

Effects of Altered Temperature and Precipitation on Desert Protozoa Associated with Biological Soil Crusts

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ABSTRACT. Biological soil crusts are diverse assemblages of bacteria, cyanobacteria, algae, fungi, lichens, and mosses that cover much of arid land soils. The objective of this study was to quantify protozoa associated with biological soil crusts and test the response of protozoa to increased temperature and precipitation as is predicted by some global climate models. Protozoa were more abundant when associated with cyanobacteria/lichen crusts than with cyanobacteria crusts alone. Amoebae, flagellates, and ciliates originating from the Colorado Plateau desert (cool desert, primarily winter precipitation) declined 50-, 10-, and 100-fold, respectively, when moved in field mesocosms to the Chihuahuan Desert (hot desert, primarily summer rain). However, this was not observed in protozoa collected from the Chihuahuan Desert and moved to the Sonoran desert (hot desert, also summer rain, but warmer than Chihuahuan Desert). Protozoa in culture began to encyst at 37 °C. Cysts survived the upper end of daily temperatures (37–55 °C), and could be stimulated to excyst if temperatures were reduced to 15 °C or lower. Results from this study suggest that cool desert protozoa are influenced negatively by increased summer precipitation during excessive summer temperatures, and that desert protozoa may be adapted to a specific desert's temperature and precipitation regime.

Key Words. Chihuahuan desert, climate change, Colorado Plateau, environmental stress, soil fauna, soil food webs, thermotolerance.

ARID and semi-arid lands, such as much of southwest United States, constitute nearly one-third of the earth's land surface area. Global circulation models predict overall temperatures in the southwest United States to increase 2–6 °C through the 21st century (The National Assessment Synthesis Team 2000). These models also project increased annual precipitation and an increase in the frequency of extreme precipitation events throughout most of this arid region. Soil moisture is increased by precipitation events, which stimulate the pulse of desert processes (Noy-Meir 1973), and temperature influences biology in general (Chown and Gaston 1999; Clarke and Fraser 2004). Furthermore, increased temperature could accelerate water loss in surface soils causing desiccation stress on both the protozoa and their food sources (Evans et al. 2001).

Biological soil crusts cover up to 70% of arid and semi-arid lands (Belnap 2003). Biological soil crust formation begins with early-colonizing fungi and cyanobacteria, which stabilize the soil surface and facilitate colonization by other species. Subsequently, lichens, green algae, and mosses establish, representing a later stage of successional maturity. Biological soil crusts provide significant ecosystem functions due to the vast land area covered (Belnap, Hawkes, and Firestone 2003): they increase both the physical stability of surfaces (Belnap and Gillette 1997, 1998; Williams et al. 1995) and soil fertility through dust entrapment (Reynolds et al. 2001), photosynthesis (Beymer and Klopatek 1991), nitrogen fixation (Jeffries et al. 1992; Rychert and Skujins 1974; Rychert et al. 1978; West and Skujins 1977), and mineral chelation and retention (Belnap and Harper 1995; Harper and Belnap 2001). Nonetheless, the autotrophic components of these crusts are highly sensitive to surface disturbances and climate changes, such as altered temperature and precipitation (Belnap 2003). For example, rapid wetting and drying cycles are detrimental to soil lichens (Belnap, Phillips, and Miller 2004).

Protozoa are important members of soil food webs as they contribute a significant portion of the heterotrophic nitrogen miner-

alization (Clarholm 1985; Darbyshire 1994; Darbyshire et al. 1994a; de Ruiter, Neutel, and Moore 1995). Many protozoa are inactive (encysted) for much of the time in soil (Foissner 1987). When active, their growth and reproduction increases with increasing temperature to a point, then stops due to encystment (Sleigh 1973). Most of the protozoa in desert biological soil crusts are cyst-forming bacterivores that are tolerant of rapid wetting and drying cycles (Bamforth 1984, 2004). However, Robinson, Bamforth, and Dobson (2002) found non-encysting protozoa in cyanobacterial crusts of Australia and proposed that these crusts offer refuge for some unique protozoa. The objectives of the present study were threefold: (1) survey the abundance of protozoan motility groups in the field at two desert types that contrast in temperature and precipitation (Colorado Plateau and Chihuahuan Desert) and beneath two types of biological soil crust cover (cyanobacteria vs. cyanobacteria plus lichen), (2) perform a field mesocosm experiment to test the hypothesis that higher temperatures and greater amounts of summer precipitation will reduce the abundance of protozoa; and (3) identify upper and lower environmental thresholds that trigger encystment or excystment of soil protozoa.

MATERIALS AND METHODS

Field site characterization. This study focused on three sites representative of southwestern deserts in the United States. The Colorado Plateau is characterized as a cool desert and the experimental site for this desert is located near Canyonlands National Park (southeastern Utah, USA). This area receives a mean annual precipitation of 215 mm, 60% occurring in the winter, and soil texture is a sandy loam. The cyanobacterium *Microcoleus vaginatus* dominates early successional stage biological soil crusts (hereafter called "cyanobacteria" crusts) of this region. Late successional stage crusts (hereafter called "cyano/lichen" crusts) have a pinnacled microtopography containing the cyanobacteria *Scytonema myochrous* and *Nostoc commune*, in addition to *M. vaginatus*. The lichen flora is often restricted to late successional stage crusts and includes *Collema tenax* and *Collema coccophorum* (Rosentreter and Belnap 2001).

The Chihuahuan Desert is characterized as a hot desert because it is about 5 °C warmer than the Colorado Plateau and receives a mean annual precipitation of 254 mm with 60% occurring in the

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summer. The site for this desert is located in the Jornada Experimental Range (southern New Mexico, USA) where the soil texture is fine loamy sand. The cyanobacteria crusts of this region have a similar microfloral community composition to those in Utah, but cyano/lichen crusts have a rugose microtopography, are composed mostly of the cyanobacterium *M. vaginatus*, and the dominant lichen is *C. coccophorum* (Rosentreter and Belnap 2001).

The Sonoran Desert is also classified as a hot desert, but mean annual temperature is an additional 5 °C warmer than the Chihuahuan desert. The site for this desert was the Desert Laboratory on Tumamoc Hill (Tucson, AZ, USA) and receives similar amounts of precipitation in the same season to that of the Chihuahuan Desert (Western Regional Climate Center, www.wrcc.dri.edu).

Field survey. In September 2002, 10 representative plots were chosen from a 0.5-ha area in each of the Colorado Plateau and Chihuahuan Desert. At each location, five plots were chosen that were dominated by cyanobacteria crusts and five plots with cyano/lichen crusts. Within each plot, representative 25-g soil samples were collected from 0 to 10 cm depth and from 10 to 30 cm depth, and sent via overnight delivery in a chilled, insulated container to Toledo, OH, for immediate processing and enumeration of protozoa.

Field mesocosms. In September 2002, 120 black PVC pots (15 cm diam.) were filled with soil: 60 from the Colorado Plateau and 60 from the Chihuahuan Desert. From each desert, half the pots ($n = 30$ per location) were filled with soil beneath and associated with cyanobacteria crust and half with soil beneath and associated with cyano/lichen crust. We maintained the integrity of the crust by wetting the surface, inserting a polyvinyl chloride (PVC) ring (15 cm diam., 10 cm deep), and then sliding a 0.5-mm-thick sheet of stainless steel under the crust in the ring. This crust ring was set aside on the sheet while the remainder of the soil for the core was excavated. We mimicked natural vertical structure by filling each pot first with soil from 20 to 30 cm depth, and then with soil taken from 10 to 20 cm depth. Half of all pots filled from Colorado Plateau (15 from cyanobacteria crusts and 15 from cyano/lichen crust) remained in the Colorado Plateau while the remaining 30 pots (15 from cyanobacteria crusts and 15 from cyano/lichen crust) were moved to the Chihuahuan Desert to be exposed to a natural, increased annual mean temperature of 5 °C. Similarly, 30 pots constructed from the Chihuahuan Desert remained in the Chihuahuan Desert while the remaining 30 pots were moved to the Sonoran Desert to achieve a 5 °C increase in mean annual temperature. The Sonoran Desert site was used only to achieve a 5 °C increase in temperature from the Chihuahuan Desert; protozoa were not sampled from crusts of the Sonoran Desert.

At each location, all pots received one of three precipitation treatments applied weekly: control, twice winter precipitation, and twice summer precipitation. Control precipitation was determined as the 50-yr median precipitation of the pots' experimental location, not original location (Western Regional Climate Center, www.wrcc.dri.edu). For example, the control precipitation for pots collected and staying in the Colorado Plateau was the 50-yr median precipitation for the Colorado Plateau, while the control precipitation for pots collected in the Colorado Plateau but moved to the Chihuahuan Desert was the 50-yr median precipitation for the Chihuahuan Desert. Twice winter precipitation was simply double the amount of weekly precipitation added from December 21 through March 21, with 50-yr median levels applied for the rest of the year (same frequency as in control). Twice summer precipitation was simply double the amount of weekly precipitation added from June 21 through September 21, with 50-yr median for rest of year (same frequency as in control). Thus, five replicate

pots represented each origin (2) × location (2) × crust type (2) × precipitation (3) combination resulting in $n = 120$ total experimental pots. All pots were insulated with foam wrap and kept under shelters using acrylite sheet OP-4 (CYRO Industries, Lin-tech International, Macon, GA), which blocked rain but transmitted >93% of natural sunlight, according to the manufacturer's specification (www.cyro.com). Thermocouples were inserted horizontally into the center of the pots at 0.5 cm depth in one representative control pot at each location to collect daily minimum, average, and maximum soil temperatures. Following 1 yr, 15-g soil samples were collected from 0 to 10 cm depth and sent via overnight delivery in a chilled, insulated container to Toledo, OH, for immediate enumeration of protozoa.

In spring of 2004, a second experiment was conducted to quantify any potential effects of experimental manipulation. Five treatments were used to progressively replicate the handling of field mesocosm pots and were chosen to test the four most likely sources of experimental disturbance that could influence the potted protozoa: excavating the soil, keeping soil in PVC pots, transporting the soil, and artificially watering the soil in covered shelters. To estimate baseline abundance, 15-g soil samples were collected in April 2004, from five different plots, just before experimental treatments were implemented. For Treatment 1, "field control," five plots were identified in April but left undisturbed for two months. For Treatment 2, "dug, no pot," soil from five plots was excavated and returned to the ground without a pot. For Treatment 3, "dug, in pot," soil was excavated, put in a PVC pot as in the field mesocosm experiment and returned to the ground. These three treatments were not beneath shelters, were not transported anywhere, and all received natural rainfall. For the final two treatments, soil was excavated from the ground, put in a PVC pot as in the field mesocosm experiment, driven around for several hours to mimic transportation, and finally stored beneath a shelter used in the field mesocosm study. The only distinction between these treatments was that Treatment 4, "natural water," was watered the day after a natural rain event with the amount of equivalent to the rain event while Treatment 5, "control water," received weekly watering that mimicked the control precipitation treatment of the field mesocosm study. After 8 wk of incubation at their assigned treatment, 15-g samples were collected from each replicate pot or plot, sent to Toledo, OH, for immediate enumeration of protozoa.

For the field survey and field mesocosm experiments, protozoa were enumerated using a most probable number (MPN) technique (Darbyshire et al. 1974) with the following modifications. Sterilized soil extract (6% w/v) prepared from each sample's native soil was used as the diluent for MPN dilutions. Nine grams of the original soil sample were mixed in 80 ml of soil extract and agitated on a rotary shaker for 5 min at room temperature. Five milliliters of this dilution was further diluted threefold, seven times (in serial dilutions), and homogenous 1-ml aliquots of each of the final six dilutions were placed in each of eight wells across a 48-well Falcon[®] tissue culture plate row, one dilution per row. Final dilutions on the plate ranged from 1:100 to 1:22,000 of the original undiluted soil. Wells positive for protozoa were recorded for each plate after 3, 10, 17, and 24 d of incubation at room temperature using a Leica DMIL inverted microscope with phase contrast at 200× or 400× magnification. Repeated observations were necessary to observe natural ecological succession in protozoan communities that developed in culture. Approximately 1 min per well per week was spent seeking each motility group throughout the entire well, resulting in a search effort of about 30–60 min per plate per week. The MPN was calculated according to Cochran (1950) for each motility group (amoebae, flagellates, and ciliates), and "total protozoa" was estimated by summing the three motility groups. The minimum detection limit was estimated

to be 7 cells/g dry soil by calculating a hypothetical MPN from a standard threefold dilution series initiated by 9.0 g soil that would contain a single positive well at the most concentrated dilution. All sample weights were corrected for gravimetric soil moisture determined by drying soil to equilibrium at 60 °C (Forster 1995) upon the samples' arrival.

Temperature adaptations. Protozoa were cultured in controlled laboratory experiments to quantify the growth of protozoa at various temperatures and test the effect of temperature shifts on protozoan activity. Wheat grass medium (2.5 g/l) was prepared from soil extract (100 g soil from each sample's native desert autoclaved 1 h in 1 L of distilled water) and diluted 3:1 (v/v) with sterile distilled water (Sonneborn 1970). Ten grams of soil from the control precipitation treatment pots, taken at the end of the 1-yr mesocosm experiments at the Colorado Plateau and Chihuahuan Desert, were used as inocula in 200 ml of wheat grass medium incubated in the dark at 15 °C. The cultures were grown for 1 wk to adapt to the laboratory conditions. The mixed protozoan culture was maintained at 15 °C and re-fed weekly by transferring half the vol. to a new flask and bringing to full vol. with fresh medium. In these conditions, cells begin to encyst at stationary phase (observed by monitoring cell abundance and activity state during growth phase), so excystment was initiated before each experiment by pouring out two-thirds of the liquid and replenishing with fresh sterile medium. The following growth curve and temperature shift experiments were replicated three times and initiated by transferring a known vol. (1–5 ml) of the mixed protozoan culture (active cells in growth phase) to a Petri dish and adjusting the cell abundances by topping up with fresh medium. Whether for monitoring the source culture or enumerating experimental units, cell abundances were determined with a hemacytometer grid or directly from the Petri dish under phase-contrast using an inverted microscope, depending on the abundances (Adl et al. 2006). Abundances at the beginning of the experiments were adjusted to low levels to provide a long period of growth before stationary phase (on the order of 1×10^4 amoebae and flagellate and 1×10^{-1} ciliate cells/ml, or as indicated on figures at time 0). Abundance was defined as living, active cells. Dead cells were not distinguished from encysted cells so a decline in abundance indicates either cell death or, more likely, cell encystment.

To establish growth curves, cell abundance was assayed daily at temperatures between 4 °C and 55 °C through 9 d. Next, three temperature shift regimes were tested to assay survival in relation to temperature shifts. For survival at varied temperatures, initially active protozoa were exposed to 15 °C for 1 d, then to 37 °C for 1 d, followed by 4 d at (a) 15 °C or (b) 26 °C. As well, active protozoa were exposed to 15 °C for 1 d, then 37 °C for 1 d, then 1-day exposure to (a) 37 °C, (b) 45 °C, or (c) 55 °C, followed by 5 d at 15 °C. For survival of cysts at low temperatures, active protozoa were exposed to 15 °C for 1 d, 4 °C for 3 d, 0 °C for 1 d, -4 °C for 2 d, and 15 °C for 2 d. Temperature shifts were conducted with incubators that adjusted to new temperatures within two hours.

Statistical analysis. Analysis of variance was performed to test the effects of treatments and environmental factors for both the field baseline and mesocosm experiments. For baseline comparisons, a three-way mixed model ANOVA (type III SS) tested the overall effects of location, crust type, vertical depth, and a two-way interaction of location and crust on total protozoa and on each motility group (i.e. amoebae, flagellates, and ciliates). Experimental units (plots) unique to each location and crust combination were treated as random variables. Field mesocosm experiments were analyzed by performing ANOVAs separately for each combination of original location and crust type. Main effects of altered temperature and precipitation and a two-way interaction of temperature and precipitation were computed using

abundances of amoebae, flagellates, and ciliates as the three dependant variables. For the potting experiment, the field control treatment was compared against all other treatments with one-sided (lower) Dunnett's test. For laboratory growth experiments and temperature shifts, means and standard errors of abundances were calculated from five samples removed from each of three replicates for each experiment. Abundance data were log-transformed [$\ln(x+1)$] to meet assumptions of normality before statistical analyses. Statistical analyses were performed using the MIXED procedure in Statistical Analysis Software (SAS Release 8.00, SAS Institute Inc., Cary, NC).

RESULTS

Field survey. Amoebae were always the most abundant group in the field, ranging from 50% to 90% of the total community abundance. Flagellates were the second most abundant group, ranging from 8% to 43% of total community abundance, and ciliates were generally sparse ranging from 0.1% to 9.0% of community abundance. Abundances of amoebae, flagellates, and ciliates were greater at 0–10 cm than at 10–30 cm depths (Table 1 and Fig. 1). Abundances of flagellates and ciliates were greater with cyano/lichen crusts than cyanobacteria crusts, but abundances of amoebae did not differ between the two crust types (Table 1).

Field mesocosms. Soil surface temperatures beneath the rain-out shelters in winter (first half of experiment) were progressively warmer from the Colorado Plateau, Chihuahuan Desert, and Sonoran Desert, as expected. According to modeled temperatures fit to a sine curve, the lowest average daily temperatures (mid-January) were 3.2 °C at Colorado Plateau, 9.5 °C at Chihuahuan Desert, and 15.7 °C at Sonoran Desert (Fig. 2). Surface temperatures for much of the summer (second half of experiment) were 5–10 °C greater than outside the shelter at all sites, but more so at Colorado Plateau. Consequently, mean summer temperatures at the Colorado Plateau were warmer than expected (36.6 °C, mid-July, Fig. 2) and nearly equal to the Chihuahuan Desert (35.5 °C), although both were lower than Sonoran Desert (41.8 °C). Daily minimum and daily maximum temperatures followed a similar pattern.

In general, abundance of protozoa in the mesocosms that were moved from the Colorado Plateau Desert to the Chihuahuan Desert declined compared with the protozoa that remained on the Colorado Plateau (Fig. 3). This was true for amoebae, flagellates, and ciliates of both crust types (ANOVA, $P < 0.0001$ in all cases). The overall decline in protozoa moved from Colorado Plateau to Chihuahuan Desert across both crust types and all precipitation treatments converts to a 50-, 10-, and 100-fold decrease in amoebae, flagellates, and ciliates, respectively.

Conversely, abundance of protozoa in soil moved from the Chihuahuan Desert to the Sonoran Desert increased compared

Table 1. Analysis of variance (F values) for selected protozoan community parameters from baseline field observations.

Variable	Location	Crust type	Location × Crust type	Depth
df	1	1	1	1
Total Protozoa	0.44	5.63*	1.57	15.19**
Amoebae	2.00	1.00	1.22	5.19*
Flagellates	5.13*	15.00**	5.13*	22.48***
Ciliates	0.01	10.37**	13.59**	55.65***

Fixed effects for the mixed ANOVA model included location, crust type, depth, and a two-way interaction of location and crust type. Experimental units unique to each location and crust type combination were treated as random variables to account for non-independence between depths within experimental units.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

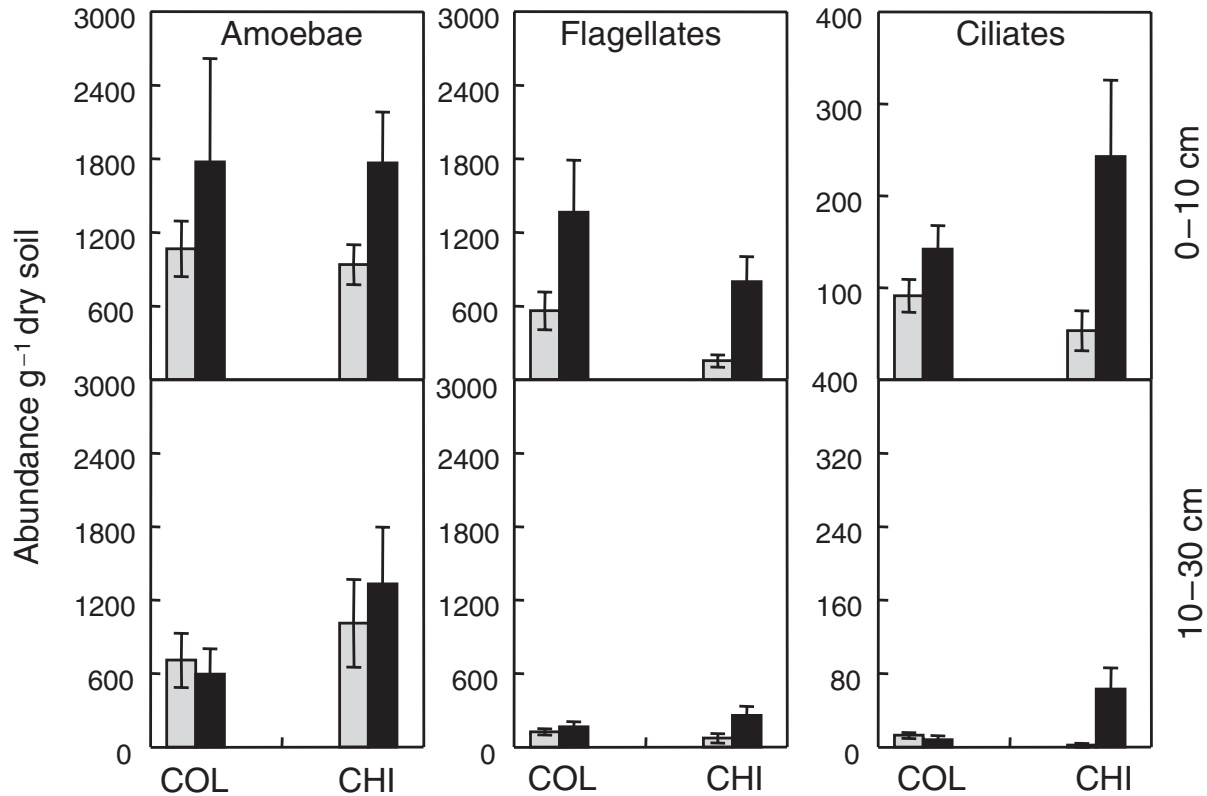


Fig. 1. Mean abundance of amoebae, flagellates, and ciliates (± 1 SE, $n = 5$) from baseline measurements determined by the most probable number technique. Bars indicate protozoa from Colorado Plateau (COL, cool desert) and Chihuahuan Desert (CHI, warm desert), beneath cyanobacteria crusts (light bars) and cyano/lichen crusts (dark bars), and shallow (0–10 cm) soil (top) and deep (10–30 cm) soil (bottom).

with protozoa in soil remaining in the Chihuahuan Desert. This was true for abundance of amoebae (ANOVA, cyanobacteria crusts: $P = 0.0002$, cyano/lichen crusts: $P = 0.0059$), flagellates (cyanobacteria crusts: $P = 0.0186$, cyano/lichen crusts: $P = 0.0056$), and ciliates (cyanobacteria crusts: $P = 0.0064$, cyano/lichen crusts: $P < 0.0001$). The overall increase in protozoa in soil moved from the Chihuahuan Desert to the Sonoran Desert, across both crust types and all precipitation treatments constituted a two-, seven-, and 28-fold increase in amoebae, flagellates, and ciliates,

respectively. There were no significant effects of precipitation or two-way interaction effects between precipitation and location for any protozoan group (ANOVA, $P > 0.05$).

In the experiment to quantify confounding effects of experimental manipulation, abundance of amoebae and flagellates in soil were unaffected by any treatment (Fig. 4). Only ciliates from Treatment 2, “dug, no pot,” were less abundant than the field control.

Temperature adaptations. Growth curves and survival through temperature shifts was identical between protozoa from the Colorado Plateau and Chihuahuan Desert, so we report the mean across both soils. Optimal range for growth was between 4°C and 26°C (Fig. 5). Above 29°C , growth rate was reduced compared with lower temperatures. Some amoebae continued to grow between 33°C and 37°C for 2–3 d, and no growth of any protozoa was sustained for more than a few hours above 37°C (observation). Species encysted or died at higher temperatures. Abundance of active cells was 0 following exposure to 37°C in the first two temperature shift experiments, indicating death or encystment of all cells (Fig. 6A and B). At least some cells encysted because abundance increased at 15°C or 26°C following exposure to 37°C for 1 d (Fig. 6A and B). Abundance grew more rapidly at exposure to 26°C than 15°C , indicating more rapid excystment or cell division (Fig. 6A). However, a 1-day exposure of cysts to greater temperatures (of 45°C and 55°C , as compared with 37°C) delayed regrowth or excystment of cells when transferred to 15°C (Fig. 6B). In the final temperature shift experiment, growth was halted at 4°C and 0°C caused death or encystment (Fig. 6C). Cysts survived 2 d at -4°C as indicated by excystment and regrowth when warmed to 15°C (Fig. 6C).

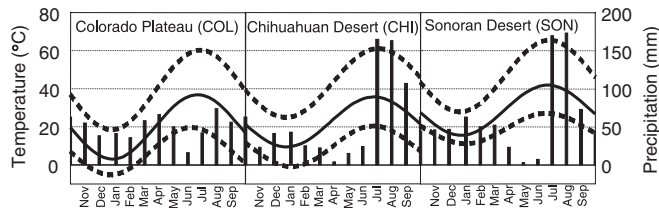


Fig. 2. Temperature and precipitation conditions experienced by experimental pots in the Colorado Plateau (COL), Chihuahuan Desert (CHI), and Sonoran Desert (SON). Mean temperature (left y-axis) and precipitation (right y-axis) are illustrated as solid lines and bars, respectively. Dashed lines represent maximum and minimum daily temperature values. Temperature curves were fit to actual data collected 0.5 cm below the soil surface during the experiment from the fall of 2002 through the winter, spring, and summer of 2003. Precipitation represents the 50-yr median precipitation for each respective desert and was applied through a water schedule to the sheltered mesocosms as a replacement of natural rainfall.

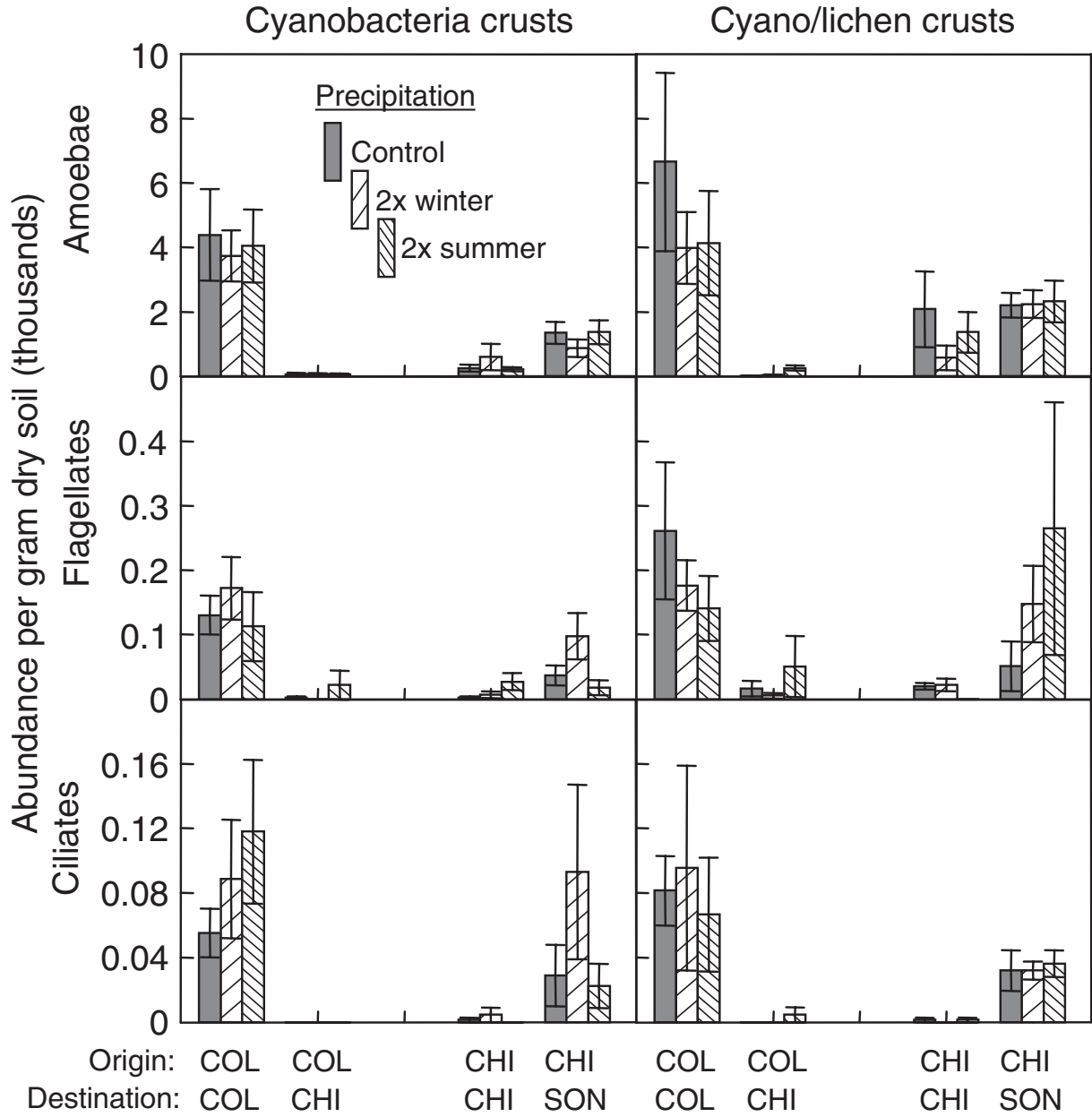


Fig. 3. Mean abundances of total protozoa (± 1 SE, $n = 5$) from a field mesocosm experiment in which soils and crusts from the Colorado Plateau site were transferred to the Chihuahuan Desert site and soils and crusts from Chihuahuan Desert were transferred to the Sonoran Desert site. Data represent mesocosms from cyanobacteria-dominated crusts (left side of figure) and cyano/lichen dominated crusts (right side of figure). The x-axis categories encode original location (COL, Colorado Plateau, CHI, Chihuahuan Desert) and experimental location (same as above, plus SON, Sonoran Desert). Shading pattern indicates precipitation treatment, with shaded bars receiving control precipitation (50-yr median throughout the year), light dashed bars receiving $2 \times$ winter precipitation (twice 50-yr median during winter, rest of year as in control) and doubly dashed bars receiving $2 \times$ summer precipitation (twice 50-yr median during summer, rest of year as in control). Note that transfer from COL to CHI significantly reduced abundances of all groups while transfer from CHI to SON significantly increased abundances. There was no impact of precipitation detected statistically.

DISCUSSION

In this study, abundance of flagellates and ciliates was greater when associated with cyano/lichen crusts than cyanobacterial crusts alone. Amoebae exhibited a similar pattern, but were not significant. Several attributes of cyano/lichen crusts probably contributed to greater protozoan abundances. Biological soil crusts dominated by cyanobacteria and lichens support greater microfloral biomass and diversity, which would provide more microflora as prey, than bare soil or soil covered by cyanobacteria only

(Rosentreter and Belnap 2001; Yeager et al. 2004). Furthermore, biological soil crusts can stabilize the soil surface (Belnap and Gillette 1997, 1998; Belnap et al. 2003; Williams et al. 1995) and reduce soil losses to wind erosion. This could limit the frequency of rapid dehydration of active protozoa during wind events. In laboratory experiments, George et al. (2003) found that soil crusts slowed evaporative water loss compared with bare soil. Cyanobacteria and lichens may retain moisture and retard drying by filling pore spaces and covering the soil surface. Thus, cyano/lichen crusts could prolong the period that levels of soil moisture

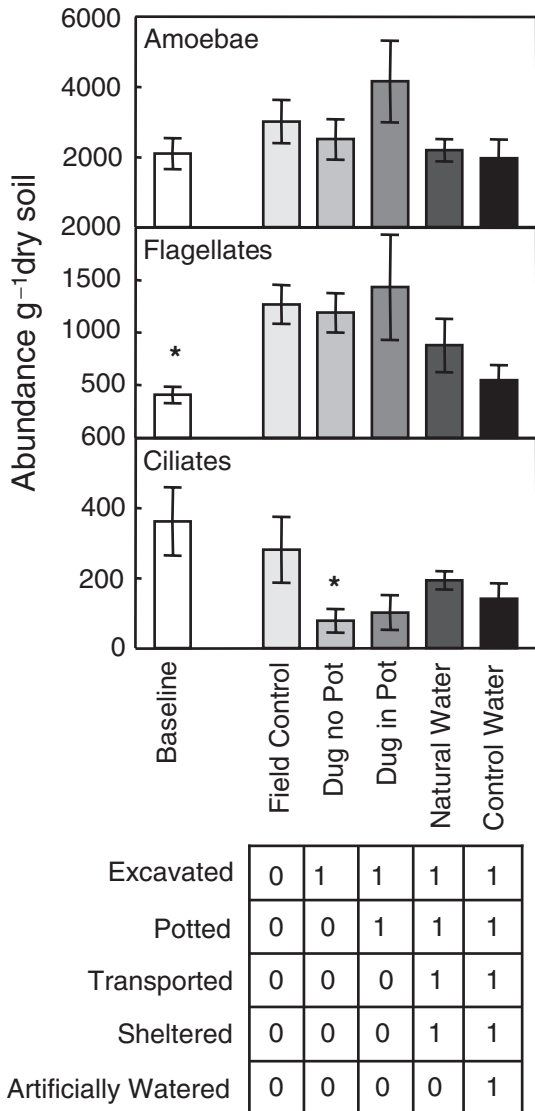


Fig. 4. Mean abundances of amoebae, flagellates, and ciliates (\pm 1 SE, $n = 5$) from a field experiment to determine if any experimental artifact affected the results of the field mesocosm experiment. Baseline estimates were collected in April, while Field Control, Dug No Pot, Dug In Pot, Natural Water, and Control Water were collected in June after 8 wk of their assigned treatment. Treatments denoted by an asterisk are significantly lower than the field control treatment (Dunnnett's one-sided comparisons). Bars are shaded by increasing number of disturbance types, and the lower grid aids in the interpretation of presence (1) or absence (0) of various disturbances.

or humidity were sufficient to maintain active protozoa in comparison with bare soil or cyanobacteria crusts alone. However, cyano/lichen crusts are darker in color and absorb energy, increase latent heat flux under certain conditions, and may accelerate evaporation over some parts of the soil surface (George et al. 2003). Understanding the influence of fine scale temperature and moisture dynamics in these soils will influence our understanding of how protozoa adapt to these nutrient-rich, but ephemeral habitats.

The field mesocosm experiment was designed to test the hypothesis that increased temperature and greater amounts of summer precipitation will reduce the abundance of protozoa. Amoebae, flagellates, and ciliates in soil moved from Colorado

Plateau (cool desert dominated by winter rain) to Chihuahuan Desert (hot desert dominated by summer rain) declined 50-, 10-, and 100-fold, respectively, in comparison with those remaining on the Colorado Plateau. Contrary to our intentions, the Colorado Plateau was warmer than expected and the only major difference in temperature between the Colorado Plateau and Chihuahuan Desert occurred during the winter. Rather, it appears that summer precipitation was the primary contrast between the two sites: control mesocosms received 50–75 mm weekly precipitation in the Colorado Plateau and 150 mm weekly precipitation in Chihuahuan Desert and Sonoran Desert throughout July and August (as prescribed by desert specific 50-yr median precipitation in the experimental design). We have ruled out the possibility that physical disturbance due to potting the soil caused an experimental artifact. Furthermore, increased temperatures experienced under the shelters during the winter were within optimum growth temperatures of cultured protozoa, and summer temperatures were within tolerance range. Thus, our results are consistent with our original hypothesis, but the results must be qualified by restating that cool-desert protozoa responded negatively to greater summer precipitation in the midst of above average summer temperatures. However, protozoa did not respond significantly to the experimentally applied precipitation treatments, and we are unable to explain this.

Based on the laboratory experiments, protozoa from our studies probably encyst as temperature increases during the day before excessive summer temperatures. The cysts, but not the active cells, can survive the upper end of daily temperatures as well as short term freezing at -4°C . This suggests a subtle and dynamic response of these cells to increases in soil temperature and decreases in soil moisture content, so that encystment occurs before fatal desiccation or freezing. This also supports a general conclusion that the activity of protozoa in these soils may be determined by a combination of moisture and temperature optima, not either alone. This has ecological ramifications because protozoa perform several roles in the cycling of soil resources, especially in arid lands. Protozoan grazers of microflora affect soil nutrient flow by altering the bacterial community and mineralizing nutrients contained in microbial biomass (reviewed in Griffiths 1994). A food web model estimates that protozoa contribute about 30% of the mineralization of nitrogen from soil fauna (Hunt et al. 1987). A change in abundance or activity of protozoa, due to altered temperature and moisture, could change their functional role in soil.

We hypothesize that the protozoan communities from each desert differ in species composition or physiology and are adapted specifically to their original desert type's temperature and precipitation regimes. Colorado Plateau protozoa declined in abundance with increased summer precipitation during high summer temperatures, but it appears that Chihuahuan Desert protozoa can tolerate increased summer temperature amidst greater summer precipitation since they did not decline in abundance when moved to the Sonoran Desert. Because amoebae from the Chihuahuan Desert were similar in original field abundances to those from the Colorado Plateau, we further hypothesize that the most likely long-term response of most protozoa, especially amoebae, to climate change would be a shift in composition rather than or in addition to a decline in standing abundance. This is supported indirectly by the observation that desert nematode communities, a functionally similar faunal group, differ markedly in species composition between the same Colorado Plateau and Chihuahuan Desert sites (Darby, Neher, and Belnap 2006). Given the complexity of interactions between protozoa and their prey, a shift in composition might also have other ecological consequences. Unfortunately, specific feeding habits of most protozoa are unknown, leaving it a primary challenge for ecologists to define their trophic habits.

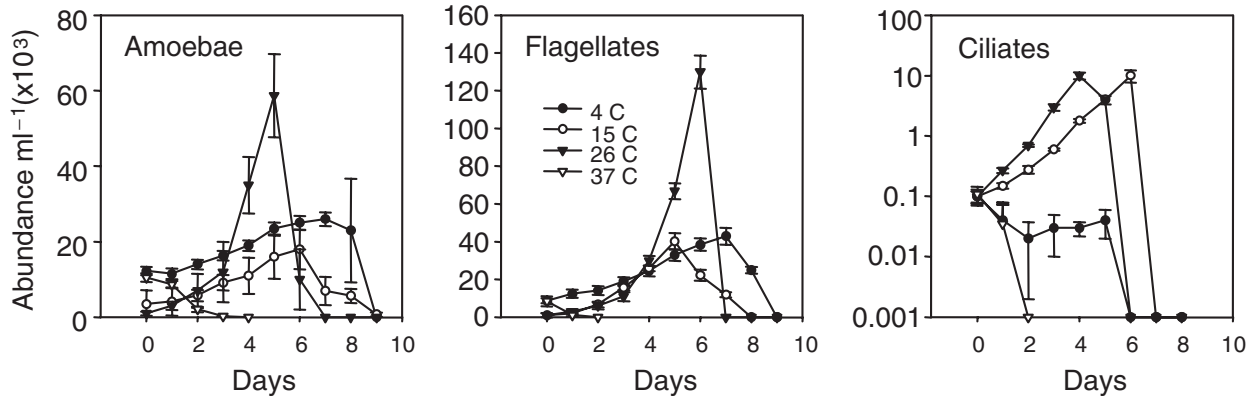


Fig. 5. Impacts of temperature on growth rates of Colorado Plateau and Chihuahuan Desert protozoa, combined, originating from liquid wheat grass cultures. Mean abundances through time of amoebae, flagellates, and ciliates (± 1 SE, $n = 3$) in culture at 4 °C (filled circles), 15 °C (open circles), ciliate abundance on \log_{10} scale, 26 °C (filled triangles), and 37 °C (open triangles). Decrease in abundances reflects death or encystment of the cells.

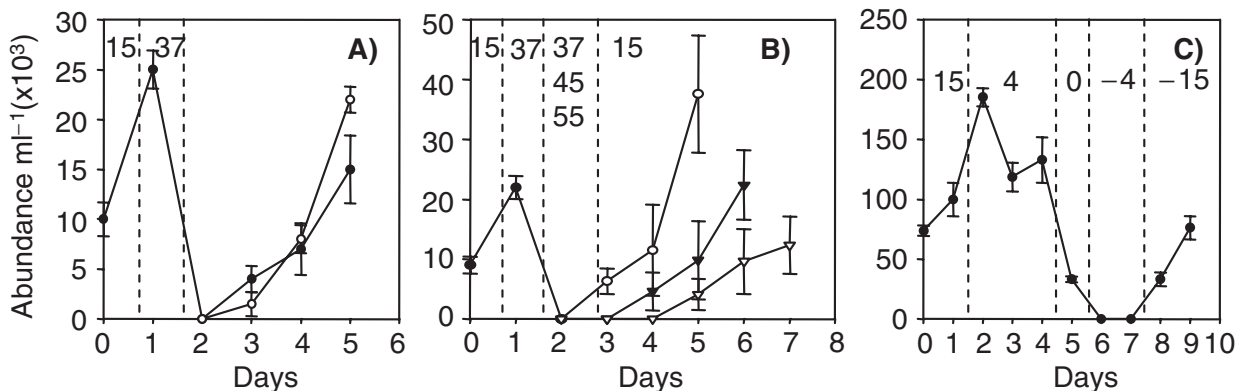


Fig. 6. Impacts of varying temperature manipulations on the encystment and excystment dynamics of Colorado Plateau and Chihuahuan Desert protozoa, combined, originating from liquid wheat grass cultures. (A) Temperature shift of 15 °C to 1 day at 37 °C followed by 15 °C (open circles) or 26 °C (filled circles). (B) Temperature shift of 15 °C to 37 °C, followed by 1 day at 37 °C (open circles), 45 °C (filled triangles) or 55 °C (open triangles), and then followed by 15 °C beginning on 4th day. (C) Temperature shift of 15 °C to 4 °C followed by 1-day exposure to 0 °C followed by 2 days exposure to -4 °C, and followed by 15 °C. Dashed lines indicate the shift in temperature and the incubation temperatures are indicated on the graph. Decrease in abundances reflects death or encystment of the cells.

We used the MPN procedure to enumerate protozoa from field samples and a direct count method to enumerate cultured cells. The MPN method will restrict enumerations to just those cells that can be observed in liquid media with limited food sources, which is probably biased toward bacterivorous protozoa (Adl 2003). The MPN method also enumerates both active and encysted cells. Surface soil is generally very dry during the summer (2–3% gravimetric moisture), so we presume that most protozoa enumerated from 0- to 10 cm are encysted; deeper soil is generally more moist (3–5%) and we presume that most protozoa from 10- to 20 cm samples could be active (Darby and Neher, unpub. data). Despite these limitations, we found the MPN procedure the most appropriate method to deal with the large number of field samples ($n = 120$) being collected within a two week period at the conclusion of the field mesocosm experiment.

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