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Global change and biological soil crusts: effects of ultraviolet augmentation under altered precipitation regimes and nitrogen additions

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Abstract

Biological soil crusts (BSCs), a consortium of cyanobacteria, lichens, and mosses, are essential in most dryland ecosystems. As these organisms are relatively immobile and occur on the soil surface, they are exposed to high levels of ultraviolet (UV) radiation and atmospheric nitrogen (N) deposition, rising temperatures, and alterations in precipitation patterns. In this study, we applied treatments to three types of BSCs (early, medium, and late successional) over three time periods (spring, summer, and spring–fall). In the first year, we augmented UV and altered precipitation patterns, and in the second year, we augmented UV and N. In the first year, with average air temperatures, we saw little response to our treatments except quantum yield, which was reduced in dark BSCs during one of three sample times and in *Collema* BSCs two of three sample times. There was more response to UV augmentation the second year when air temperatures were above average. Declines were seen in 21% of the measured variables, including quantum yield, chlorophyll *a*, UV-protective pigments, nitrogenase activity, and extracellular polysaccharides. N additions had some negative effects on light and dark BSCs, including the reduction of quantum yield, *β*-carotene, nitrogenase activity, scytonemin, and xanthophylls. N addition had no effects on the *Collema* BSCs. When N was added to samples that had received augmented UV, there were only limited effects relative to samples that received UV without N. These results indicate that the negative effect of UV and altered precipitation on BSCs will be heightened as global temperatures increase, and that as their ability to produce UV-protective pigments is compromised, physiological functioning will be impaired. N deposition will only ameliorate UV impacts in a limited number of cases. Overall, increases in UV will likely lead to lowered productivity and increased mortality in BSCs through time, which, in turn, will reduce their ability to contribute to the stability and fertility of soils in dryland regions.

Keywords: climate change, cyanobacteria, deserts, drylands, lichens, microbiotic soil crusts, semiarid

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Introduction

Biological soil crusts (BSCs), composed primarily of photosynthetic cyanobacteria, algae, lichens, and mosses, play a key role in semiarid and arid ecosystems around the world (Belnap & Lange, 2003). They can constitute up to 70% of the living ground cover (Belnap *et al.*, 2003). They reduce both wind and water erosion (Belnap, 2003a; Warren, 2003) and influence many processes that determine soil fertility. They are often the dominant source of nitrogen (N) in these systems (Evans & Ehleringer, 1993; Evans & Belnap, 1999), while also contributing substantial amounts of carbon (C; Beymer & Klopatek, 1991). They secrete chelating metals and growth-promoting compounds (reviewed

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in Belnap *et al*., 2003), and the surface roughness associated with BSCs aids in the capture of nutrient-rich dust (Reynolds *et al*., 2001).

Studies have shown many cyanobacterial processes are sensitive to ultraviolet (UV) under laboratory conditions (reviewed by Castenholz & Garcia-Pichel, 2000). Despite extensive research, the impact on these organisms from current and enhanced levels of UV is far from clear. This may be due to the frequent use of excessive short-wavelength irradiation, which is not found in the solar spectrum even under substantial ozone depletion. These short wavelengths cause extensive damage to biological systems, making extrapolation to field situations tenuous. Field-based experiments on decomposition processes show a variety of UV influences on fungal and bacterial communities (Zepp *et al*., 2007), suggesting the possibility of a general sensitivity to UV among microbes. Using phytoplankton as a proxy for microbes, Day & Neale (2002) concluded that photosynthetic damage from UV occurs to a greater extent in phytoplankton than in vascular plants, as plants have various protective and repair mechanisms which appear to prevent direct UV damage under conditions of simulated ozone depletion (Caldwell & Flint, 1994). An explanation for the potentially greater UV sensitivity of cyanobacteria, compared with plants, is the shorter distance between the cell surface and tissues affected by UV. In addition, terrestrial cyanobacteria are, in general, less protected and have less time for repair and pigment production than higher plants (Castenholz & Garcia-Pichel, 2000; Day & Neale, 2002).

Many habitats, including alpine areas and deserts, naturally have high UV. Thinned ozone allows additional UV to reach the soil surface. Whereas ozone thinning is being mitigated by bans on many chlorofluorocarbons in developed countries, the expanding use of these compounds in developing countries may be slowing this recovery (McKenzie *et al*., 2007).

Surface-dwelling poikilohydric organisms in deserts are relatively immobile and metabolically active only when wet. Thus, although they are exposed to high UV levels, conditions suitable for repair activities are limited. For example, BSCs are subjected to UV about 4400 h annually in Moab, UT, USA. However, the C fixation needed for repair, maintenance, and production of tissue can only occur in light and when soils are wet, conditions that exist <500 h annually (Belnap *et al*., 2005). This makes the amount and timing of precipitation critical in determining BSCs' ability to withstand high UV radiation.

Soil organisms have three main strategies to cope with UV radiation: avoidance, repair, and protection (Cockell & Knowland, 1999). Avoidance means migrating deeper into the soil than UV can penetrate (Bebout & Garcia-Pichel, 1995; Quesada & Vincent, 1997). Repair requires replacing UV-damaged cellular components (Castenholz & Garcia-Pichel, 2000). Protection most often means synthesizing UV-protective pigments that occur in one of three groups: scytonemins, mycosporine-like amino acids (MAAs), and carotenoids-xanthophylls. Scytonemin is a colored pigment that prevents UV from entering the cell (Garcia-Pichel *et al*., 1992; Dillon & Castenholz, 1999). MAAs absorb electrons within the cell and also provide UV screening to DNA (Cockell & Knowland, 1999). Carotenoids and xanthophylls include echinenone, canthaxanthin, β-carotene, myxoxanthophyll, lutein, and zeaxanthin. These compounds quench free radicals within the cell and thus provide protection from all wavelengths (Kieft *et al*., 1987).

There are three major BSC types that occur in many deserts worldwide that represent successional stages. 'Light' BSCs, referring to their light coloration, are dominated by early successional species such as the cyanobacterium *Microcoleus vaginatus*. This species is large, filamentous, and highly mobile, with little UV-protective pigmentation. It resides just below the soil surface until sufficient moisture allows the filaments to glide to the soil surface (Garcia-Pichel & Pringault, 2001). As the surface begins to dry or UV exposure becomes too great, the filaments move downwards. Thus, this species uses an avoidance strategy for UV protection (Castenholz, 2004).

'Dark' BSCs are dominated by mid-successional, nonmotile, heavily pigmented cyanobacteria such as *Scytonema myochrous* and *Nostoc commune* (Quesada & Vincent, 1997; Sinha & Häder, 1998). These species occur on the soil surface, with the more motile *M. vaginatus* residing just below them, using them as a sunscreen (Karsten *et al*., 1998). Because dark BSCs are a mixture of motile and nonmotile species, they use a combination of protective pigments and mobility for UV protection.

Collema BSCs are a late-successional crust type. They are dominated by the lichen *Collema* (which contains the cyanobacterium *N. commune*) and also contain *S. myochrous*, *N. commune*, and *M. vaginatus*. *Collema* receives higher radiation than the cyanobacteria which grow on or below the soil surface, as it receives both direct radiation and radiation reflected from the soil surface. As *Collema* is immobile, it cannot move to avoid UV, but instead, the photosynthetic cells are buried within 2–4 mm of heavily pigmented fungal tissue, reducing UV by $>90\%$ (Dodds, 1989; Büdel *et al*., 1997).

In addition to increased UV, atmospheric N deposition is increasing (Vitousek, 1994). N deposition is likely to affect the structure and function of BSCs, as those species that fix N are likely to be differentially affected

relative to those that do not fix N. Studies have shown that while N additions to lichens and cyanobacteria suppress fixation, other processes, such as photosynthesis and growth, are often stimulated (Hällbom & Bergman, 1979; Singh *et al.*, 1986–1987). Therefore, N additions might partially offset the stress of UV augmentation.

This study was designed to examine the sensitivity of BSCs to simulated ozone depletion both alone and combined with altered precipitation frequency and N additions. We measured the effects of these treatments on dark-adapted quantum yield, nitrogenase activity, concentrations of photosynthetic and UV-protective pigments and extracellular polysaccharide content.

Materials and methods

All three BSC types, plus an uncrusted sand control, were field-collected during the last week of March 1999 and March 2000 from Canyonlands National Park in SE Utah, USA. This is a cool desert with bimodal rainfall (35% of annual precipitation occurs during summer). Soils were sandy loams typical of this area. The sand control was sterilized. Samples were collected as plugs (3 cm^2 surface area, 3 cm deep) such that the soil surface and the vertical profile were not disturbed at any time during treatments or measurements. These plugs were then divided among treatments that ran approximately 2.5 months in the spring (April 1 to June 15, hereafter referred to as 'spring'), approximately 2.5 months in the summer (approximately June 15 to August 31, hereafter referred to as 'summer'), or 6–7 months from approximately April 1 to October 31 (hereafter referred to as 'spring–fall'). The experiments began 1 April and ended in October (1999) or December (2000).

The experiment was conducted adjacent to the area where the samples were collected. Samples were placed under clear, UV-transparent covers [Aclar type 22A, Honeywell (formerly Allied-Signal), Pottsville, PA, USA] to prevent wetting by natural precipitation. Supplemental UVB was provided by filtered fluorescent UVB lamps suspended from racks above the cores (referred to as the +UV treatment; UVB313, Q Panel Co., Cleveland, OH, USA). The lamps were controlled by a modulation system which electronically adjusted the UVB supplement to track ambient UVB levels (Caldwell *et al.*, 1983). Lamps were filtered with cellulose diacetate (which blocks UVC and short wavelength UVB radiation), and spectral irradiance measurements were made with a double-grating spectroradiometer (Optronic model 742, Orlando, FL, USA) modified for field use (Barnes *et al.*, 1995). We simulated a 30% ozone depletion using a weighting function approximating the inhibition of photosynthesis in cyanobacteria (Häder *et al.*, 1995), but otherwise the lamp intensity adjustment was similar to previous techniques (Barnes *et al.*, 1995). (This weighting function yielded a considerably greater UV dose than is typically used for experiments with higher plants. The higher plant experiments have typically used a weighting function which only takes into account the shorter UVB wavelengths, but recent evidence suggests a weighting function similar to the one used here may be more appropriate; Flint & Caldwell, 2003.) On another rack of lamps, individual fluorescent tubes were wrapped with clear polyester to block UVB. This provided a control plot with no supplemental UVB (referred to as the −UV treatment). Lamps were calibrated the first week of April and rechecked in the middle of June. Lamp filters were changed every 10–14 days and samples rotated every 7–10 days to assure equal exposure. Lamp banks were also 'switched' once a month by alternating the filters, and the BSCs were moved into position under the appropriate light treatment.

During 1999, three precipitation treatments were applied to the different BSC types with 10 replicates per treatment. While all samples received total precipitation equal to the weekly 50-year average for this area, we applied rainfall at three different frequencies: (1) 50% below the 50-year weekly average (referred to as the low precipitation treatment), (2) average (average precipitation treatment), and (3) 50% above (high precipitation treatment) the 50-year weekly average. Because the total amount of precipitation was held constant, the amount of rainfall applied in a given event was 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). Samples were watered at mid-day, using a gentle spraying of rainwater. Clouds were not always present during watering, and thus samples watered on cloudless days undoubtedly dried more quickly than they would have under natural conditions. Air temperatures during the experimental time were continuously measured 0.5 km away.

In 2000, new samples were field-collected, and 10 replicates received one of the two UV treatments described above in addition to either no N (referred to as the −N treatment) or applied N (referred to as the +N treatment). The N was applied as an aqueous solution with a 4 : 1 NO_3 : NH_4 ratio (based on current atmospheric deposition ratios). The solution was made by mixing 1.75 g $NaNO_3$ and 0.55 g NH_4NO_3 (both 99.99% pure) into 1 L of rainwater. BSC samples were fertilized approximately every 2 weeks, with controls receiving rainwater of the same volume. Between fertilizations, water was applied such that the total amount of precipitation simulated the 50-year average amount and frequency for the study's different months.

At the end of the treatments, samples were transported to the laboratory for immediate analyses. For quantum yield measurements, samples were watered with 1 mm precipitation equivalent (Lange *et al*., 1998) and left in the dark at 22 °C for 12 h. They were then again brought to 1 mm precipitation equivalent before and during measurement at the same temperature. Quantum yield was measured with a portable pulse amplitude fluorometer (PAM-2000, Walz Inc., Effeltrich, Germany) using the saturation pulse method (Bilger *et al*., 1995). Samples were run in a random order at light levels of $<25\,\mu mol\,m^{-2}\,s^{-1}$. At least three measurements were taken per sample.

In 2000, steady-state photosynthesis and respiration rates were measured in the laboratory on the *Collema* BSC plugs that received the UV, but not the N, treatments. Unmixed samples that maintained soil surfaces and vertical profiles intact were placed in a growth chamber set to 26 °C with 25% relative humidity and photosynthetically active radiation (PAR) of $500\,\mu mol\,m^{-2}\,s^{-1}$, and once again brought to a moisture content of 1 mm precipitation equivalent. After 2 h in the growth chamber, samples were removed individually, watered to equal 1 mm precipitation equivalent, and placed in the moss/lichen cuvette of a CIRAS portable gas-exchange system (PP Systems, Haverhill, MA, USA). The cuvette was placed under a Philips Warm White $\times 2700$ light held at $PAR = 500\,\mu mol\,m^{-2}\,s^{-1}$, where samples were allowed to equilibrate to the cuvette environment of 400 ppm carbon dioxide (CO_2) and 26 °C. Once CO_2 exchange rates became steady, ambient net photosynthetic rates were measured. The cuvette was then darkened, allowed to equilibrate, and dark measures were taken. Gross photosynthetic rates were calculated as the difference between the light measurement (net photosynthesis) and the dark measurement (respiration). Rates are reported on a surface area basis.

Immediately following either the fluorometer or gas-exchange measurements, nitrogenase activity was measured with the acetylene-reduction method. Samples were placed in clear, gas-tight tubes, wetted evenly with distilled water, and injected with enough acetylene to create a 10% acetylene atmosphere. After injection, samples were incubated for 4 h at 26 °C in a chamber lighted with Chroma 50 (5000 K), cool white fluorescent bulbs (General Electric, Fairfield, CT, USA). Subsamples (0.25 mL) of the head space were then analyzed for acetylene and ethylene content on a Shimadzu FID gas chromatograph (Kyoto, Japan) equipped with a 2.4 m, 8% NaCl on alumina column, using helium as the carrier gas ($30\,mL\,min^{-1}$). Calibration with ethylene standards was done at each measurement time. Results are reported in $nmol\,C_2H_2\,m^{-2}\,h^{-1}$.

After assessment of nitrogenase activity, the top 2 mm of the sample were analyzed for pigment concentrations. Dried, ground samples were extracted with acetone and analyzed with a Waters HPLC (Franklin, MA, USA; Karsten & Garcia-Pichel, 1996). Results were compared with commercially obtained standards. As scytonemin standards are not commercially available, scytonemin was quantified using the 436 nm peak area and modifying the 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). The xanthophylls (zeaxanthin, lutein, and myxoxanthophyll – hereafter referred to as the xanthophyll subgroup) were grouped on the basis of similar function, absorbance spectra, retention times and/or difficulty distinguishing between two of the pigments (lutein and zeaxanthin). Concentrations are reported on a dry weight basis.

In 2000, exopolysaccharides were measured on plugs not receiving N. Samples were ground, extracted, and analyzed using a Hewlett Packard 8452A Diode-Array Spectrophotometer (Palo Alto, CA, USA) at 480, 486, and 490 nm. A standard curve of glucose solutions was obtained by plotting glucose concentration vs. absorbance. Results are expressed as glucose equivalents per gram of dried sample (Dubois *et al*., 1956).

Data normality was tested using the Shapiro-Wilk test. When normal, the data were analyzed using an Independent *t*-test (for two-level analyses) or one-way ANOVA (for multilevel analyses) if variances were equal (determined by Levene's test) or a Kruskal–Wallis H ANOVA if variances were not equal. If three or more means were being compared, a Tukey's HSD test was used to determine significant differences if variances were equal. If not, a Dunnett's T-3 test was used. When datasets were not normally distributed and could not be transformed, nonparametric tests were used: the Mann–Whitney *U*-test for comparisons of two datasets, and the Kruskal–Wallis H test for comparison of three or more datasets. Kruskal–Wallis H tests were also followed with Dunnett's T-3 tests to determine significant differences. A Wilcoxon's signed-rank test was used to compare across all treatments for a given variable by season. Statistics were run using SPSS version 12 (SPSS, 2003; SPSS Inc., Chicago, IL, USA). Differences with $P<0.10$ are reported as significant. This was justified given the wide range of species and biomass among individual samples, which imparted a large variability to the results obtained.

Results

As expected, sterile sand showed no quantum yield, nitrogenase activity, or pigments, regardless of treatment.

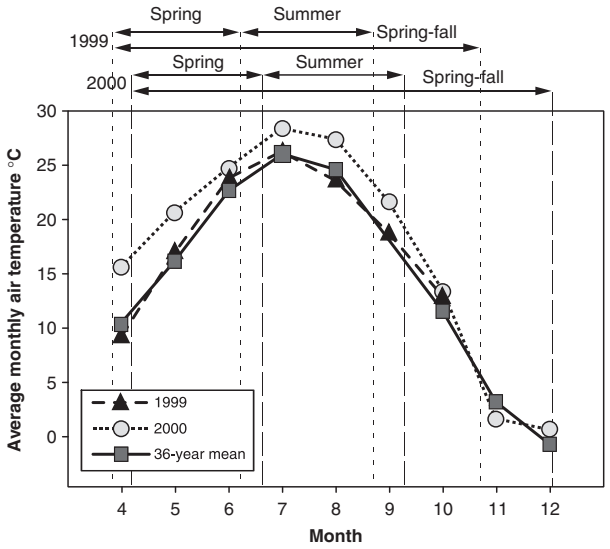

Fig. 1 Average monthly air temperatures over the duration of the experiments for 1999, 2000, and the entire period of record (1965–2001) at the Needles District, Canyonlands NP, Utah, USA. The starting and ending dates of each experimental period are shown by lines above the figure.

Climate

Air temperatures during the 1999 and 2000 study periods are shown in Fig. 1. Average monthly temperatures in the 1999 experimental period were close to the 36-year average. However, average monthly temperatures in the 2000 experimental period were substantially higher than the long-term average. The samples were under a total rainout shelter, and thus we controlled the amount of water they received.

Effects of altered precipitation without UV augmentation

The effects of altered precipitation treatments alone (with no UV or N manipulations) were reported in Belnap *et al*. (2004). In summary, increasing the frequency of precipitation had a negative effect on quantum yield and pigment concentrations in light, dark, and *Collema* BSCs. The majority of these effects occurred in the spring–fall samples.

Effects of UV augmentation under average precipitation conditions

Under average precipitation conditions, dark-adapted quantum yield was the variable that responded most frequently to augmented UV. In 1999, UV augmentation under average precipitation conditions resulted in changes in quantum yield in summer and spring–fall dark BSCs and in summer and spring–fall *Collema* BSCs (thus, four of nine possible cases or 44%; Fig. 2, top panel, middle set of bars within each season). In 2000 (when all samples received average precipitation), UV augmentation without N additions (thus comparing −N + UV to −N−UV) resulted in a decline in quantum yield in spring and spring–fall light BSCs and spring–fall dark BSCs (33% of possible cases), while there was an increase in quantum yield in summer *Collema* BSCs. Most changes in quantum yield were quite dramatic.

There was almost no response to UV additions for 1999 in pigment concentrations, nitrogenase activity, or polysaccharide content under the average precipitation treatment, as only scytonemin in spring light BSCs responded (Figs 3–5, Table 1). More variables responded to UV additions in 2000 (under average precipitation conditions; −N + UV vs. −N−UV), although the response was still limited (13 of a possible 72 responses, or 18%; Figs 3–5, Table 2). In 2000, declines were seen in chlorophyll *a* in spring–fall light BSCs, summer dark BSCs, and summer *Collema* BSCs (Fig. 3). As with quantum yield, observed declines in chlorophyll *a* concentrations were large, averaging 40%. Canthaxanthin declined in spring and summer *Collema* BSCs (Fig. 4). Echinenone declined in spring–fall light BSCs, spring and summer dark BSCs, and spring and summer *Collema* BSCs (Fig. 5). As with quantum yield and chlorophyll *a*, declines in pigment concentrations were large when they occurred. Nitrogenase activity declined in summer light BSCs and spring–fall dark BSCs and extracellular polysaccharides declined in spring–fall light BSCs (Table 2). Gross photosynthetic rates in 2000 declined 56% in *Collema* BSCs with UV augmentation relative to the UV controls (Table 3), while dark BSCs did not show a statistically significant response.

Fewer variables responded in 1999 (five variables) to UV augmentation under average conditions than responded in 2000 (17 variables; Figs 2–5, Tables 1 and 2). The 1999 responses were mixed, with three variables increasing with UV and two variables showing a decrease. In contrast, 16 of the 17 responding variables decreased in 2000. Air temperatures were also higher in 2000, indicating that this or other environmental variables were likely interacting with the UV augmentation treatments to make them more stressful.

Effect of simultaneously increasing UV and precipitation frequency (1999)

A simultaneous increase in UV and precipitation frequency (thus, differences found when comparing + UV with low precipitation vs. + UV with average or high precipitation frequency) resulted in the most variables

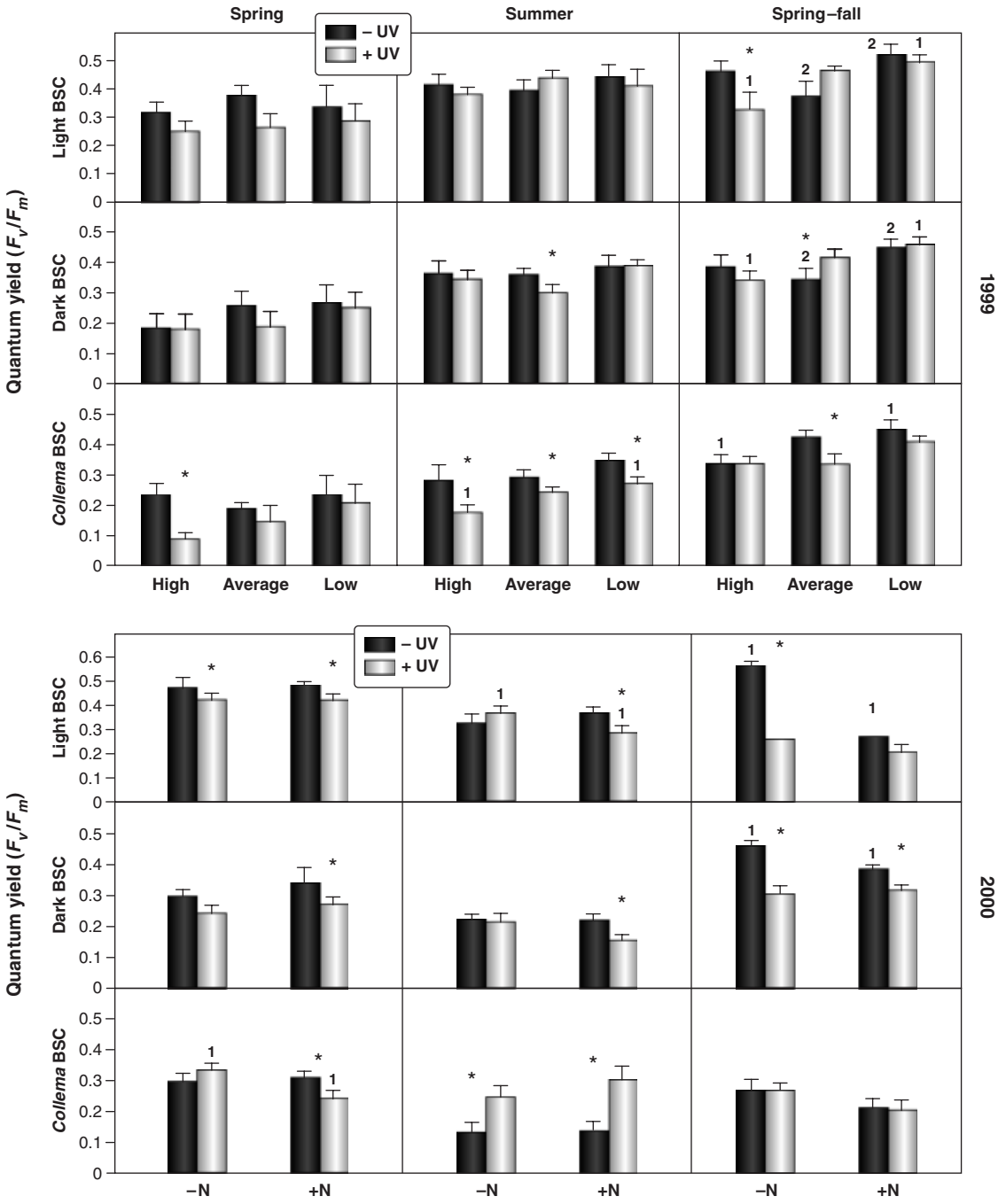

Fig. 2 Response of quantum yield to treatments. Top panel, 1999 experiments: effects of UV (+, −) and altered precipitation frequency (high, average, and low) on three biological soil crust (BSC) types. Bottom panel, 2000 experiments: effects of UV and nitrogen addition. Values are means ± standard error ($N = 10$). Comparisons are made only within each season. *The paired bars are significantly different ($P < 0.10$). Significant differences among all bars within a season are denoted by the same, unique number. For example, light BSC/spring–fall treatment: in the high-frequency precipitation treatment, −UV is significantly higher than +UV (denoted by *); +UV/high precipitation treatment was lower than +UV/low precipitation frequency treatment (denoted by #1s); and the −UV/average precipitation frequency treatment is significantly lower than the −UV/low precipitation frequency treatment (denoted by #2s). Bottom panel, 2000 experiments: effects of UV and nitrogen addition. Notation of significant differences is the same as the top panel.

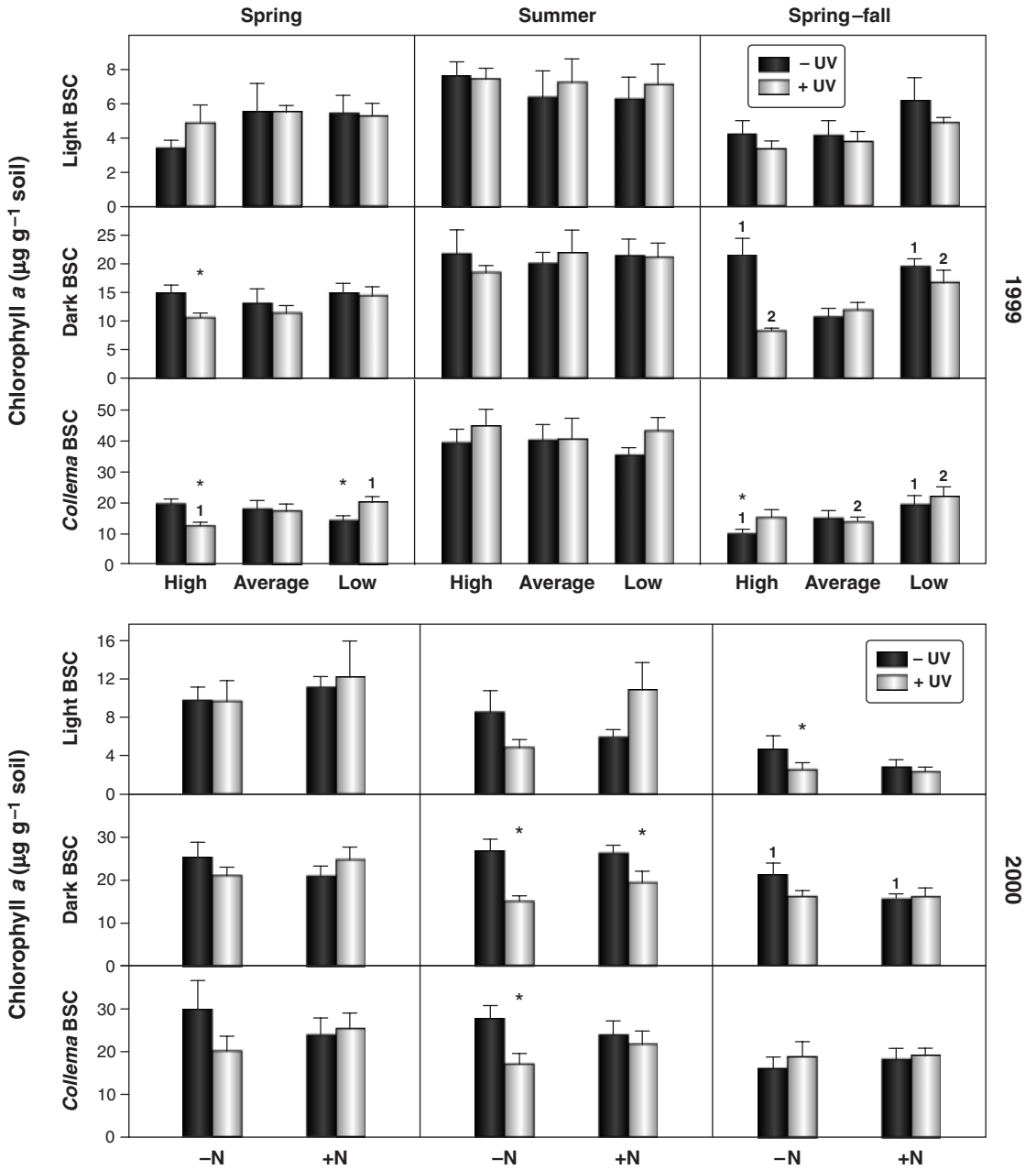

Fig. 3 Response of chlorophyll *a* to treatments. Top panel, 1999 experiments: effects of UV (+ , −) and altered precipitation frequency (high, average, and low) on three biological soil crust (BSC) types. Bottom panel, 2000 experiments: effects of UV and nitrogen addition. Values are means ± standard error ($N = 10$). Comparisons are made only within each season. Notation of significant differences is the same as in Fig. 2.

responding after the longer spring–fall treatment (12) when compared with the spring (7) or summer (1) treatments (Figs 2–5, Table 1). Under augmented UV, increasing precipitation frequency (therefore, more but smaller rain events) resulted in more stress for the sample organisms, as indicated by lower values for measured variables when compared with samples receiving lower precipitation frequency and thus more infrequent, larger rain events. This can be seen in quantum yield (spring–fall light BSCs, spring–fall dark BSCs, and summer *Collema* BSCs; Fig. 2), chlorophyll *a* (spring–fall dark and spring and spring–fall *Collema* BSCs; Fig. 3), canthaxanthin (spring–fall light and spring *Collema* BSCs; Fig. 4), echinenone (spring–fall light BSCs, spring and spring–fall dark BSCs, and spring *Collema* BSCs; Fig. 5) and xanthophylls (spring

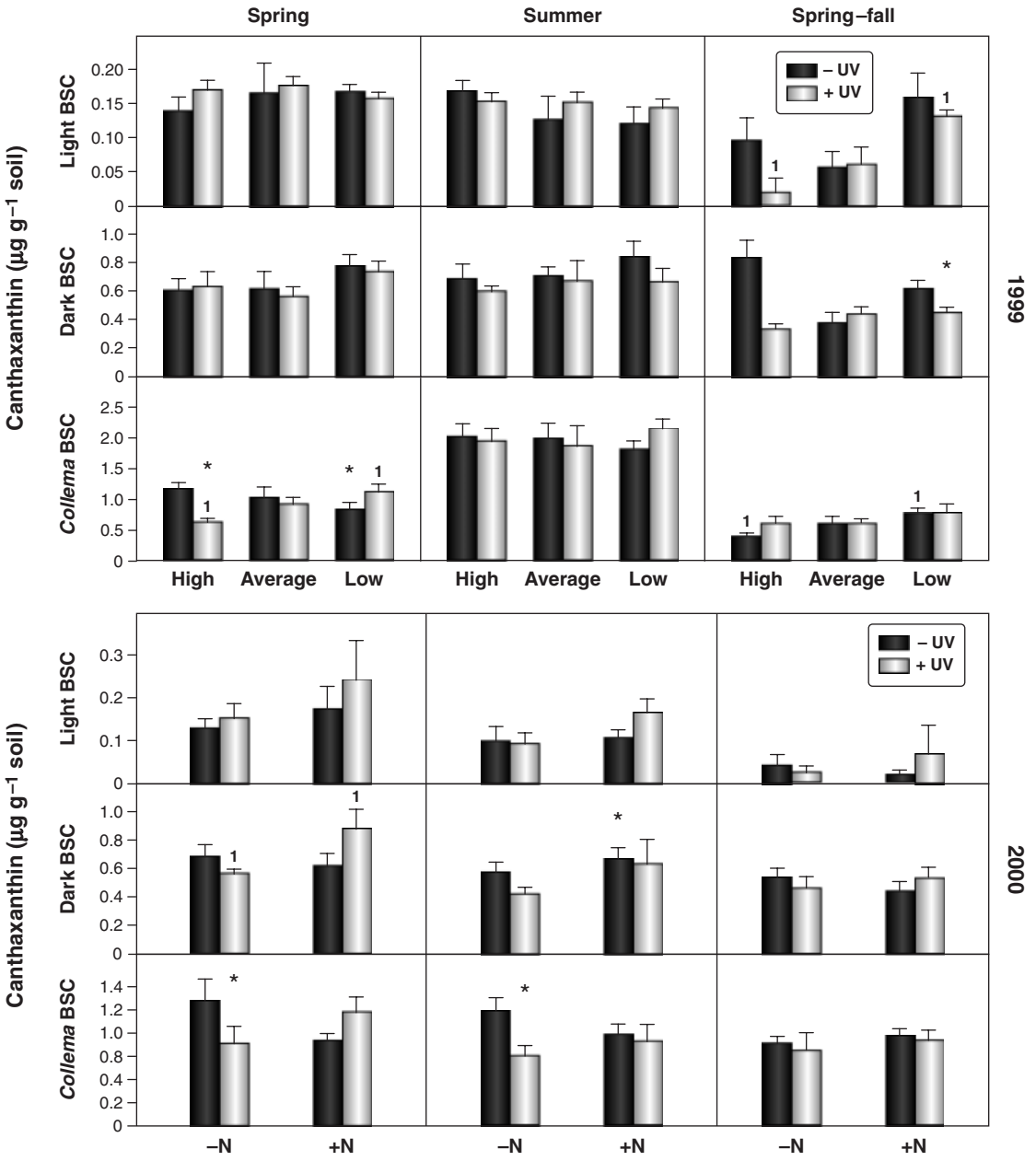

Fig. 4 Response of canthaxanthin to treatments. Top panel, 1999 experiments: effects of UV (+ , −) and altered precipitation frequency (high, average, and low) on three biological soil crust (BSC) types. Bottom panel, 2000 experiments: effects of UV and nitrogen addition. Values are means ± standard error ($N = 10$). Comparisons are made only within each season. Notation of significant differences is the same as in Fig. 2.

and spring–fall light and *Collema* BSCs; Table 1). The exception to this was scytonemin, which was higher with increased precipitation frequency (spring–fall light BSCs; Table 1). *Collema* BSCs had a greater number of variables respond to the combination of augmented UV and increased precipitation frequency than light BSCs or dark BSCs. However, given the number of treatments, BSC types, and variables measured, the overall number of variables responding to these treatments was quite low.

Effect of N additions without UV additions (2000)

When comparing the −UV−N to the −UV + N treatments (thus adding N but no UV), there was little response in the measured variables (Figs 2–5, Table 2).

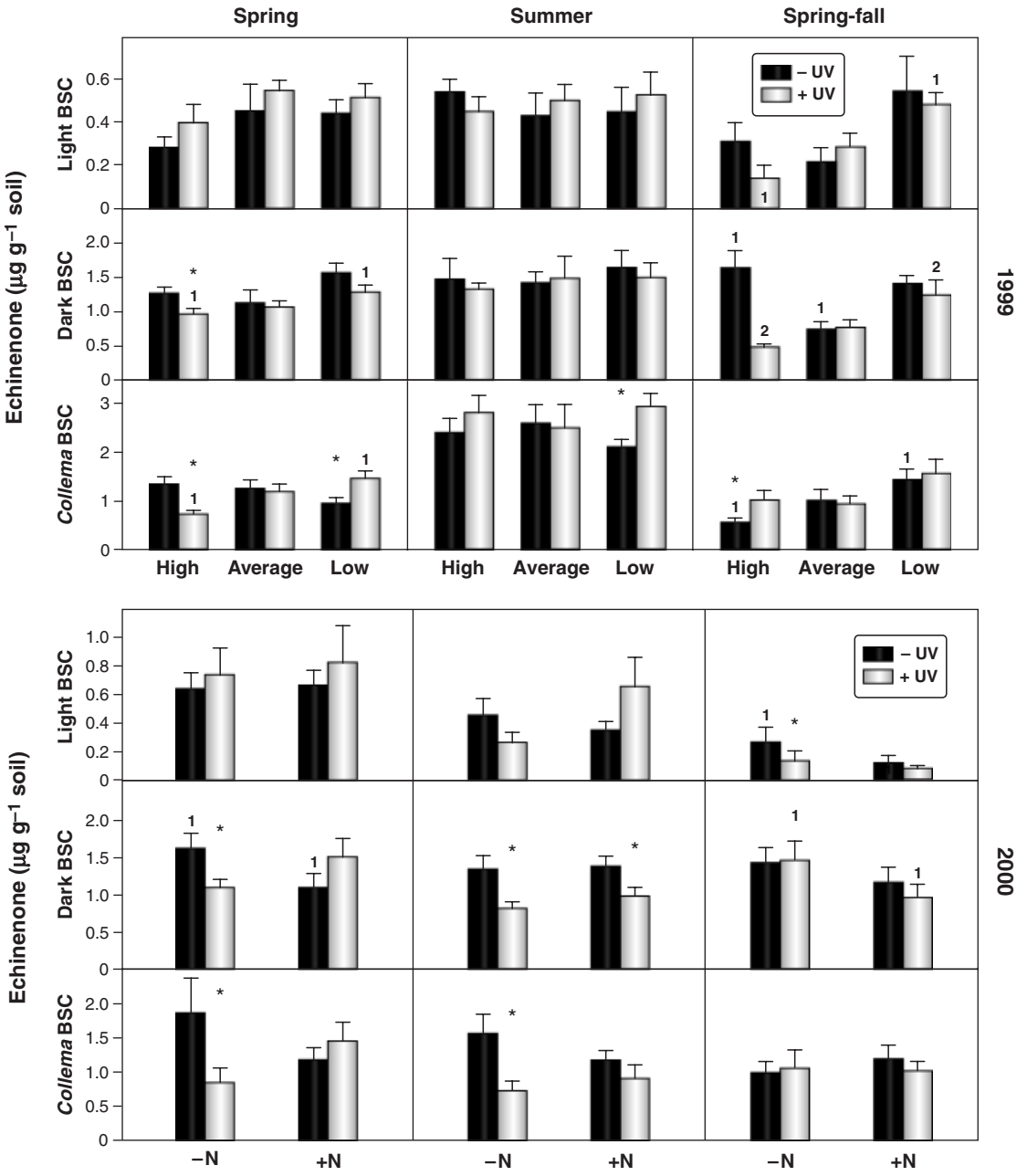

Fig. 5 Response of echinenone to treatments. Top panel, 1999 experiments: effects of UV (+ , −) and altered precipitation frequency (high, average, and low) on three biological soil crust (BSC) types. Bottom panel, 2000 experiments: effects of UV and nitrogen addition. Values are means ± standard error ($N = 10$). Comparisons are made only within each season. Notation of significant differences is the same as in Fig. 2.

When all crust types and seasons were combined, only eight of 81 possible cases showed any response to N additions. Contrary to expectations, seven of these eight responses were negative. Quantum yield declined in spring–fall light and dark BSCs (Fig. 2). Chlorophyll *a* declined in spring–fall dark BSCs (Fig. 3), and echinenone declined in spring–fall light BSCs and spring dark BSCs (Fig. 5). Xanthophylls declined in summer dark BSCs (Table 2). A decline in nitrogenase activity was observed in spring–fall dark BSCs, but an increase was seen in summer dark BSCs (Table 2).

Effect of N plus UV additions

We saw more responses when UV and N were added together than when N but no UV was added (thus,

Table 1 Response to 1999 UV augmentation and precipitation frequency (high, average, and low) treatments in three BSC types for scytonemin, xanthophylls, β-carotene, and nitrogenase activity

Season	Precipitation frequency	UV treatment	Mean ± SE Scytonemin ($\mu g\,g^{-1}$ soil)	Xanthophylls ($\mu g\,g^{-1}$ soil)	β-carotene ($\mu g\,g^{-1}$ soil)	Nitrogenase activity (nmol ethylene $m^{-2}\,h^{-1}$)
Light BSC						
Spring	High	−UV	8.3 ± 4.4	0.62 ± 0.14	0.28 ± 0.06	0.005 ± 0.005
		+UV	7.8 ± 2.6	0.57 ± 0.15 1	0.47 ± 0.15	0.000 ± 0.000
	Average	−UV	5.3 ± 1.1 1	0.92 ± 0.24	0.43 ± 0.15	0.000 ± 0.000
		+UV	10.4 ± 1.9 1	1.03 ± 0.07	0.44 ± 0.06	0.000 ± 0.000
	Low	−UV	7.3 ± 4.1	1.01 ± 0.15	0.52 ± 0.12	0.005 ± 0.005
		+UV	7.1 ± 1.2	0.89 ± 0.15 1	0.60 ± 0.14	0.007 ± 0.007
Summer	High	−UV	21.8 ± 3.7	0.59 ± 0.20	0.58 ± 0.10	0.041 ± 0.017
		+UV	29.4 ± 5.0	0.37 ± 0.21	0.55 ± 0.12	0.016 ± 0.016
	Average	−UV	24.9 ± 6.5	0.25 ± 0.15	0.57 ± 0.20	0.075 ± 0.037
		+UV	22.8 ± 3.4	0.37 ± 0.26	0.53 ± 0.16	0.022 ± 0.013
	Low	−UV	16.3 ± 4.3	0.61 ± 0.26	0.56 ± 0.17	0.028 ± 0.014
		+UV	16.6 ± 2.5	0.29 ± 0.15	0.62 ± 0.19	0.031 ± 0.015
Spring–fall	High	−UV	5.7 ± 1.0 1	0.31 ± 0.14	0.28 ± 0.10	0.000 ± 0.000
		+UV	13.6 ± 4.2 2	0.02 ± 0.02 1	0.12 ± 0.06 1	0.859 ± 0.456
	Average	−UV	19.8 ± 6.2	0.13 ± 0.10	0.22 ± 0.10	1.396 ± 0.706
		+UV	10.8 ± 2.0	0.12 ± 0.07	0.24 ± 0.12	0.529 ± 0.466
	Low	−UV	12.7 ± 2.7 1,3	0.47 ± 0.22	0.64 ± 0.19	1.514 ± 0.616
		+UV	5.7 ± 0.5 2,3	0.36 ± 0.12 1	0.54 ± 0.09 1	0.425 ± 0.383
Dark BSC						
Spring	High	−UV	124.9 ± 23.3	3.05 ± 0.54	2.27 ± 0.23 1	0.014 ± 0.008
		+UV	105.6 ± 15.6	3.87 ± 0.72	1.36 ± 0.16 1	0.060 ± 0.042
	Average	−UV	118.9 ± 23.0	2.32 ± 0.50 1	1.79 ± 0.35	0.113 ± 0.055
		+UV	83.8 ± 9.4	3.19 ± 0.47	1.62 ± 0.17	0.016 ± 0.010
	Low	−UV	116.1 ± 14.8	4.16 ± 0.52 1	2.04 ± 0.31	0.164 ± 0.143
		+UV	126.7 ± 18.5	3.78 ± 0.56	1.94 ± 0.20	0.029 ± 0.029
Summer	High	−UV	104.1 ± 11.1	0.58 ± 0.42	1.95 ± 0.38	0.232 ± 0.219
		+UV	104.6 ± 19.4	0.70 ± 0.42	1.69 ± 0.12	0.022 ± 0.011
	Average	−UV	101.0 ± 18.0	1.92 ± 0.68	1.76 ± 0.25	0.582 ± 0.566
		+UV	110.9 ± 24.9	0.51 ± 0.46	1.78 ± 0.32	0.475 ± 0.322
	Low	−UV	90.4 ± 10.8	2.07 ± 0.66	2.08 ± 0.44	0.040 ± 0.039
		+UV	106.0 ± 26.8	1.08 ± 0.59	1.79 ± 0.37	0.086 ± 0.052
Spring–fall	High	−UV	90.4 ± 10.8	2.07 ± 0.66	2.08 ± 0.44 1	0.040 ± 0.039
		+UV	146.0 ± 20.3	0.00 ± 0.00	0.45 ± 0.07 2	1.050 ± 0.546
	Average	−UV	140.3 ± 18.7	0.71 ± 0.37	1.05 ± 0.21	0.437 ± 0.344
		+UV	180.0 ± 26.8	0.25 ± 0.25	1.12 ± 0.25	1.648 ± 0.708
	Low	−UV	190.1 ± 20.8 1	0.89 ± 0.42	2.21 ± 0.31 1	0.000 ± 0.000
		+UV	141.2 ± 13.8 1	0.71 ± 0.42	2.27 ± 0.42 2	1.540 ± 1.001
Collema BSC						
Spring	High	−UV	91.3 ± 18.8	3.43 ± 0.65	1.41 ± 0.20 1	0.06 ± 0.046
		+UV	96.1 ± 19.1	3.03 ± 1.08 1	0.92 ± 0.10 1, 2	0.02 ± 0.011
	Average	−UV	98.5 ± 16.4	4.60 ± 0.96	1.28 ± 0.30	0.02 ± 0.008
		+UV	82.5 ± 19.2	4.33 ± 0.68	1.61 ± 0.36	0.04 ± 0.021
	Low	−UV	75.9 ± 10.5	3.44 ± 0.66	1.33 ± 0.41	0.65 ± 0.451
		+UV	72.9 ± 11.1	4.13 ± 0.70 1	1.88 ± 0.43 2	0.19 ± 0.504
Summer	High	−UV	213.0 ± 36.8	3.05 ± 1.41	2.15 ± 0.44	0.06 ± 0.026
		+UV	163.5 ± 28.0	4.82 ± 1.60	2.62 ± 0.33	0.11 ± 0.050
	Average	−UV	195.4 ± 33.5	4.08 ± 1.26	1.99 ± 0.27	0.12 ± 0.065
		+UV	169.0 ± 24.2	3.23 ± 1.21	2.19 ± 0.46	0.06 ± 0.027

Continued

Table 1. (*Contd.*)

Season	Precipitation frequency	UV treatment	Mean ± SE Scytonemin ($\mu g\,g^{-1}$ soil)	Xanthophylls ($\mu g\,g^{-1}$ soil)	β-carotene ($\mu g\,g^{-1}$ soil)	Nitrogenase activity (nmol ethylene $m^{-2}\,h^{-1}$)
	Low	−UV	148.2 ± 26.6	2.98 ± 0.99	1.50 ± 0.21 1	0.28 ± 0.223
		+UV	195.6 ± 23.1	3.99 ± 1.89	2.54 ± 0.30 1	0.15 ± 0.076
Spring–fall	High	−UV	100.2 ± 12.5 1	0.42 ± 0.21	0.66 ± 0.23 1	2.02 ± 0.799
		+UV	166.4 ± 19.9 1	0.36 ± 0.27 1	1.01 ± 0.23	0.72 ± 0.382
	Average	−UV	122.2 ± 14.3	1.27 ± 0.56	1.04 ± 0.24	0.98 ± 0.487
		+UV	146.5 ± 13.0	0.64 ± 0.32	1.04 ± 0.20	0.69 ± 0.686
	Low	−UV	126.5 ± 15.2	1.26 ± 0.40	1.88 ± 0.41 1	0.72 ± 0.486
		+UV	130.9 ± 17.6	2.12 ± 0.67 1	1.70 ± 0.31	2.04 ± 1.158

Values are means ± SE ($n = 10$). Significant differences ($P < 0.10$) within a season are denoted by the same, unique number. For example, scytonemin in light BSC/spring/average precipitation treatment: −UV values were significantly lower than +UV values (denoted by #1 s). For scytonemin response in the light BSC/spring–fall treatment: −UV high-frequency precipitation treatment is significantly lower than −UV low precipitation frequency treatment (denoted by #1); +UV/high precipitation frequency treatment is significantly higher than +UV low precipitation frequency treatment (denoted by #2); and −UV/low precipitation is significantly higher than +UV/low precipitation (denoted by #3 s).
UV, ultraviolet; BSC, biological soil crusts.

comparing the 'control' −UV + N to the 'treatment' +UV + N). Quantum yield showed the most response, with seven of the nine possible cases responding, with six of these responses negative (spring and summer light BSCs; spring, summer, and spring–fall dark BSCs; and spring *Collema* BSCs; Fig. 2) and one positive (summer *Collema* BSCs).

Few pigments showed any response to the simultaneous addition of N and UV (thus, comparing the 'control' −UV + N to the 'treatment' +UV + N; Figs 3–5, Table 2). Summer dark BSCs showed the largest response, with declines in chlorophyll *a* (Fig. 3), canthaxanthin (Fig. 4), echinenone (Fig. 5), and β-carotene (Table 2). Xanthophylls increased in summer light and *Collema* BSCs. Nitrogenase activity declined in spring–fall dark BSCs and increased in *Collema* BSCs (Table 2).

There were a few cases in which the addition of N appeared to mitigate the decline in function seen with UV augmentation. In 2000, there were 15 cases in which the addition of UV resulted in a decline of the measured variable (comparing +UV−N with −UV−N treatments). Of these, 11 showed less of a decline when N was added (comparing +UV treatments with and without N). While none of these variables were statistically different when considered alone, the N additions did significantly mitigate the negative UV effect ($P < 0.05$) when all the variables together were compared with a Wilcoxon's signed-rank test. Curiously, five of the variables that declined with UV additions without N additions were in the N-fixing *Collema* BSCs, and all five showed improvement with N additions when considered together ($P < 0.04$).

Effects of year, season, and BSC type

Year. When all BSC types and treatments were pooled, there were pronounced differences in the type of response seen in 1999 compared with 2000, showing a distinct effect of year (Table 4). In 1999, values of the responding variables generally increased through the spring and summer season (spring vs. summer values) and overall during the experiment (spring vs. spring–fall). However, values dropped significantly when the summer samples were compared with the spring–fall samples. In contrast, in 2000, almost all responding variables declined significantly in all season comparisons (spring vs. summer, summer vs. spring–fall, and spring vs. spring–fall) compared with the early spring measures. The exceptions to this were concentrations of xanthophylls, which declined significantly in all seasons in both years, and nitrogenase activity, which increased in most seasons (except spring vs. summer, 2000) in both years.

Season by crust type. In 1999, when all treatments were combined, more variables in light and dark BSCs responded in a statistically significant way to added UV and changes in precipitation frequency during the longer spring–fall experimental period than the other two shorter seasons (Figs 2–5, Table 1). In *Collema* BSCs, spring and spring–fall had about the same number of responding variables. In 2000, there was no obvious pattern among the crust types (Figs 2–5, Table 2).

Crust type. In 1999, *Collema* BSCs showed the greatest response when results from the UV and altered

Table 2 Response to 2000 UV and nitrogen additions in the three BSC types for scytonemin, xanthophylls, β-carotene, nitrogenase activity, and polysaccharides

		Mean ± SE				
Season	Treatment	Scytonemin ($\mu g\,g^{-1}$ soil)	Xanthophylls ($\mu g\,g^{-1}$ soil)	β-carotene ($\mu g\,g^{-1}$ soil)	Nitrogenase activity (nmol ethylene $m^{-2}\,h^{-1}$)	Polysaccharide content (μg glucose g^{-1} soil)
Light BSC						
Spring	−UV−N	3.7 ± 1.7	0.30 ± 0.15	1.17 ± 0.20	0.02 ± 0.01	84.4 ± 14.5
	+UV−N	7.8 ± 2.5	0.82 ± 0.32	0.90 ± 0.24	0.05 ± 0.04	125.5 ± 25.7
	−UV+N	10.2 ± 4.0	0.24 ± 0.12	1.32 ± 0.23	0.03 ± 0.01	
	+UV+N	36.3 ± 27.7	1.33 ± 0.86	1.31 ± 0.48	0.01 ± 0.00	
Summer	−UV−N	6.2 ± 1.7	0.94 ± 0.42	0.72 ± 0.26	0.04 ± 0.01 1	47.6 ± 14.2
	+UV−N	3.3 ± 0.8	0.47 ± 0.17	0.23 ± 0.10 1	0.01 ± 0.00 1	70.1 ± 24.6
	−UV+N	3.4 ± 1.3	0.37 ± 0.16 1	0.38 ± 0.07	0.03 ± 0.01	
	+UV+N	4.8 ± 1.7	1.48 ± 0.52 1	0.95 ± 0.36 1	0.03 ± 0.01	
Spring–fall	−UV−N	1.0 ± 1.0	0.56 ± 0.38	0.22 ± 0.15	0.14 ± 0.01	118.2 ± 70.8 1
	+UV−N	1.0 ± 1.0	0.02 ± 0.02	0.06 ± 0.06	0.15 ± 0.01	64.6 ± 54.9 1
	−UV+N	0.0 ± 0.0	0.11 ± 0.09	0.05 ± 0.05	0.14 ± 0.01	
	+UV+N	1.3 ± 1.3	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.01	
Dark BSC						
Spring	−UV−N	157.5 ± 23.9	2.96 ± 0.99	2.53 ± 0.47	0.05 ± 0.02	244.3 ± 27.9
	+UV−N	139.0 ± 22.1	1.60 ± 0.83 1	2.47 ± 0.26	0.02 ± 0.01	245.3 ± 32.4
	−UV+N	186.1 ± 19.6	3.36 ± 1.03	2.36 ± 0.38	0.01 ± 0.00	
	+UV+N	195.3 ± 27.1	5.10 ± 1.39 1	2.41 ± 0.57	0.03 ± 0.01	
Summer	−UV−N	184.0 ± 23.1	2.13 ± 0.84 1	2.13 ± 0.41	0.01 ± 0.01 1	208.4 ± 29.6
	+UV−N	172.6 ± 24.2	1.09 ± 0.71	1.55 ± 0.18	0.01 ± 0.00 2	254.0 ± 22.0
	−UV+N	261.2 ± 38.8	0.74 ± 0.62 1	2.40 ± 0.32 1	0.05 ± 0.01 1	
	+UV+N	213.3 ± 29.0	0.93 ± 0.52	1.38 ± 0.14 1	0.03 ± 0.01 2	
Spring–fall	−UV−N	86.4 ± 27.1	0.64 ± 0.57	1.24 ± 0.36	0.53 ± 0.31 1,2	244.3 ± 27.9
	+UV−N	115.6 ± 20.2	0.05 ± 0.05	1.18 ± 0.26	0.07 ± 0.04 1	245.3 ± 32.4
	−UV+N	129.5 ± 20.3	0.08 ± 0.05	1.11 ± 0.24	0.04 ± 0.01 2,3	
	+UV+N	121.5 ± 31.9	0.34 ± 0.24	0.69 ± 0.16	0.01 ± 0.00 3	
Collema BSC						
Spring	−UV−N	126.2 ± 14.7	6.20 ± 1.66	2.17 ± 0.75	0.12 ± 0.11	316.6 ± 43.0
	+UV−N	130.4 ± 20.1	2.52 ± 1.02	1.46 ± 0.29	0.09 ± 0.04	270.2 ± 20.4
	−UV+N	136.5 ± 30.3	3.25 ± 1.35	1.55 ± 0.44	0.02 ± 0.01	
	+UV+N	152.6 ± 31.0	4.45 ± 0.78	1.44 ± 0.36	0.02 ± 0.01	
Summer	−UV−N	126.7 ± 21.3	3.37 ± 1.21	1.35 ± 0.28	0.05 ± 0.01	265.8 ± 41.0
	+UV−N	152.5 ± 24.3	1.04 ± 0.40	0.91 ± 0.22	0.09 ± 0.05	197.8 ± 18.4
	−UV+N	172.9 ± 23.1	0.85 ± 0.52 1	0.79 ± 0.17	0.03 ± 0.01	
	+UV+N	151.0 ± 28.7	2.53 ± 0.89 1	0.93 ± 0.14	0.01 ± 0.01	
Spring–fall	−UV−N	74.0 ± 20.1	1.21 ± 0.70	0.63 ± 0.23	0.53 ± 0.34	254.5 ± 30.5
	+UV−N	154.5 ± 42.8	0.47 ± 0.26	0.85 ± 0.24	0.33 ± 0.19	274.9 ± 39.2
	−UV+N	55.2 ± 9.9	0.43 ± 0.23	0.50 ± 0.11	0.18 ± 0.01 1	
	+UV+N	119.4 ± 34.9	0.44 ± 0.18	0.45 ± 0.10	0.22 ± 0.08 1	

Values are means ± standard error ($n = 10$). Significant differences ($P < 0.10$) within a season are denoted by the same, unique number, as explained in the Table 1 legend.
UV, ultraviolet; BSC, biological soil crusts.

precipitation frequency treatments were combined, with 42% of the measured variables responding (30 responses from a possible 72; nine treatments × eight variables). Light BSCs showed only 12 significant differences (17%) and dark BSCs 17 differences, or 24% (Figs 2–5, Table 1). However, in 2000, the dark BSCs were the most responsive crust type, with 43% of the variables showing a significant difference when treatments were applied (23 of a possible 54 responses; six treatment combinations × nine variables). *Collema* BSCs had 19% of the variables respond (10/54) and light BSCs had 22% of the variables respond (12/54; Figs 2–5, Table 2).

Table 3 Effect of UV augmentation on the mean maximum gross photosynthetic rates (net photosynthesis – respiration) of 2000 spring–fall samples of *Collema* and dark BSCs. Samples were from the control (–N) treatments

Mean maximum net photosynthetic rates				
Crust type	UV added?	$\mu mol\, m^{-2} s^{-1}$	SE	*P*
Collema BSC	No	2.02	0.22	0.002
	Yes	0.87	0.18	
Dark BSC	No	1.15	0.2	
	Yes	0.83	0.34	0.4

UV, ultraviolet; BSC, biological soil crusts.

Discussion

Treatment effects on quantum yield, chlorophyll a*, gross photosynthesis, and nitrogenase activity*

The literature reports many instances in which UV augmentation was damaging to BSC organisms. Studies with terrestrial cyanobacteria show that photosynthesis, respiration, N_2-fixation, and nutrient uptake can be strongly reduced by UV radiation (reviewed in Castenholz & Garcia-Pichel, 2000) although there are exceptions (Solheim *et al.*, 2006). Other studies have confirmed that damage can occur, showing declines in quantum yield (Jackson & Seppelt, 1997; Underwood *et al.*, 1999), chlorophyll *a* (Quesada & Vincent, 1997; Roos & Vincent, 1998; Bowker *et al.*, 2002), and photosynthesis (Quesada & Vincent, 1997; Prasad & Zeeshan, 2005), as well as the deactivation of nitrogenase (Newton *et al.*, 1979; Tyagi *et al.*, 1992; Sinha *et al.*, 1995; Kumar *et al.*, 1996). Garcia-Pichel & Castenholz (1994) showed that exposure to UVB resulted in photoinhibition of *Microcoleus* spp. intertidal mats. Preliminary studies indicate that *M. vaginatus* in Colorado Plateau BSCs migrates to lower depths in the BSC when exposed to UVB supplements, and at these lower depths, photosynthetic rates are depressed (Garcia-Pichel & Belnap, 1996).

Unlike most published studies, we saw little negative effect of UV in 1999 on quantum yield, chlorophyll *a*, or nitrogenase activity, despite exposure of the three BSC types to relatively high UV levels. In contrast, we saw a pronounced reduction in quantum yield and gross photosynthesis in all three BSC types in 2000, with and without N additions. Temperatures were average during the 1999 experimental period, while temperatures during the experimental period in 2000 were well above average. This suggests that temperature can influence the BSC response to UV augmentation: UV additions under average temperatures result in little negative effect, while UV additions under higher than average temperatures have a much more pronounced effect. As BSCs are only metabolically active when hydrated, and higher air temperatures result in a shorter time of soil moisture and BSC hydration, it is likely that BSCs in the 2000 experiment had less activity time

Table 4 Observed increases or decreases in a given variable when all treatments and BSC types were combined (1999: three precipitation treatments × two UV treatments × three BSC types = 18 possible outcomes; 2000: two UV treatments × two N treatments × three BSC types = 12 possible outcomes) and when samples from different seasonal pairs were compared (e.g. values from spring treatment samples compared with values from summer treatment samples, spring values compared with spring–fall values, summer values compared with spring–fall values)

	Spring vs. summer				Spring vs. spring through fall				Summer vs. spring through fall			
	Total '+'	*P*	Total '–'	*P*	Total '+'	*P*	Total '–'	*P*	Total '–'	*P*	Total '–'	*P*
	1999		2000		1999		2000		1999		2000	
Quantum yield	+18/18	<0.001	−11/12	<0.001	+17/18	<0.001	−7/12	0.05	−5/18	0.003	−4/12	0.001
Chlorophyll *a*	+18/18	<0.001	−10/12	0.03	+8/18	ns	−12/12	<0.001	−18/18	<0.001	−10/12	<0.001
Scytonemin	+13/18	0.03	−4/12	ns	+16/18	<0.001	−11/12	<0.001	−11/18	ns	−11/12	<0.001
Xanthophylls	+1/18	0.002	−9/12	0.002	+0/18	<0.001	−11/12	<0.001	−17/18	<0.001	−12/12	<0.001
Canthaxanthin	+12/18	0.02	−10/12	0.01	+0/18	<0.001	−11/12	<0.001	−17/18	<0.001	−9/12	0.01
Echinenone	+16/18	0.001	−11/12	0.001	+6/18	0.06	−8/12	<0.001	−17/18	<0.001	−7/12	ns
β-carotene	+15/18	0.006	−11/12	<0.001	+5/18	0.03	−12/12	<0.001	−14/18	0.005	−12/12	<0.001
Polysaccharide	–	–	−5/6	0.05	–	–	−2/6	ns	–	–	−3/6	ns
Nitrogenase activity	+14/18	0.10	−5/12	ns	+15/18	<0.001	−1/12	<0.001	−8/18	0.001	−2/12	<0.001

A '+' means the value was greater in the second of the paired seasons (e.g., if '+' appears in the spring to summer comparison, the value was higher in summer than the spring). A '–' means the value was lower in the second of the paired comparisons. Data were analyzed with the Wilcoxon signed-rank test. *P*-values are reported when $P < 0.10$; all others are denoted 'ns' for not significant. Note that most response comparisons were positive in 1999, whereas all response comparisons in 2000 were negative.
UV, ultraviolet; BSC, biological soil crusts.

in which to repair damage to the photosynthetic apparatus that occurred during times they were dry or during times of high UV exposure. In addition, *M. vaginatus* would have less photosynthetically active time at the soil surface and less time to migrate downwards to avoid UV damage.

UV protective pigment production

Previous studies have repeatedly shown that UV exposure increases concentrations of scytonemins, MAAs, xanthophylls, and carotenoids in a wide variety of cyanobacterial and lichen taxa from varying habitat types (Paerl, 1984; Garcia-Pichel & Castenholz, 1991; Leisner *et al*., 1994; Ehling-Schulz *et al*., 1997; Quesada & Vincent, 1997; Odmark *et al*., 1998; Roos & Vincent, 1998; Underwood *et al*., 1999; Castenholz & Garcia-Pichel, 2000; Lange, 2003). There are a few reported exceptions to this pattern (Odmark *et al*., 1998). Polysaccharide production has also been observed to increase with UV exposure, presumably as a UV screen (Ehling-Schulz *et al*., 1997).

We found almost no increase in pigment concentrations in response to augmented UV in either year of our study. In 1999, only scytonemin increased in one crust type during one season (out of three crust types × three seasons × three precipitation treatments × five pigments). There was a greater response in 2000 when crust type, season, and N treatments were combined: of 90 possible responses, we saw decreases in pigment concentrations in 10 cases (canthaxanthin, three instances; echinenone, six instances; β-carotene, one instance) and increases in two cases (xanthophylls). These results stand in direct contrast to previous studies: not only were there very few responses to augmented UV, but when there was a response, it was mostly a decline, not an increase, in UV-protective pigment production. However, most studies have been short-term (i.e. on the order of days or weeks), while our experiments occurred over a period of weeks to months. Our UV augmentations were based on realistic projections of levels that may occur in the field. Perhaps most importantly, our study material was exposed to naturally occurring levels of temperature and precipitation, whereas most laboratory experiments occur at room temperature, and the experimental organisms are watered frequently.

Our inability to detect changes in UV-protective pigment concentrations in response to augmented UV may be explained in two ways: (1) levels of UV-protective pigments in the BSCs we tested were adequate to the level of UV they were exposed to and were being replaced such that we could not detect production of pigments or (2) neutral or deficit C balances of the test organisms precluded them from increasing UV-protective pigments in response to the augmentation of UV. We believe that the second explanation best explains the results of this study.

The production of UV-protective pigments is dependent on acquisition of adequate C and N by the crust organisms, and such acquisition is ultimately controlled by the availability of soil moisture, as BSCs show no activity when dry (Belnap, 2003b; Lange, 2003). Therefore, the amount of precipitation and the air/soil temperatures at which it falls are critical in determining the amount of C and N fixed by BSCs. Monitoring of soil moisture at a nearby field site exposed to natural precipitation during the 1999 and 2000 study periods showed that 3 mm events (the average event size for this region and slightly more than the low-frequency precipitation treatment in this study) in late spring through early fall seldom wetted soils for more than 20–30 min. Based on laboratory gas exchange curves, this is not long enough for *Collema* to reach its net compensation point (when respired C is replaced with newly fixed C) and is barely long enough for cyanobacteria to reach their net compensation point (Lange *et al*., 1998; J. Belnap, unpublished data). Thermal limits to photosynthesis ($\sim$ 26–30 °C) further limit C gains during summer (Lange, 2003). Therefore, the interplay of many factors (amount of precipitation, air temperature, light levels, and organism characteristics) can determine whether a species experiences C loss or gain during a given precipitation event.

It is likely that the combination of (1) the relatively low precipitation amounts used in our experiment and the low event size in the high precipitation frequency treatment, (2) the average and above-average air temperatures during the experimental time, and (3) the declines we observed in quantum yield, chlorophyll *a*, and gross photosynthetic rates resulted in low or no C gain during 1999 and no C gain or C loss during 2000 in the UV augmentation treatments. Limited C availability would require the organisms to make allocation 'choices' between maintenance or repair of C acquisition systems (e.g. photosystem II, chlorophyll *a*) and the production of new tissue (e.g. UV-protective pigments). Under stress, it is likely C would first be allocated toward the photosynthetic machinery and only when additional C was available, toward new construction of UV-protective pigments.

Our hypothesis that C limitation prevented BSC organisms from responding to the increased radiation stress is supported by several lines of evidence. First, the concentration of most pigments during a year of average temperatures (1999) increased after the spring treatment (when the most moisture, and therefore the most C, would have been available during our experimental period) but declined after spring–fall and summer treatments (when temperatures were higher and

less C would have been available), despite continued exposure to high UV. Second, in contrast to 1999, pigment concentrations declined across all seasons in all BSC types and all treatments in 2000, a year of above average temperatures. Third, it has been well documented that levels of UV pigments in cyanobacteria increase when exposed to increased UV (e.g. Ehling-Schulz *et al*., 1997; Castenholz, 2004) although this production can be limited by osmotic stress, temperature, or photo-oxidative stress (Dillon *et al*., 2002). Fourth, samples receiving the high-frequency precipitation treatments responded more negatively than those receiving the low-frequency precipitation treatments. BSCs in the high-frequency precipitation treatment would have received smaller rain events, which dried more quickly and resulted in less C gain, than those in the low-frequency precipitation treatments. Lastly, a field experiment was conducted at a nearby site during 1999 under natural conditions (Bowker *et al*., 2002). Rainfall during the experimental period was 78% above average and temperatures were average: thus, for BSCs, this was a wet time period. Light and dark BSCs were harvested at times equivalent to the spring and spring–fall harvest of this experiment. Under these favorable wet conditions, a large increase in most pigments was documented, in contrast to our observations of no increase in pigments under less rainfall (1999) or a decline in pigments under less rainfall and higher temperatures. Therefore, the comparison of these studies would indicate that the BSCs will make UV protective pigments when they have the resources to do so: that is, in years of above average rainfall and/or average or below average temperatures. This also implies that as global temperatures increase, these organisms are less likely to survive drought years, as they will be unable to make sufficient protective UV pigments and/or maintain a positive C balance. Increased temperatures have already been shown to result in high mortality for *Collema* in SE Utah (Belnap *et al*., 2006).

Effects of N and UV additions

Studies in the literature report cyanobacteria and lichens generally respond to N additions (reviewed in Stewart, 1964; Nash, 1996). These responses include the inhibition of heterocyst formation and a decrease in nitrogenase activity in the N-fixing species and an increase in growth in most species (Hällbom & Bergman, 1979; Singh *et al*., 1986, 1987). In nature, light BSCs fix much less N than dark or *Collema* BSCs (Belnap, 2003b). Based on this, we expected that N additions would have the greatest effect on N fixation in dark and *Collema* BSCs and the least in light BSCs. However, no such pattern emerged. Contrary to our expectations and reports from the literature, N additions without UV augmentation more often resulted in declines of quantum yield and pigment concentrations, whereas adding UV often resulted in an increase in these factors. The reason for this response is not obvious. It is possible that the BSC organisms acclimated to N additions after a short time, and environmental conditions then overrode any effects of N additions. Thus, our longer-term study conducted under natural temperatures may have elicited a very different response from the shorter-term laboratory studies done under more optimal conditions. Still, this does not explain why access to increased N would act as an additional stressor to these organisms. However, this result was consistent enough across BSC types and seasons to warrant further investigation.

Conclusion

Based on our results, BSCs appear able to tolerate UV augmentation under average temperature and moisture conditions. However, they may be at risk of C deficits during times when air temperatures are high and moisture is limited. The C needed for repair and growth is reduced under these conditions and this, if continued longer than our relatively short experimental period, would likely impair physiological functioning in these organisms. Many future climate scenarios could exacerbate this situation, including increases in temperatures or UV or changes in rainfall timing, amount, and intensity. Given currently rising temperatures, BSCs will likely experience added stress in the future. Our results indicate that the effect of atmospheric N deposition will be conditional and not necessarily compensate for UV stress. Effects on BSCs are expected to reverberate through ecosystems via effects on other soil biota, nutrient availability, and soil stability. Because they appear so vulnerable to climate change, it is critical they be studied when addressing climate change in arid ecosystems in long-term, field-based studies.

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