, we expected *Collema* BSCs to be the most affected by our increased precipitation frequency treatments, dark BSCs the next most affected, and light BSCs the least affected. This was corroborated by our results (Tables 1, 2, 3). Therefore, significant changes in precipitation characteristics may lead to changes in species composition. This alteration, especially when lichens are replaced with cyanobacteria, will have many profound effects on ecosystem function, as lichens fix more nitrogen and C, better stabilize soils, and support more complex and diverse soil food webs than cyanobacteria (Belnap and Eldridge 2003). In addition, these findings may partially explain why globally it has long been noted that the cover and species richness of lichens decline as amounts of summer precipitation increase. Summer rains may be an advantage only to BSC organisms that can reach net compensation points quickly upon hydration due to short moisture times (e.g., cyanobacteria), whereas species which take longer (e.g., lichens) may suffer C deficits with summer rains.

Effects of increasing precipitation frequency on carbon balances

Most previous studies among a wide variety of cyanobacterial taxa from varying habitat types show that protective pigment production increases with radiation exposure (Neinow et al. 1988; reviewed in Castenholz and Garcia-Pichel 2000). However, manufacturing protective pigments comes at a cost. MAAs in *Nostoc commune* may be 10% of the colony dry weight (Robinson et al. 2000) and scytonemin can be 15% of cyanobacterial dry biomass (Büdel et al. 1997). In addition, pigments are constantly degrading and need replacement. Therefore, times of manufacture must also be times of high and positive C balance. However, incoming solar radiation and subsequent radiation-induced damage to PSII (as indicated in this study by quantum yield) and photosynthetic pigments (as indicated in this study by chlorophyll *a*) peaks during summer. This is the same time period when low soil moisture and thus limited activity time reduces the ability of BSC organisms to maintain a positive C balance.

Most previously reported studies examining the effect of radiation on BSC organisms have been short-term (i.e., days or weeks) and most importantly, conducted in the laboratory under water and temperature regimes favorable for positive C balances. Our spring short-term exposure also occurred when temperatures, and thus water relations, were generally favorable for a positive C balance. Similar to the previous studies, we also observed an increase in pigment concentrations, regardless of precipitation treatment. In contrast, our longer-term exposure (spring-fall) included many days of high temperatures and water stress that likely resulted in a negative C balance. Under these conditions we observed dramatic declines in pigment production despite the high radiation levels present during this time. Increased precipitation frequencies also resulted in large declines in quantum yield and chlorophyll *a* concentrations, indicating that photosynthetic efficiency was also being reduced. Because our rainout shelters reduced radiation loads by about 10%, the effects we observed are likely even more pronounced under natural conditions.

It is the interplay of many factors (amount of precipitation, air temperature, length of time with sufficient light, and organism characteristics) that determines whether an individual crust type experiences C loss or gain during a given precipitation event. Our treatments ranged from ~2 mm (high frequency) to 6–10 mm (low frequency) per watering event. Monitoring of soil moisture at a nearby field site during the study time showed that, due to high air temperatures, events less than 3 mm often resulted in soils being wet for less than 30 min. In addition, during much of this time, the study organisms experienced high air temperatures, high radiation loads, and water contents less than optimal for photosynthesis. Therefore, we hypothesize that the increased precipitation frequency treatments during our long spring-fall exposure resulted in significant C losses for all three BSC types. Limited C would require allocation choices between maintenance, repair, and/or production of tissues required for C acquisition (e.g., PSII, chlorophyll *a*) and that of protective pigments. Under these conditions, it is likely that photosynthate would first be allocated towards insuring C acquisition. Only after that was accomplished, would resources be allocated towards protective pigments, despite the on-going exposure of the organism to high radiation levels. This scenario best explains the results of this experiment.

There are multiple lines of evidence to support our hypothesis that our experimental material suffered C losses under the increased precipitation frequency treatment. First, after seasons when both quantum yield and chlorophyll *a* values did not change or increased (post-spring, post-summer), most pigments did not change or increased as well. In contrast, when quantum yield and chlorophyll *a* declined (post spring-fall), production of almost all other pigments also declined. The second piece of evidence that the C balance of BSCs influences their ability to produce radiation-protective pigments comes from a field-based experiment conducted near our study site at the same time as this study (Bowker et al. 2002). Light and dark crusts in both experiments were harvested at approximately the same time for the post spring (June) and post spring-fall (October) treatments. In contrast to our results, Bowker et al. (2002) observed increases in the quantum yield and concentrations of chlorophyll *a*, scytonemin, myxoxanthophyll, canthaxanthin, and echinenone post spring-fall compared to post spring. Different C availability likely explains the different results between these two studies. The Bowker et al. (2002) study samples received natural rainfall that was 78% above-average in amount and slightly above-average in frequency. Greater

moisture availability would have meant longer activity times and greater C gain.

Thirdly, the spring-fall increased precipitation frequency treatment had a greater number of pigments decline, with larger declines, than the shorter spring or summer increased precipitation frequency treatment. As temperatures and incoming solar radiation were higher in spring and summer compared to fall, these greater declines were likely due to greater accumulated C losses from longer exposure to the stress of increased precipitation frequency treatment. As with the other comparisons discussed above, the greater declines in quantum yield and chlorophyll *a* were accompanied by greater declines in protective pigment production. It could be argued that pigments declined because fall was a time of less radiation stress. However, quantum yield and chlorophyll *a* declined as well, indicating the organisms were under stress. In addition, the Bowker et al. (2002) study did not see such a decline after fall, despite falling radiation levels. Fourthly, rewetting is associated with leakage of C, N, and electrolytes (Kieft et al. 1987). Thus, organisms that are wetted more often are likely to lose more C than those receiving less frequent precipitation.

There is one additional line of evidence to support our hypothesis. *Collema* occurs in the reflected light zone and receives more radiation stress than any organisms in the cyanobacterial BSCs, a situation that requires higher pigment concentrations than the other two BSC types to prevent radiation damage. However, our data shows that *Collema* had a greater decline in pigment concentrations than the other two BSCs. Thus it appears that despite the greater need for pigments, *Collema* was less able to produce them than the other two BSC types. This likely occurred because *Collema* suffered the greatest C losses, due to its faster drying (as it occurs above the soil surface) and higher moisture and net compensation points than the other two BSC types.

Both our data (Tables 1, 2) and the Bowker et al. (2002) data suggest that radiation-protective pigments are synthesized in response to increased radiation if there is sufficient C. These data also imply that BSCs live "on the edge" in relation to their ability to make protective pigments. If the amount or frequency of precipitation is average or below-average, it appears that protective pigment production cannot be continued past the summer season, despite the continued need for radiation protection. With above-average amount or frequency of precipitation, high concentrations of these protective pigments can be supported throughout the fall. Even with pigment production, solar radiation can still be a source of significant mortality for BSC species. The Bowker et al. (2002) study showed large mortality over summer and fall (*Microcoleus* 11%; *Nostoc* 37%; *Scytonema* 21%), despite concomitant increases in pigment production. Therefore, the inability of BSCs to produce protective pigments in our increased precipitation frequency treatment and spring-fall treatments undoubtedly resulted in even higher mortality rates, although this was not measured.

Conclusion

Climate change scenarios predict significant changes in precipitation timing, intensity, and interannual variability in the aridlands of the western United States (Schlesinger et al. 1990; Weltzin and McPherson 2003). Based on results from this study, predicted changes in both seasonal intensity and interannual variability of precipitation events will likely impact on the cover, species composition, and C cycles in BSCs. The picture that emerged from this study was one of BSC organisms "living on the edge" during drier and hotter times of the year. During this time, all crust types appear vulnerable to stress imposed by increased rainfall frequency. This study was unusual in that it was relatively long-term and under more natural conditions than most studies reported in the literature. The value of the quasi-field approach becomes apparent when comparing the results of our study with previously published studies: whereas short-term laboratory studies show cyanobacteria and lichen organisms respond to radiation stress by increasing protective pigment production, our results indicated that these organisms may often lack the resources for this production under many, even average, field conditions. This is especially true for the immobile *Collema*, a species that lives on and above the soil surface. Given that climate change is likely to affect the timing and amount of precipitation and that individual species will respond differentially, the species composition of BSCs is expected to change. These changes could have large effects on the fertility and stability of desert soils. Changes in precipitation timing and amount are also likely to directly influence the ability of crust organisms to respond to other stresses such as increased UV, land use changes, and increasing temperature, warranting further long-term studies to assess the impact of climate change on BSC organisms.

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