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Effects of Recreational Impacts on Soil Microbial Communities

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ABSTRACT / The functional diversity of soil microbial communities in heavily impacted subalpine campsites and adjacent undisturbed areas was compared using the Biolog method of carbon utilization profiles. Principal components analysis of patterns and level of microbial activity indicate that microbial communities differentiate in response to disturbance in the top 6 cm of soil, while below 6 cm there were no recognizable differences between disturbed and undisturbed soil communities. Analysis of the factors that differentiate the upper microbial communities between disturbed and undisturbed sites revealed that the percent of total carbon sources utilized was significantly less in the disturbed (54%) than in undisturbed areas (95%). Carbon substrates important in the discrimination between soil communities include plant, invertebrate, and microbial derivatives that could not be metabolized by microbial communities from disturbed sites. Comparisons of total culturable actinomycetes, bacteria, and fungi reveal no difference in overall number of colony forming units (CFU) on disturbed and undisturbed sites, but a marked decrease in actinomycetes on disturbed sites. Biolog and spread-plate data combined indicate a shift in the structure and function of the microbial community in campsite soils, which may be a useful indicator of soil community disturbance.

Soil microorganisms play key roles in determining the structure and function of plant communities. They are responsible for nitrogen fixation, nutrient cycling, immobilization of essential nutrients, and production of phytohormones (Perry and Amaranthus 1990, Turkington and others 1988, Curl and Truelove 1986). In addition, the composition of the soil microbial community can affect plant growth and the competitive outcome between plants, altering species composition, as well as directly affecting a species' ability to colonize an area (Bever 1994, Ingham 1994, Chanway and others 1991, Turkington and others 1988, Tranqullini 1979).

Despite its ecological significance, the response of soil microbial communities to disturbance is poorly understood. Mycorrhizae have been shown to be sensitive to anthropogenic disturbances (Allen and Friese 1992), but few data exist on microbial community structure response to disturbance (Zak and others 1992). Disturbance that includes loss of vegetation can have a significant impact on soil systems, leaving soils open to wind and water erosion. Loss of surface organic matter and plant root exudates decreases carbon substrate availability for microbial communities. If compaction of the soil is combined with devegetation, the loss of aggregate stability and reduction of pore space can affect soil microbial communities through their effect on water-holding capacity and aeration of the soil (Schimel and Parton 1986, Parton and others 1987, Wardle 1992).

Our research compares soil microbial communities of heavily impacted campsites to those of adjacent undisturbed sites. The criteria used to distinguish heavy impacts is borrowed from Marion and Merriam (1985): severe compaction of soils, loss of vegetation and organic matter, and exposed soil. Studies in natural areas have documented an increase in bulk density on campsite soils ranging from 15% (Cole 1982) to 34% (Monti and Mackintosh 1979) compared to adjacent sites. The physical changes in the soil environment that could affect microbial communities include the elimination of or decrease in pore space (Marshall and Holmes 1979), and the accompanying changes in soil gas and moisture relationships (Kuss 1986).

Functional diversity of microbial communities can be measured with carbon utilization profiles (Zak and others 1994, Garland and Mills 1991, Biolog, Inc. 1993). Standardized soil slurries are incubated with 95 separate carbon sources and a control solution with no carbon. Microbial activity is indicated by the reduction of a tetrazolium dye, which can be quantified with spectrophotometric measurements. This method has been used to measure differences in community structure in soils from beech forests and adjacent meadows (Winding 1994); in comparisons of soil, freshwater, and

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hydroponic systems; in a coastal lagoon–ocean inlet gradient (Garland and Mills 1991); in a basalt aquifer (Zheng and Kellogg 1992); in soils from six arid plant communities (Zak and others 1994); and in soils from the rhizosphere of three different tree species and adjacent root-free soil (Grayston and others 1994).

The site of this work was Heart Lake, in Mineral County, on the Superior Ranger District of the Lolo National Forest, western Montana, USA (46°57′N, 114°58′W). Heart Lake is a subalpine lake (1770 m elevation) at the base of a cirque basin in the northern Bitterroot Mountains of the Rocky Mountain Range. It is typically free of snow between July and October and receives an average annual precipitation of 208 cm with a mean annual temperature of 2.2°C. The soils in the area are Andic Cryochrepts–Loamy Skeletal, Mixed of the Belt geological formation. The dominant tree species is subalpine fir (*Abies lasiocarpa*), with lodgepole pine (*Pinus contorta*), whitebark pine (*P. albicaulis*), western white pine (*P. monticola*), Engelmann spruce (*Picea engelmannii*), and mountain hemlock (*Tsuga mertensiana*) present in smaller quantities. The understory near the study sites is dominated by beargrass (*Xerophyllum tenax*) and huckleberry (*Vaccinium globulare*). Vegetation structure consists of scattered clumps or single trees with a dense shrub layer and patches of open meadow.

Methods

The functional diversity of microbial assemblages was measured from soil collected from four campsites and adjacent undisturbed areas in October 1994, at the end of the growing season. One soil core, 2.2 cm in diameter and to the depth of impenetrable soil (ca. 18 cm), was extracted from the core area of each of the four campsites (sites 1, 3, 5, and 7) and undisturbed areas adjacent to the campsites (sites 2, 4, 6, and 8). Cores were divided into an upper and a lower layer, based on two distinctly colored horizons: dark brown upper layer (A horizon) and yellow–brown lower layer (Bw horizon). The average thickness of the upper layer was 6 cm and of the lower layer was 11.3 cm. Samples were aseptically collected and placed in sterile plastic bags on ice.

Ten grams (wet weight) of composited soil from each layer of each core were removed, placed in sterile Whirl-Pak bags containing 90 ml of 0.1% (w/v) sodium pyrophosphate, and sonicated (Branson 2200, Branson Equipment Co., Shelton, Connecticut) at a low level for 10 min at 4°C. Aliquots were diluted in sterile phosphate-buffered saline (pH 7.5) and plated by spread plate onto a low nutrient agar (R2A Agar, Difco Laboratories, Detroit, Michigan) to estimate the numbers of culturable bacteria and actinomycetes. After 36 h of incubation at 25°C, the number of colony-forming units (CFU) was determined, and these values were used to standardize the amount of inoculum added to each well of Biolog GN plates (Biolog Inc. Hayward, California).

For inoculation, a second soil sample (10 g) from each core layer was sonicated and diluted as described above to give approximately 1.0×10^4 CFU/well. This dilution also minimized the color of the soil solution. One-hundred-microliter aliquots of diluted sample were added to each of the 96 wells of the plate. Microbial activity in a well causes the reduction of the tetrazolium dye, which results in an increase in absorbance, as measured by optical density. The microtiter plates were incubated at 20°C in a covered plastic container, and the absorbance (570 nm) was read at 24- to 48-h intervals for 10 days, until mold began to grow in some of the wells. Previous studies with Biolog plates use a shorter observation time—50 (Garland and Mills 1991) to 72 h (Zak and others 1994). Zak and others (1994) recommended the following criteria to establish the length of the observation period: (1) the rate of color development; (2) the number of substrates used; (3) a color change in the control well; and (4) fungi observed growing in the wells. In this study, the length of observation period was established by the first appearance of fungus growing in the wells. Our objective was to measure activity of microbial populations that might be present in small numbers, resulting in a lag time before activity is detected.

Principal components analysis (PCA) was used to examine the relationship between patterns of microbial activity (as indicated by absorbance readings) on disturbed and undisturbed sites (Goldbeck 1986). PCA is an indirect gradient analysis that reduces a multidimensional data set into a smaller number of axes. Based on correlations between patterns of activity in soil samples, data points are plotted along principal components axes, highlighting the relationship between samples (Pielou 1984, Gauch 1982, Chapter 4). Since no environmental data are included in the analysis to further distinguish sites, further interpretation is necessary to determine whether ordination axes have biological meaning. Average well color development (AWCD) was calculated by averaging absorbance for each of the 95 carbon sources on a single plate (Garland and Mills 1991). The absorbance of the control well was subtracted from the 95 readings to account for dye reduc-

Table 1. Total viable counts (CFU/g) on R_2A agar, number of colony types after 14 days of incubation (20°C), and actinomycetes (%)

Upper layers	CFU/g	Colony types (N)	Actinomycetes (%)	Lower layer	CFU/g	Colony types (N)	Actinomycetes (%)
Campsites							
1	6.6×10^6	6	<2.0	1	9.9×10^6	7	33
3	5.5×10^5	5	<1.0	3	6.7×10^5	7	<1.0
5	7.8×10^6	8	<1.0	5	8.6×10^6	7	25
7	1.1×10^7	10	<1.0	7	5.6×10^5	4	<1.0
Mean	6.5×10^6	7.25	<1.25	Mean	4.9×10^6	6.25	~15
Undisturbed sites							
2	2.1×10^7	10	53	2	4.7×10^6	9	82
4	4.3×10^5	5	<1.0	4	1.9×10^6	5	76
6	8.2×10^5	8	13	6	2.8×10^6	6	80
8	1.2×10^6	7	45	8	1.4×10^6	8	39
Mean	5.9×10^6	7.5	~30.75	Mean	2.7×10^6	7.0	69

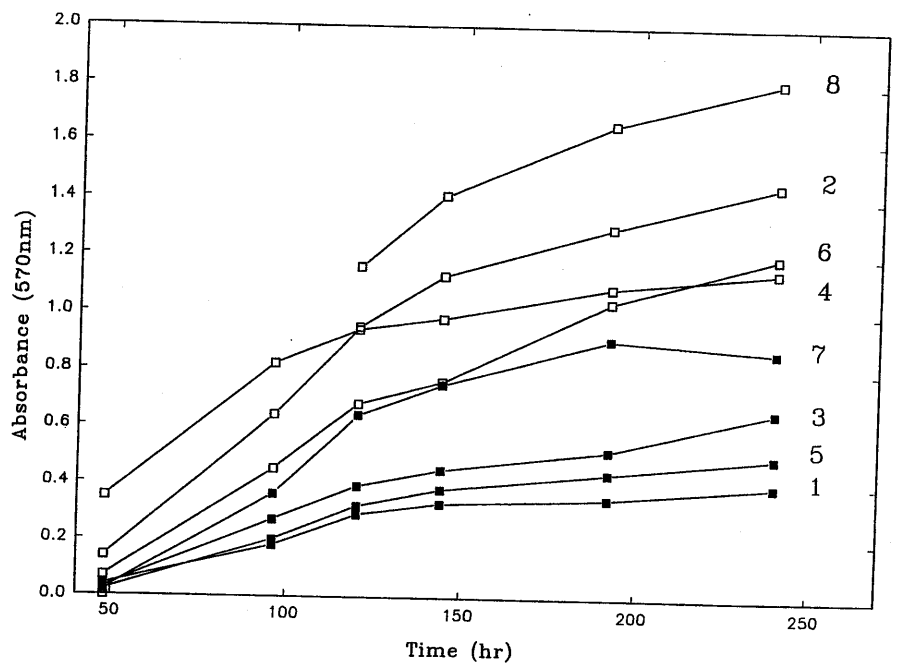

Figure 1. Average well color development in disturbed (■) and undisturbed (□) upper soil layers.

tion from the possible introduction of exogenous nutrients.

Results

There were no significant differences in numbers of colony forming units (CFU) between campsites and undisturbed sites ($F = 0.22$, $P = 0.65$), or between upper and lower layers ($F = 0.60$, $P = 0.45$) (Table 1). There were also no significant differences in number of colony types between campsite and undisturbed sites ($F = 0.61$, $P = 0.45$), or between upper and lower layers ($F = 0.26$, $P = 0.61$). Actinomycete densities declined significantly with disturbance ($F = 9.25$, $P = 0.01$), and with disturbance ($F = 20.06$, $P = 0.001$). The percent of CFU that were actinomycetes was 30.75% in the upper layer of undisturbed sites and about 1.25% in the upper layer of disturbed sites, while in the lower layers the percentage dropped from 69.25% on undisturbed sites to 15% on campsites.

Figure 1 summarizes AWCD for microbial communities from the upper layer of campsites and undisturbed sites at 48, 96, 120, 144, 192, and 240 h of incubation time. Microbial activity, as measured by AWCD, contin-

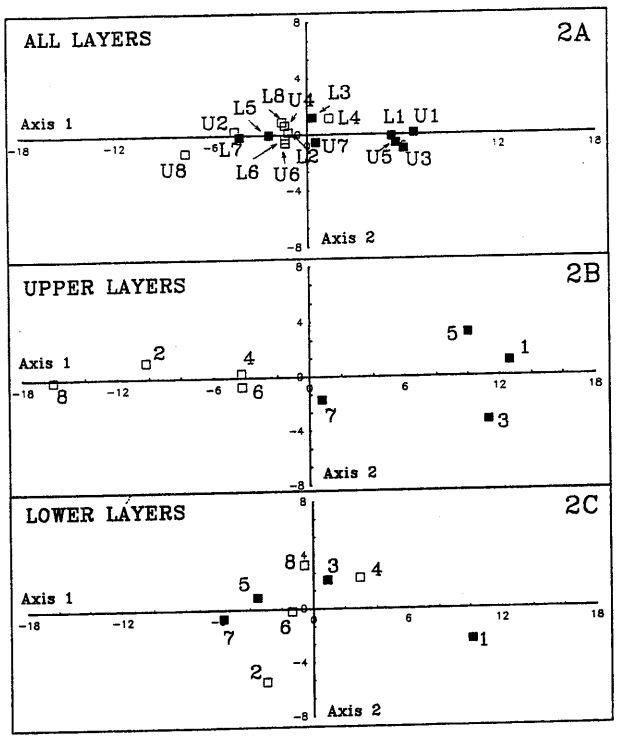

Figure 2. Principal components analysis of carbon utilization profiles from **(A)** upper (U) and lower (L) soil layers combined, **(B)** upper soil layers, and **(C)** lower soil layers of disturbed (■) and undisturbed (□) sites.

ued to increase with time even at the later sampling points and was consistently higher for undisturbed sites than for campsites. A decline in AWCD from site 7 samples between 192 and 240 h was the result of a color change in the control well between 192 and 240 h. Analyses of microbial activity were done on OD readings at 192 h to maximize microbial population growth and before interference from external sources affected absorbance measurements.

Principal components analysis, done on samples from both upper and lower layers of soil after 192 h of incubation time, showed 30% of the variance between sites accounted for on the first axis, and an additional 9.4% accounted for on the second axis (Figure 2A). All but one of the samples to the right of axis 2 were from disturbed sites, although samples from two disturbed sites fell to the left of axis 2. Comparing microbial activity from only the upper layer of soil, the first axis accounted for 43.5% of the variance between sites, and the second axis accounted for an additional 14.2% (Figure 2B). Samples from disturbed sites clustered to the right of axis 2, and samples from undisturbed sites clustered to the left of axis 2. In PCA on samples from the lower layer of each soil core, the first axis accounted for 26% of the variance, and the second axis accounted for an additional 18%. There was no distinction between disturbed and undisturbed microbial communities in lower layers of the soil.

Table 2. Carbon sources responsible for PCA pattern for surface samples, incubated 192 hours[a]

Carbon source	r	Sites with OD reading >0.5
m-Inositol	−0.976	2, 4, 6, 7, 8
L-Arabinose	−0.973	2, 4, 6, 7, 8
N-Acetyl-D-glucosamine	−0.954	4, 6, 8
D-Glucosaminic acid	−0.930	2, 4, 6, 7, 8
Formic acid	−0.929	2, 4, 6, 7, 8
Hydroxy-1-proline	−0.911	2, 4, 6, 7, 8
D-Gluconic acid	−0.903	2, 4, 6, 7, 8
D-Psicose	−0.902	2, 4, 6, 7, 8
Adonitol	−0.883	2, 4, 6, 7, 8
Uridine	−0.873	2, 4, 6, 7, 8
Glucose-6-phosphate	−0.870	2, 6, 8
cis-Aconitic acid	−0.853	2, 4, 6, 7, 8
Glycyl-L-glutamic acid	−0.846	1, 2, 4, 6, 7, 8
D-Arabitol	−0.844	1, 2, 4, 5, 6, 8
Xylitol	−0.839	2, 6, 7, 8
τ-Hydroxybutyric acid	−0.838	2, 8
Glycogen	−0.833	2, 6, 8
2-Amino-ethanol	−0.830	2, 4, 5, 6, 7, 8
d-Glucuronic acid	−0.821	2, 6, 7, 8

[a]Carbon source with Pearson product moment coefficient of correlation (r) with ordination axis 1. Sites 1, 3, 5, and 7 are campsites; sites 2, 4, 6, and 8 are adjacent undisturbed areas.

Carbon sources with the greatest contribution to the patterns illustrated in Figure 2B were identified by the Pearson product moment coefficient of correlation (r), which indicates the strength of the linear relationship between the carbon source and axis 1 (Table 2). For all carbon sources listed, r was negative, meaning that color development, hence microbial activity, was higher for samples taken from undisturbed sites than it was for disturbed sites. A list of sites for which the optical density reading for each of the substrate wells was greater than 0.5 (which represents the maximum color change in any of the control wells), revealed greater microbial activity from undisturbed sites and campsite 7 (Table 2).

Absolute microbial activity, based on color change in microtiter wells that was at least 0.1 absorbance units or 0.05 units greater than the color change in the control well, showed that microbial activity was significantly higher in soils from the upper layer of undisturbed sites relative to the campsites. The average number of carbon sources utilized by soil microbes from the surface layer of campsites was 51.5 as compared to 91.3 used by communities from undisturbed sites (Table 3). The soil microbial community from site 7 utilized more substrates (83) than soil communities from other camp-

Table 3. Total number of carbon sources utilized and average well color development (AWCD) for each site

Surface layer site number	Active wells	AWCD	Lower layer site number	Active wells	AWCD
Camp sites					
1	37	0.35	1	59	0.50
3	45	0.52	3	84	0.88
5	41	0.44	5	81	1.16
7	83	0.91	7	91	1.31
Mean	51.5	0.56		78.8	0.96
Undisturbed sites					
2	93	1.31	2	82	0.98
4	94	1.09	4	65	0.82
6	92	1.04	6	89	1.11
8	86	1.66	8	76	1.10
Mean	91.3	1.28		78.0	1.00

sites (37–45). At lower layers of soil, the number of carbon sources utilized by the microbial communities was comparable on and off campsites (78.8 on disturbed sites versus 78.0 on undisturbed sites). Analysis of variance shows a significant disturbance effect ($F = 8.02$, $P = 0.015$), no significant layer effect ($F = 1.03$, $P = 0.329$), and a significant intereaction effect ($F = 8.65$, $P = 0.012$). The same patterns were reflected in AWCD, with a decline in activity from microbial communities from campsites as compared to undisturbed sites at the surface layers, and no difference in samples from lower layers of soil (Table 3). ANOVA on AWCD data showed no significant difference in disturbance ($F = 2.59$, $P = 0.134$) or depth ($F = 0.02$, $P = 0.892$), but a significant interaction ($F = 5.84$, $P = 0.032$).

Discussion

Comparison of the carbon utilization and activity profiles of the soil community from campsites and adjacent intact sites provides a measurable and useful analysis of community differences. Principal components analysis revealed a distinction between disturbed and undisturbed sites in the upper layer of soil, but not the lower layer. Differences in AWCD reflect a loss of microbial activity in the upper layer of campsite soils, as is indicated by the decrease in the number of substrates used by different communities. The relatively long incubation period of soil samples in Biolog plates (192 h) should have allowed for the growth of even very small microbial populations. The loss of ability to utilize substrates despite the long incubation time represents a significant decline in functional diversity of microbial communities from campsite soils. While the number of CFU did not vary on and off disturbed sites, the decline in percent actinomycetes suggests that there are structural changes in microbial communities. The data from the spread plate and Biolog plate analyses combined suggest structural and functional shifts in microbial communities in response to recreational impacts.

Differences between campsites and intact sites that may cause microbial community shifts include physicochemical changes in soils and the total or near total lack of vegetation on campsites, as compared to the dense vegetative cover on undisturbed sites. Plant exudates and sloughed root cells are a primary source of carbon for rhizosphere communities (Rovira 1965). The loss of plant cover on the campsites represents a loss of substrates that support a number of microbial species. The carbon utilization patterns observed in this study may reflect the occurrence of specific compounds in the undisturbed and disturbed soils (Table 2). Inositol, for example, is a plant growth regulator, and the ability to metabolize it is only present in the microbial communities from undisturbed sites and from site 7. Arabinose is abundant in the hemicellulose fraction of most plants and therefore represents a significant source of carbon and energy for the heterotrophic soil microbiota (Alexander 1977, pp. 163–172). The absence of vegetation and associated hemicellulose in the disturbed sites may account for the absence of arabinose degradation activity. *N*-Acetyl-glucosamine is a common component of bacterial (peptidoglycan) and fungal (chitin) cell walls and the exoskeleton (chitin) of insects. That none of the microbial communities from campsites were able to metabolize it suggests that bacterial and fungal components of the soil community have been severely disrupted. This is further supported by data in Table 1 showing the severe decline in culturable actinomycetes (or conidiospores) in the disturbed sites. The actinomycetes are thought to be important in the decomposition of more complex organic matter (Alexander 1977, pp. 47–51), and their loss in the disturbed areas suggests either the reduced presence of complex plant organic matter or a decline due to soil compaction and associated changes in soil water infiltration and O_2 concentration.

The work reported here demonstrates the utility of carbon source community profiles in characterizing disturbance. Although microbial biomass is recommended as a useful ecological indicator of stress caused by anthropogenic activities (Wardle 1992), our data show that even though there was not a significant difference in microbial numbers based on plate counts, there was a loss of structural and functional diversity of microbial communities on and off disturbed sites in surface layers. Furthermore, carbon source profiles are relatively easy and inexpensive to use, and show great

potential as an ecological indicator of impact levels and perhaps restoration potential. The information we gather from studies that compare disturbed and undisturbed sites can be used to form baseline data for restoration projects.

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