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Supplementary Materials

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Materials and Methods
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Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities

Ferran Garcia-Pichel,[1]* Virginia Loza,[1,2] Yevgeniy Marusenko,[1] Pilar Mateo,[1,2] Ruth M. Potrafka[1]

Global warming will likely force terrestrial plant and animal species to migrate toward cooler areas or sustain range losses; whether this is also true for microorganisms remains unknown. Through continental-scale compositional surveys of soil crust microbial communities across arid North America, we observed a latitudinal replacement in dominance between two key topsoil cyanobacteria that was driven largely by temperature. The responses to temperature of enrichment cultures and cultivated strains support this contention, with one cyanobacterium (*Microcoleus vaginatus*) being more psychrotolerant and less thermotolerant than the other (*M. steenstrupii*). In view of our data and regional climate predictions, the latter cyanobacterium may replace the former in much of the studied area within the next few decades, with unknown ecological consequences for soil fertility and erodibility.

Plant interspaces in arid lands can be colonized by cryptic photosynthetic assemblages known as biological soil crusts (biocrusts) (*1*, *2*). These largely microbial communities help stabilize the soil against erosion (*3*), are net exporters of biologically fixed carbon and nitrogen (*4*, *5*), and modify the hydrological properties of soil (*6*), all of which are crucial roles for the fertility and sustainability of desert ecosystems. Because of the extent of arid lands, the biogeochemical contributions of soil biocrusts are important globally and not only locally (*7*). Some of the macroscopic biotic components typical of old-growth biocrusts, such as lichens or mosses, are known to display geographic patterns of distribution (*8*), which likely drive the patterns in biocrust fungi recently detected by using molecular surveys (*9*). As with most free-living microorganisms (*10*, *11*), the geographic distributions of biocrust microbes remain largely undefined, even for the pioneer primary producer microbes that start biocrust formation.

We undertook a continental-scale survey of biocrust bacterial diversity based on 16*S* rRNA gene diversity in community DNA (Fig. 1) and characterized each site by a range of parameters related to soil type, geochemistry, and texture, as well as geography and climate (table S1). An initial analysis of bacterial community composition resolved at the phylum level yielded neither statistically discernible geographic patterns nor any strong taxon-parameter associations (table S2). This was perhaps not surprising given the large functional diversity contained in many bacterial phyla (*12*). For the Bacteria in general it was not possible to achieve deeper taxonomic resolution.

Because much prior descriptive work was available for Cyanobacteria (*13*–*17*), which made up the biocrust's dominant phylum, we could develop ad hoc bioinformatic algorithms that allowed robust assignment of the large majority of sequences to well-defined generic or subgeneric entities (Fig. 1). The phototroph community was dominated by two oscillatorian (filamentous, non-heterocystous) cyanobacteria: *Microcoleus vaginatus* and *M. steenstrupii*. The former is considered to be the most common and widespread cyanobacterium in biocrusts (*13*) (*18*), but the second is known to dominate at least some locations of the Lower Sonoran region (*14*). In spite of sharing a generic name, these two cyanobacteria are not closely related phylogenetically and in fact likely belong in different families. But both species are rope-formers, a convergent trait that helps them to stabilize the soil on contact (*17*) and to become biocrust pioneers. They are in this sense keystone species. Other phototrophs detected include several heterocystous (N_2-fixing) cyanobacteria and also eukaryotic algae (as plastid rRNA sequences), most of which were closely allied to *Klebsormidium* spp. (*Streptophyta*), a known inhabitant of some biocrusts (*19*). In fact, the algal contribution overwhelmingly came from just two sites (9 and 12), where streptophytes were dominant. Similarity ordination of the phototroph community structure revealed three groups of self-similar sites (Fig. 1, bottom). One was defined by the dominance of streptophytes (sites 9 and 12). Most of the other sites could be cleanly split into those dominated by *M. steenstrupii* (12 sites, mostly southern latitudes), and those dominated by *M. vaginatus* (8 sites, mostly northern). This is consistent with prior molecular surveys carried out in a site of the Sonoran (*14*) versus one in the Colorado Plateau (*15*). Similarity in community composition between sites did not correlate well with geographic distance between them ($R^2 = 0.2$).

A canonical correspondence analysis (CCA) ordinated most sites along a line on a plane, the axes of which explained 82 (60 and 22) % of the variability in site community composition (Fig. 2). Algae-dominated sites mapped away from this line. *M. steenstrupii* was among the community members characterizing one end of the main distribution, whereas *M. vaginatus* mapped at the opposite end. CCA also identified several climatic parameters related to temperature as important community composition drivers; precipitation and geochemical factors were much less influential (Fig. 2). Simple correlation and multiple regression analyses applied to the distribution of individual taxa (table S3 also showed the relevance of temperature). This influence was most conspicuous for the pioneer, crust-forming oscillatorian cyanobacteria: Among all parameters, *M. steenstrupii* (Pearson coefficient = 0.81) and *M. vaginatus* (–0.56) abundances correlated best with mean annual temperature (MAT) (Fig. 2). In fact, there was a replacement in relative representation between these two cyanobacteria along the MAT range, with a tipping range around 13° to 15°C. This dominance shift was not absolute: Both cyanobacteria were detected in all samples, precluding a lack of dispersal as an explanation

[1]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA. [2]Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid, Spain.
*Corresponding author. E-mail: ferran@asu.edu

for the replacement. Rather, environmental selection likely lead to niche separation.

One of the comparative advantages of studying climate and geography patterns in microbes is that ecophysiological hypotheses can be tested directly by using cultures, adding a capacity for causal attribution to the correlative patterns detected statistically (*20*). To test the temperature segregation hypothesis, we used two cultivation avenues: the outcome of enrichment cultures as a function of incubation temperature and the determination of thermophysiological traits of newly isolated, pedigreed strains. We hypothesized, on the basis of results in Fig. 2, that incubation of field samples in standard minimal media maintained below the apparent inflexion range of 13° to 15°C should result in a preferential enrichment of *M. vaginatus* over *M. steenstrupii* regardless of the geographic origin of inoculum and that the opposite outcome would result from incubations at higher temperature. Results of such enrichments (Table 1) confirmed this prediction. A collection of 22 oscillatorian, rope-forming isolates (fig. S1) were pedigreed and determined to fall into three of the phylotypes recognized in the field communities: *M. vaginatus* (5 strains), *M. steenstrupii* (12 strains), and a minor group that could be assigned to the morphogenus "*Schizothrix*" (5 strains). These strains were subjected to a battery of tests designed to highlight any significant differences in their thermal physiology. All strains survived freezing, so this was not quantified further. By contrast, we found a differential response to high temperature. No *M. vaginatus* survived incubation for 12 days at 40°C, bleaching completely and losing viability, whereas all other strains remained viable (Fig. 3). Because of their clumpy, adherent, and slow mode of growth, standard microbiological growth curves based on subsampling were not possible; instead we carried out sacrificial, end-point-yield determinations. Although there was some level of interstrain variability, trends did appear. At low temperature (10°C, Fig. 3), *M. vaginatus* strains grew more than most strains of *M. steenstrupii*, the latter scoring poorly. In the psychrophylic range (5° to 15°C), average yields of all *M. vaginatus* were significantly higher ($P < 0.05$; *t* test) than those of *M. steenstrupii*. The situation reversed at high temperature, where four of the five best-performing strains at 35°C belonged to *M. steenstrupii* and *M. vaginatus* performed poorly or failed to grow. Between 30° and 35°C, *M. steenstrupii* strains grew significantly ($P < 0.05$; *t* test) more than those of *M. vaginatus*. Strains belonging to the *Schizothrix* clade, by contrast, performed uniformly well, indicating that this organism was not as influenced by temperature. The overall inference from our culture studies is that, as predicted by their geographic distribution, *M. vaginatus* represents an inherently more psychrotolerant taxon than *M. steenstrupii*, whose members are more thermotolerant.

The distributional and ecophysiological evidence gathered here allows us to logically predict that a few degrees of temperature increase

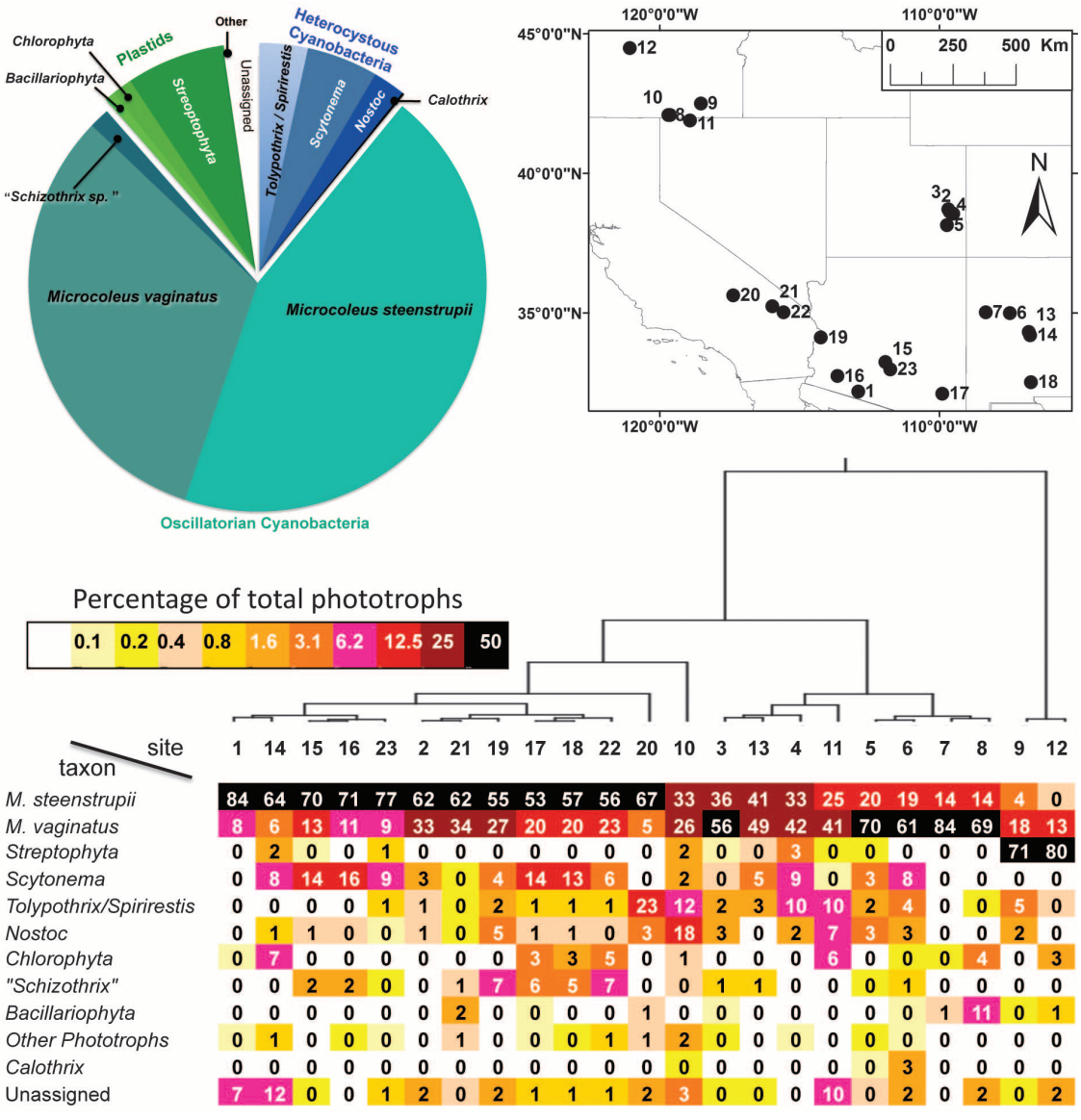
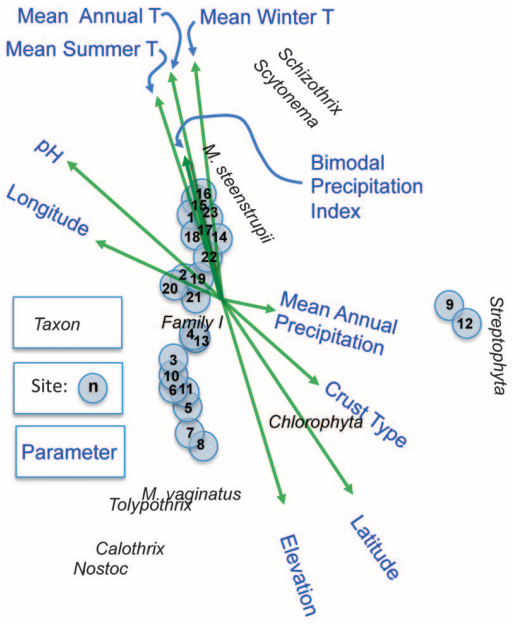


Fig. 1. Community composition of microbial phototrophs in arid soil biocrusts of the U.S. southwestern region. Site locations depicted as points in map (**top right**) encompassed several biogeographic regions (the Sonoran, Mojave, and Chihuahuan deserts, as well as the Great Basin and the Colorado Plateau). The pie chart (**top left**) shows an average distribution of phylotypes for all sites, each given equal weight. The explicit community composition for each site is in the heat map table (**bottom**), with sites arranged horizontally according to a similarity analysis (the similarity tree crowns the table heading).



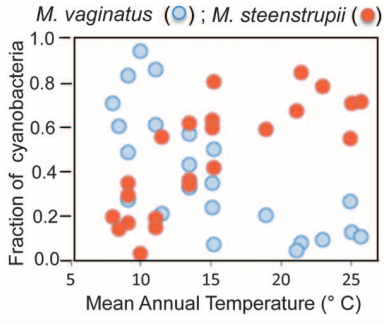


Fig. 2. Environmental factors driving community structure. CCA of the compositional and environmental data set for the survey of sites (**left**) yields a significant model (trace P = 0.008), where (with the exception of communities dominated by algae, sites 9 and 12) most communities ordinate along a continuum driven most strongly by vectors related to temperature and typified by the presence of one of the two major oscillatorian phylotypes at opposite ends in the distribution. CCA used data from all 23 sites and from 10 environmental variables preselected among those that showed some level of correlation with community composition in preliminary tests. An explicit depiction at the abundance of these two phylotypes as a function of site mean annual temperature (**right**) reveals a pattern of mutual exclusion or niche separation, with a tipping range around 13° to 15°C.

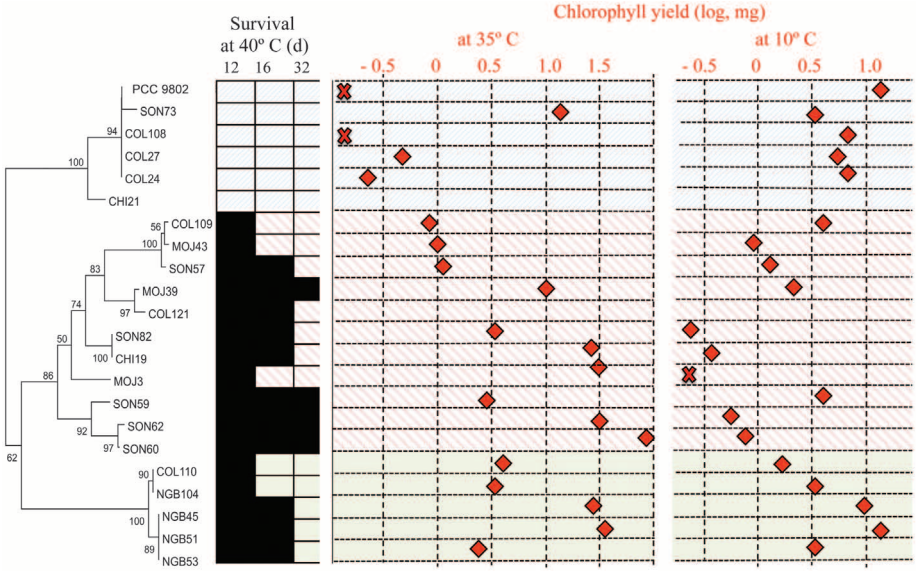


Fig. 3. Temperature physiology of biocrust-relevant strains. Strains tested are arranged vertically according to phylogenetic placement (a simplified tree is shown at left, with bootstrap support at the nodes, a full tree is in fig. S1): strains of *M. vaginatus* are at the top (data in blue background), those of *M. steenstrupii* in the middle (pink background), with "*Schizothrix*" strains below (green background). Charts show strain sensitivity to extreme heat (Survival at 40°C, positive for survival is darkened) and their ability to grow at low (yield at 10°C) and high (yield at 35°C) temperatures. Crosses indicate a value lower than the detection limit.

Table 1. Ratio of *M. vaginatus* over *M. steenstrupii* attained in enrichment cultures after 1 to 2 months of incubation as a function of temperature and inoculum source. Original ratios have their basis in data for the sites (shown in Fig. 1).

Inoculum source	Ratio			
	Original	After enrichment		
		at 4°C	at 15°C	at 30°C
Site 7 (Colorado Plateau)	6.00	27	11	0.20
Site 3 (Colorado Plateau)	1.55	48	35	0.16
Site 15 (Sonoran Desert)	0.19	9	2	0.03
Site 20 (Mojave Desert)	0.07	*	20	0.17

*Insufficient biomass development for counts

can result in a replacement in the dominance of *M. vaginatus* by *M. steenstrupii* on the cooler side of the current boundary (Fig. 2). In fact, warming in the southwestern United States is among the most marked on record (*21*), and a variety of climate models predict further increases in aridity and a warming in the order of about a degree per decade (*22*, *23*). This rate should translate in a complete replacement of *M. vaginatus* dominance from the area studied here in some 50 years. In this sense, terrestrial cyanobacteria respond differently than macroscopic vegetation in the area, whose biogeography is most heavily influenced by rainfall patterns, as is the sensitivity to global change of biocrust mosses (*24*). Given that both cyanobacterial species are found through the study range, populational shifts should proceed unimpeded by dispersal. Although some of the physiological and genetic properties of *M. vaginatus*, long recognized as important, have been determined (*25*–*28*), practically nothing is known about *M. steenstrupii*. Therefore, the consequences of replacement in such keystone species cannot be predicted at this time. In the short term, *M. steenstrupii* should also be considered as inoculum by land managers, in addition to *M. vaginatus*, for soil management and restoration efforts in arid lands.

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Supplementary Materials

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Materials and Methods
Fig. S1
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References (*29*–*42*)

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Mechanism of Eukaryotic RNA Polymerase III Transcription Termination

Soren Nielsen, Yulia Yuzenkova, Nikolay Zenkin*

Gene expression in organisms involves many factors and is tightly controlled. Although much is known about the initial phase of transcription by RNA polymerase III (Pol III), the enzyme that synthesizes the majority of RNA molecules in eukaryotic cells, termination is poorly understood. Here, we show that the extensive structure of Pol III–synthesized transcripts dictates the release of elongation complexes at the end of genes. The poly-T termination signal, which does not cause termination in itself, causes catalytic inactivation and backtracking of Pol III, thus committing the enzyme to termination and transporting it to the nearest RNA secondary structure, which facilitates Pol III release. Similarity between termination mechanisms of Pol III and bacterial RNA polymerase suggests that hairpin-dependent termination may date back to the common ancestor of multisubunit RNA polymerases.

Termination of transcription is an obligatory step after synthesis of the transcript, which leads to dissociation of RNA polymerase (RNAP) and the transcript from the template DNA. However, evolutionarily conserved multisubunit RNAPs from bacteria and Archaea and three eukaryotic RNAPs use different mechanisms to terminate transcription (*1*–*3*). Eukaryotic polymerase III (Pol III) terminates after synthesis of a poly-U stretch (*4*, *5*), and most studies have focused on the efficiency of recognition of the poly-T (on the nontemplate strand) termination signal (*6*, *7*). However, the events leading to termination on the poly-T signal; that is, dissociation of Pol III from the template, are not known.

We used in vitro–assembled elongation complexes, which have been successfully used to investigate various RNAPs (*8*–*11*), to examine this problem. These complexes—assembled with purified RNAP, synthetic complementary template and nontemplate DNA strands, and RNA—allow us to skip the initiation step, therefore excluding any accessory factors from the reaction. We immobilized complexes on streptavidin beads through biotin on the 5′ end of the nontemplate strand, and we labeled RNA by incorporation of radioactive nucleoside monophosphates (scheme in Fig. 1A) (*12*). We analyzed transcription through poly-T signals of various lengths by purified *Saccharomyces cerevisiae* Pol III. As seen in Fig. 1, A and B, transcripts finishing after T_6 to T_{10} were transcribed (compare to fig. S1).

To test if transcripts ending with a poly-U stretch were released from the template as a result of termination, we analyzed RNA in the

Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, Baddiley-Clark Building, Richardson Road, Newcastle upon Tyne, NE2 4AX, UK.
*Corresponding author. E-mail: n.zenkin@ncl.ac.uk

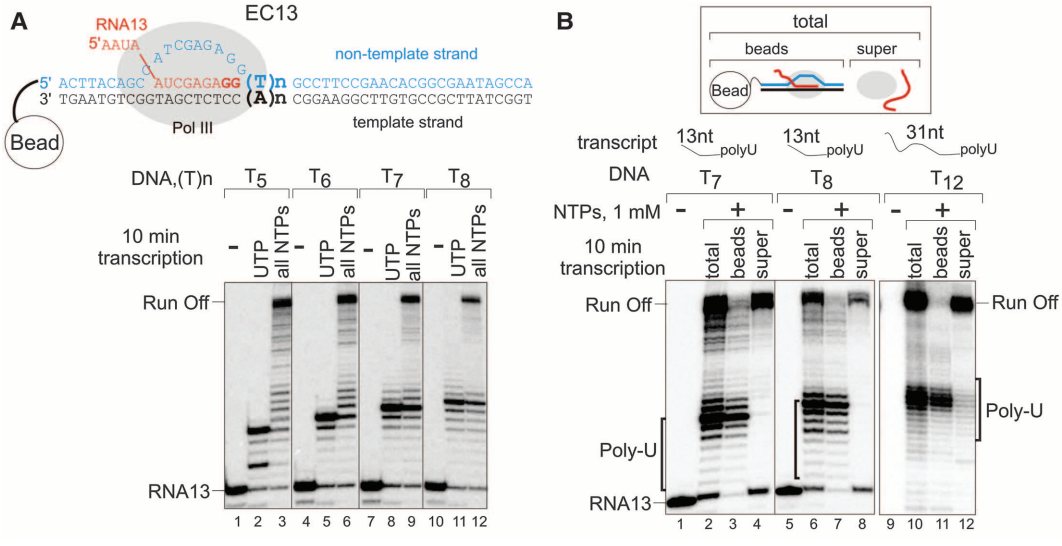

Fig. 1. Pol III pauses on the poly-T signal but does not terminate. (**A**) Scheme of assembled elongation complexes (EC13) containing 13-nt-long RNA (RNA13) is shown at the top. RNA was radiolabeled at the 3′ end of guanosine monophosphates (bold) (*12*). Complexes were immobilized on beads through biotin on the 5′ end of the nontemplate strand. Transcription occurred for 10 min on templates with poly-T signals of different lengths in the presence of 1 mM of either uridine triphosphate (UTP) or all nucleoside triphosphates (NTPs). Hereafter, black vertical lines separate parts of a single gel that were brought together. (**B**) After 10 min of transcription on the templates depicted above the gels (*12*), released transcripts (supernatant, "super") were separated from transcripts that remained in the immobilized complexes ("beads") (scheme in the frame above the gels). The length of RNA preceding the poly-U tract is depicted above the gels.

Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities

Ferran Garcia-Pichel, Virginia Loza, Yevgeniy Marusenko, Pilar Mateo and Ruth M. Potrafka

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Desert Soil Shuffle

Soil microorganisms make up a substantial fraction of global biomass, turning over carbon and other key nutrients on a massive scale. Although the soil protects them somewhat from daily temperature fluxes, the distribution of these communities will likely respond to gradual climate change. **Garcia-Pichel *et al.*** (p. 1574, see the cover; see the Perspective by **Belnap**) surveyed bacterial diversity across a range of North American desert soils, or biocrusts—ecosystems in which photosynthetic bacteria determine soil fertility and control physical soil properties such as erodability and water retention. Most of the sites were dominated by one of two cyanobacterial species, but their relative proportions were controlled largely by factors related to temperature. Laboratory enrichment cultures of the two species at different temperatures also showed temperature as a primary determining factor of bacterial diversity. It is unknown if temperature will affect the distribution of other soil microorganisms, but the marked shifts of these two keystone bacterial species suggest further change is in store for these delicate ecosystems.