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Microbial spatial heterogeneity in microbiotic crusts in Colorado National Monument. I. Algae

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MICROBIAL SPATIAL HETEROGENEITY IN MICROBIOTIC CRUSTS IN COLORADO NATIONAL MONUMENT. I. ALGAE

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ABSTRACT.—Spatial heterogeneity in visually similar sites under Utah juniper canopy in Colorado National Monument was examined. Sample sites were arranged in a transect 24 m long, such that distances between samples were 0.013 m, 0.03 m, 1.0 m, 12 m, and 24 m. Twenty-five taxa of algae were observed, mostly belonging to Cyanophyta. Algal density varied by more than an order of magnitude within the 46 samples examined. The coefficients of variation for each distance class were very similar, demonstrating that algal patchiness can be as significant on a scale less than 0.013 m as it is on a scale of 24 m. Goodall's random pairing analysis of spatial pattern supported this conclusion by indicating that the minimal area for sampling soil algal crust populations at this site was equal to or less than 0.013 m. Because of the microscale heterogeneity in algal communities in this study, we recommend that future researchers take composite samples if they wish to quantify algae of microbiotic crusts.

Key words: algae, soil; cryptogamic crusts; microbiotic crusts; heterogeneity; pinyon-juniper; Colorado National Monument.

In the last half century numerous ecologists have studied natural distribution patterns of plants. Spatial distributions of populations are regular, random, or aggregated, with most species having aggregated distributions (Ludwig and Reynolds 1988). An issue of key concern to those interested in distribution is the size (length, area, or volume) of the sample taken to study distribution. Normally one wishes to use the smallest sample size possible that will still be representative of the population as a whole. In plant populations, where samples are usually two dimensional, this representative sample size is called the minimal area (Goodall 1952, 1961). This area should be reasonably homogenous with regard to the species being studied, such that variation among replicate samples will be independent of the distance between them (Goodall 1961).

Despite the numerous papers on quantification of plant patterns and/or appropriate sample sizes for vascular plant communities (Bartlett 1964, Fisser 1969, Goodall 1974, Greig-Smith 1952, 1961, 1964, Morisita 1959, Pielou 1964), little attention has been given to the study of heterogeneity in soil algal communities or the related question of appropriate sample size. Soil microbial ecologists have long been aware of heterogeneity in bacterial populations in the soil and generally take composite samples to avoid this problem (James and Sutherland 1939). In a recent study of soil algae of crusted soils of the Lower Columbia Basin (Johansen et al. 1993) we observed considerable heterogeneity in algal communities in undisturbed sites. In particular, we found that samples taken within 5 cm of each other appeared to be as different as samples taken several meters away. There was no correlation over time between algal densities at proximal sites, even though vascular plant cover demonstrated very clear patches on a scale much larger than 5 cm.

This study is an outgrowth of the work conducted in shrub-steppe in Washington. We had two goals at the outset of the project: (1) to document the scale of algal heterogeneity in soils that macroscopically appeared to be homogenous, and (2) to report on the floristics of the soil algal community in a pinyon-juniper community of Colorado National Monument. We were not seeking to discover the variability that existed in crusted vs. noncrusted soils, or the range in density under various types of vascular plant cover; rather we wished to estimate

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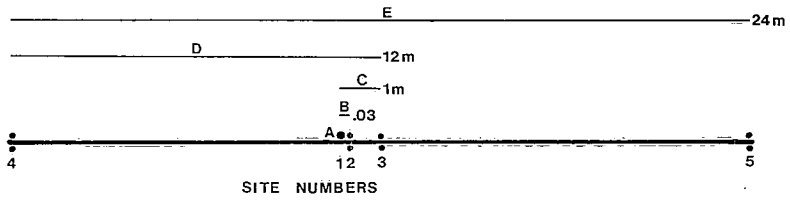

Fig. 1. Study site in canopy of Utah juniper in Colorado National Monument, Colorado. Site numbers (1–5) are given under the transect; groups of samples (A–E) and distances are given above the transect. Site 1 contained 16 samples; sites 2–4 contained 8 samples (two clusters of 4 at each point); site 5 contained 6 samples (two clusters of 3).

the algal heterogeneity within what one would consider in the field to be identical sites. A companion study on the spatial heterogeneity of bacteria and actinomycetes from the same site is reported by Wheeler et al. (1993). Recommendations regarding soil crust sampling methodology follow from this study.

MATERIALS AND METHODS

The study site is located in a pinyon-juniper community in Colorado National Monument, Colorado, USA. Sampling was conducted in early January 1989. Five plots on a transect 24 m long were chosen such that (1) they all contained pediceled, nonlichenized, soil algal crusts of similar appearance; (2) they all were under partial canopy of *Juniperus osteosperma* (Torr.) Little (Utah juniper); and (3) they were positioned such that plots or groups of plots were increasingly further apart, with distances increasing progressively such that the smallest and farthest distances differed by more than three orders of magnitude. These plots were designated sites 1–5 (Fig. 1). In each plot 8–16 replicate samples were removed by depressing a sterile plastic test tube (13-mm diameter) into the soil to remove a minimally disturbed core.

Numerous methodologies have been used to estimate abundance of algae in microbiotic soil crusts. These methods include chlorophyll *a* content (Beymer and Klopatek 1991, Klopatek in press), direct counts using fluorescence microscopy (Johansen and Rushforth 1985, Tchan 1952), dilution plate techniques (Johansen et al. 1993, Rayburn et al. 1982), and the moistened soil method (Johansen et al. 1984, 1993, St. Clair et al. 1986). All methods have advantages and disadvantages, some of which have been discussed elsewhere (Metting 1981). In this study, floristics was studied using several methodologies, while heterogeneity was examined using the dilution plate technique.

To make dilution plate cultures, the cores were returned to the laboratory. The plastic tubes were broken open such that 0.5 g of surface crust could be subsampled from each tube. These subsamples were subsequently plated on solidified Bold's Basal Medium (Bold 1949) in 10^3 and 10^4 dilutions. Viable counts of algae were made after 30 days of incubation. Although we could not enumerate by species, algal colonies were identified as either belonging to Cyanophyta or Chlorophyta/Xanthophyceae. In all cases the 10^3 dilution plates were used.

Site 1 contained 16 samples and was plated within one month of collection, with 3 replicate plates per sample. The other sites were plated within two months of collection, with 4 replicates per sample. Dominant algae were isolated from the dilution plates and subsequently identified using standard texts (Ettl 1978, Geitler 1930–32, Komarek and Fott 1983). Additional cyanobacteria were identified from soils moistened with Chu 10 Medium (Chu 1942). Diatoms were identified from permanent Naphrax mounts of selected soil samples (Johansen et al. 1982).

Individual sites were compared using analysis of variance (ANOVA) for an unbalanced, nested design (samples nested in sites) using the SAS GLM procedure (SAS Institute, Inc. 1985). Tukey's Honest Significant Difference (HSD) procedure was used to compare means when the ANOVA model was significant. Sites were progressively grouped to study heterogeneity of different-sized sample areas (Fig. 1). The first group (A) included samples from site 1, which were collected adjacent to each other such that samples were 0.013 m apart (the diameter of the sample tubes). The second group (B) included samples from sites 1 and 2 and represented a sample distance of 0.03 m. The third group (C) included samples from sites 1, 2, and 3 (sample distance = 1 m), the fourth group (D) included

TABLE 1. Algal taxa found in soils under canopy of Utah juniper in Colorado National Monument. Identifications were made from unialgal isolations from dilution plates (BBM), soil moistened with Chu's 10 media (Chu), and permanent diatom mounts (Naphrax).

Taxon	BBM	Chu	Naphrax
CYANOPHYTA			
Arthrospira jenneri (Kuetz.) Stitz.	X		
Calothrix parietina Thuret		X	
Chroococcus lithophilus Ercegovic		X	
Lyngbya allorgei Fremy		X	
Lyngbya nordgardhii Wille	X	X	
Microcoleus vaginatus (Vauch.) Gom.		X	
Nostoc microscopicum Carmichael	X		
Nostoc paludosum Kuetzing	X	X	
Nostoc punctiforme (Kuetz.) Hariot		X	
Oscillatoria foreaui Fremy		X	
Oscillatoria hamelii Fremy	X	X	
Plectonema radiosum (Schiederm.) Gomont	X		
Pseudoanabaena africana Schwabe & Simon.	X		
Schizothrix calcicola (Ag.) Gomont		X	
Schizothrix fragilis (Kuetz.) Gomont	X	X	
Scytonema burmanicum Skuja	X	X	
Scytonema coactile Montagne	X		
CHLOROPHYTA			
Chlorella protothecoides Krug.	X		
Chlorhormidium flaccidum (Kuetz.) Fott	X		
Friedmannia israelensis Chant. & Bold	X		
Lobococcus irregularis (Boye-Pet.) Reis.	X		
CHRYSOPHYCEAE			
Chrysophyte cysts			X
XANTHOPHYCEAE			
Chloridella Pascher	X		
BACILLARIOPHYCEAE			
Hantzschia amphioxys (Ehr.) Grunow		X	X
Navicula mutica Kuetzing		X	X
Navicula mutica var. *cohnii* (Hilse) Grunow			X
Pinnularia borealis Ehrenberg		X	X
Pinnularia borealis var. *rectangularis* Carlson			X

samples from sites 1, 2, 3, and 4 (sample distance = 12 m), and the fifth group (E) contained all samples (sample distance = 24 m).

The degree of aggregation in the algal populations of each of the above-mentioned groups was measured by determining the index of dispersion (variance-to-mean ratio; see Ludwig and Reynolds 1988). The coefficient of variation (standard deviation-to-mean ratio) was also determined for each group of samples. The random pairing technique for analysis of spatial pattern designed by Goodall (1974) was applied to the data. This technique is mathematically related to grid analysis or hierarchical analysis of variance (Goodall 1961, Greig-Smith 1952). It entails randomly selecting pairs of samples that are set distances apart until all samples are assigned to pairs. The mean variance of all pairs belonging to a distance class is computed for each class. Three randomizations were conducted with the data from this study.

RESULTS

A total of 25 taxa were observed, mostly cyanobacteria (Table 1). Most of these algae are typical soil forms, although some have not been reported in other floristic surveys of soil algae in the Great Basin and Colorado Plateau (Anderson and Rushforth 1976, Ashley et al. 1985, Johansen et al. 1981, 1982, 1984). The use of several different methods likely increased the number of taxa observed, even though we did not make enough isolations to ensure that we identified all chlorophytes and xanthophytes present.

The density of algae varied by an order of magnitude between individual samples, ranging from 7.0×10^3 to 9.2×10^4 cells/g dry weight soil. This is fairly homogenous compared to studies in which soils under a number of different canopy types are taken. For example, Johansen et al. (1993) found a range spanning three orders of magnitude in an undisturbed sagebrush community in Washington. However, it should be remembered that the data reported in the present study are from a single sample date rather than from multiple sample dates throughout the year as in the Washington study.

Analysis of variance revealed significant differences between the five sites, as well as significant variation between samples ($p < .001$). The Tukey multiple range test showed that site 1 had significantly greater algal density than sites 2, 3,

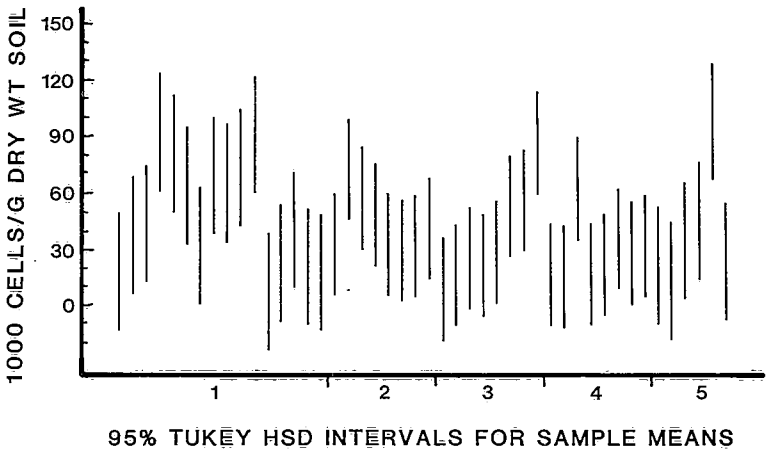

Fig. 2. Tukey Honest Significant Difference confidence intervals for counts of total algae in all samples from sites 1–5. Pairs of sample means are significantly different when their confidence intervals do not overlap.

and 4. Site 5 was not significantly different from any site. Two other ANOVAs were run, one using densities of Chlorophyta/Xanthophyceae, the other using densities of Cyanophyta. Results identical to those using total algal density were obtained.

Although there were significant differences between sites, it is difficult to consider these differences meaningful. Samples were taken from very similar sites with no visible differences. Prior small-scale impacts, such as trampling by grazing animals or defecation, may have led to the heterogeneity between sites. Runoff patterns may also have influenced crust development. The results of the Tukey multiple range test for individual samples demonstrate the variability that can exist between adjacent samples within sites (Fig. 2).

The index of dispersion (variance to mean ratio) is a measure of randomness of distribution. A value of 1.0 indicates a totally random distribution. Values much greater than 1.0 indicate aggregation. In this study, groups of all sizes (groups A–E; see Fig. 1) showed a high degree of clumping, with Cyanophyta having a more aggregated distribution than Chlorophyta and Xanthophyceae (Table 2).

The coefficient of variation (standard deviation to mean ratio) can be used to make comparisons between the amount of heterogeneity within one site and that within other sites, even though the sites may have different sample sizes and means. The larger the coefficient, the greater the degree of heterogeneity. If heterogeneity increased as the distance between samples increased, then the coefficient of variation would increase as one included samples from increasingly larger areas. Our data were surprising in that the coefficient of variation remained nearly constant for both Chlorophyta and Cyanophyta for all sample groups (Table 2). This result implies that the heterogeneity among samples 0.013 m apart (group A) was as great as that among samples taken from a transect 24 m long (group E). This indicates that soil algal patchiness can be as significant on a scale of 0.013 m as it is on a scale of 24 m (over three orders of magnitude).

TABLE 2. Index of dispersion and coefficient of variation for Chlorophyta/Xanthophyceae (CHLOR) and Cyanophyta (CYANO) for the five groups of samples.

	Index of dispersion		Coefficient of variation	
Group—Distance	CHLOR	CYANO	CHLOR	CYANO
A—0.013 m	6.4	13.6	0.46	0.86
B—0.03 m	5.2	11.4	0.43	0.78
C—1.0 m	4.8	13.7	0.43	0.88
D—12 m	4.7	13.9	0.43	0.93
E—24 m	5.2	14.2	0.45	0.95

The 16 samples comprising site 1 (group A) were collected from a four-by-four grid. By illustrating the density of organisms in thousands of cells per gram dry weight soil, we could examine aggregation patterns in a square 5.2 × 5.2

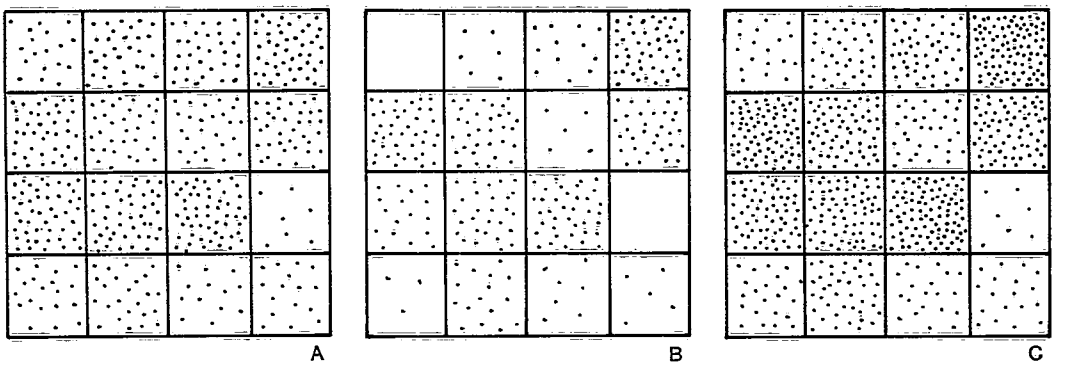

Fig. 3. Mean soil algal density for 16 adjacent samples in site 1 (group A): A, Chlorophyta and Xanthophyceae; B, Cyanophyta; C, total algae.

TABLE 3. Analysis of spatial pattern using Goodall's random pairing technique. Three separate analyses were run using density data for total algae.

	First analysis		Second analysis		Third analysis	
Spacing	df	Variance	df	Variance	df	Variance
0.013 m	5	296.77	7	242.92	4	13.24
0.03 m	4	134.49	4	170.65	5	79.66
1.0 m	6	60.78	4	118.29	5	139.55
12 m	4	172.40	4	34.00	4	25.38
24 m	3	147.30	3	191.86	4	110.12

cm (Fig. 3). It appears that some adjacent samples had similarly high densities (five samples in the middle left, two samples in the upper right). However, grid size is too small to evaluate patchiness on this scale with confidence.

Our sampling design lent itself to analysis of spatial pattern by random pairing of samples, a method proposed by Goodall (1974) as being superior to grid analysis. Our data are dissimilar to data generally used in this type of analysis in that they were not collected at random throughout the pinyon-juniper community, but were rather collected in homogenous crusted sites under juniper canopy. Thus, the large-scale patterns of algal density that may be associated with differences in vascular plant cover would not be revealed in our data. We considered the analysis useful inasmuch as it provided a completely different approach to the question of heterogeneity in algal densities.

Goodall's technique suggests that minimal area is represented by that distance class having the largest mean variance. Normally mean variance will increase as the distance class increases until the minimal area is reached, at which point mean variance levels off or even drops with increasing distance. Two of the three random pairing analyses indicated that the minimal area for algal density in the soil crusts of our study was 0.013 m or less (Table 3). The third randomization indicated that the scale of patchiness was about 1.0 m. The third randomization may either be erroneous or suggest that the minimal area lies between 0.013 m and 1.0 m.

DISCUSSION

Most evidence from this study indicates that the minimal area for sampling soil algal crust populations is about equal to or less than 0.013 m, and that even in seemingly identical sites algal abundance can vary by more than an order of magnitude when this minimum area is used. When dissimilar sites are examined, other scales of patchiness and greater ranges of algal abundance probably would be observed. Four scales of patchiness are likely: (1) the microscale patchiness within similar sites observed in this study, (2) a small scale of patchiness that reflects the type and extent of vascular plant cover, (3) a large-scale pattern that reflects differences in disturbance levels due to grazing livestock, off-road vehicles, or rangefire, and (4) macroscale patterns due to differences in soil type, altitude, precipitation, annual temperature regimes, and type of vascular plant communities resulting from the sum of these factors.

In past studies researchers have been most interested in eliciting the two largest-scale patterns (Anderson and Rushforth 1976, Cameron 1960, Johansen and St. Clair 1986,

Johansen et al. 1982, 1984, 1993). However, to accurately determine patterns on a large scale, adequate and representative samples must be taken. Consequently, an understanding of the smaller scales of patchiness is necessary before a proper sampling procedure can be selected. This study is preliminary in nature because only the smallest scale of pattern is examined. The significant differences existing between sample sites indicate that errors could easily be made in other studies because of seemingly random (or at least unexplained) microscale differences in algal abundance.

Sampling of microbiotic crusts is destructive, and so taking large samples that would lessen the problem of microscale patchiness is undesirable. Large surface soil samples are also more difficult to process and subsample. We recommend that future workers take composite samples consisting of numerous samples of similar area and depth. If the small-scale patterns of algal abundance associated with vascular plant cover are to be explored, composite samples should consist of subsamples taken at random intersections of a sampling grid placed over large quadrats. If the larger-scale patterns are under study, then it may be better to sample systematically or randomly along long transects, combining numerous small samples taken along the transect into the composite samples to be examined. Regardless of the method of quantification (chlorophyll *a*, direct counts, dilution plate counts, or moistened soil method), this type of composite sampling would be superior to taking small or large individual samples from the different areas of interest.

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