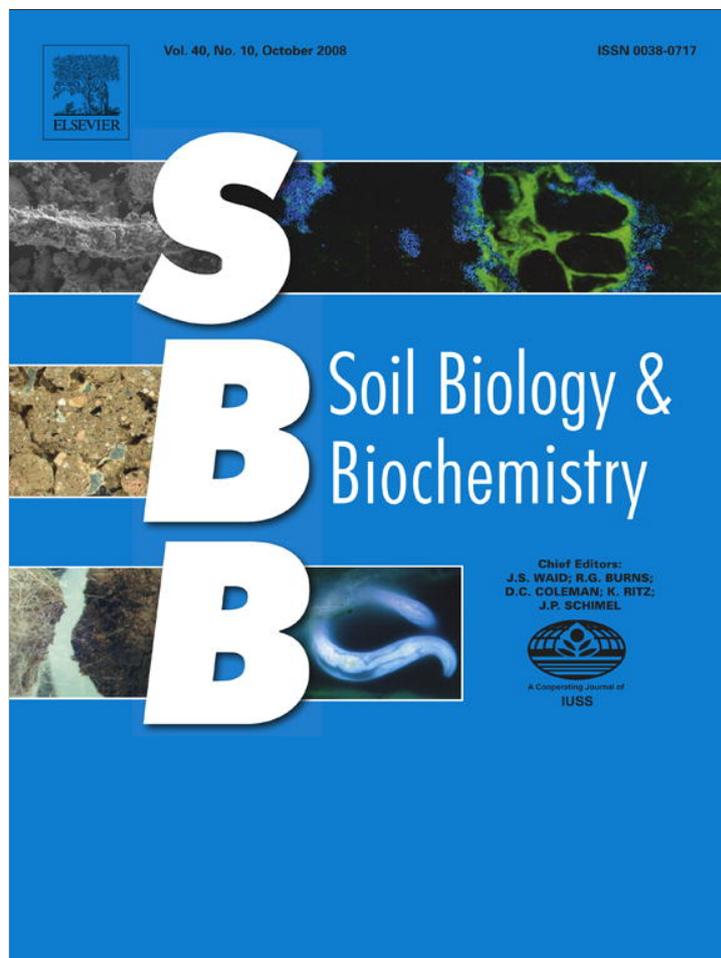


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## High levels of microbial biomass and activity in unvegetated tropical and temperate alpine soils

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## ARTICLE INFO

## Article history:

Received 25 January 2008

Received in revised form 29 April 2008

Accepted 30 June 2008

Available online 9 August 2008

## Keywords:

Alpine

Extracellular enzymes

Subnival

Microbial biomass

Talus

## ABSTRACT

Barren high-altitude soils are among the most extreme terrestrial environments on Earth. The present study was undertaken to quantify broad-scale patterns of total microbial biomass in unvegetated soils in the subnival zone of the Colorado Front Range and the high Andes of Perú. In order to better understand the limiting factors and substrates used by this community, we measured microbial biomass C, dissolved organic carbon (DOC), total organic carbon (TOC), soil gravimetric water content, and extracellular enzyme activity. To further investigate substrate limitation in these alpine soils, respiration after substrate addition was measured for samples from three sites in Colorado. In general, the abundance of microbes in these soils is positively correlated with soil water content. However, Perú talus soils had higher average microbial biomass than Colorado soils despite the Perú soils being higher in altitude and drier than the Colorado sites. Furthermore, the activity of the heterotrophic portion of the microbial community appears to be limited first by carbon and then by phosphorus as indicated both by results from extracellular enzyme assays and substrate addition experiments.

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### 1. Introduction

Barren high-altitude soils are common in high mountain ranges on Earth and occur above the zone of continuous vegetation (e.g. above tundra in the Colorado Rockies) and below the “nival zone” (zone of year-round snow or ice). These barren areas have variously been called the “subnival zone” (Europe), “puna brava” (Central Andes) or “high mountain desert” and “frost-debris zone” in general terminology (Troll, 1973). Barren subnival soils occur at much higher elevations in drier mountain ranges such as the Andes and Rockies compared to the well-studied Alps. For example the subnival zone of the Bavarian Alps starts at about 2000 m in elevation compared to 4700–5000 m in the Andes of Southern Perú and Northern Bolivia (Troll, 1973). The global extent of the subnival zone has increased significantly in recent years due to rapid retreat of glaciers and snow fields at high elevations (Zemp et al., 2006), yet we know very little about the organisms that inhabit these seemingly barren areas.

Barren high elevation soils are also of interest because they are among the most extreme terrestrial environments on Earth. The subnival zones of the Rockies and Andes are characterized by low oxygen pressure, cold temperatures, low humidity, low levels of liquid water, high levels of solar insolation and UV-B, and extreme

temperature cycling across the freezing point. These physical extremes in turn lead these high altitude systems to be low in nutrients and have been presumed in the past to be almost devoid of measurable life.

While high altitude environments are not necessarily hard to study, unvegetated subnival soils have received surprisingly little attention (Ley et al., 2004). These systems consist of boulder fields and scree slopes interspersed with deceptively young unweathered soils and are extensive at high elevations in the Rocky Mountains of Colorado (Shroba, 1977, PhD Thesis University of Colorado). Although many of these surfaces have been exposed for thousands of years, the accumulation of deep, late melting snowpack creates an environment that inhibits colonization by lichen and plants and presumably inhibits soil development beyond the barren mineral soil stage. Despite the barren appearance of these soils, recent studies indicate that they have unexpectedly high levels of carbon and nitrogen cycling (Bieber et al., 1998; Ley et al., 2001).

Efforts to characterize the microbial communities in the same soils studied by Bieber et al. (1998) and Ley et al. (2001) have yet to yield an understanding of the abundance and diversity of microbes in these soils. Recent work conducted in our lab stands as the most comprehensive study to date (Ley et al., 2004), and describes marked seasonal patterns of heterotrophic microbes that mineralize labile (e.g. amino acids) and more recalcitrant compounds (e.g. phenols). In addition, Ley and Schmidt (2002) showed that fungi dominated the mineralization of organic compounds during snow melt, whereas bacteria dominated mineralization processes

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in the summer. While the focused studies of Ley et al. (2002, 2004) yielded proof that dynamic microbial communities exist in these soils, we still do not have a robust estimate of the overall abundance and diversity of microbes in these extreme soils. The present study was undertaken to quantify broad-scale patterns of total microbial biomass in the same soils previously studied by Ley et al. (2002, 2004) and in other barren high-elevation soils of Colorado and the high Andes of Perú. In order to better understand the limiting factors and substrates used by this community, we measured microbial biomass C, dissolved organic carbon (DOC), total organic carbon (TOC), soil gravimetric water content, and extracellular enzyme activity.

## 2. Methods

### 2.1. Sampling scheme

Our goal was to investigate this understudied environment at both large and small scales through extensive sampling. At each study area, samples were collected in a nested triangle scheme at distances of 0.01 m, 0.1 m, 1 m, 10 m, 100 m, and 1000 m (Fig. 1). While this scheme does not cover the entire landscape, it does help us to understand how the variability in microbial activity is correlated with scale.

### 2.2. Study sites

In order to expand upon our previous work we used the same sites studied by Ley et al. (2004), two other sites in the Colorado Front Range and sites in the Sibinococha Basin, Perú. All of our sites are located on the eastern slope of their respective ranges. They are united in that they are all in the subnival zone as defined by Troll (1973), have similar dry season rain event dependence, prevailing dry season wind direction, snow pack dependence, and lack of vegetation. They are also some significant differences between the sites in their climate, dry season rain frequency, altitude, parent material, and time since glaciation. Detailed descriptions of all sites can be found in Williams et al. (1997) and Seimon et al. (2007).

### 2.3. Colorado sites

In September of 2002, samples were collected from three south-facing talus slopes in the Colorado Front Range at an average

elevation of 3800 m. We collected samples from two sites in the Green Lakes Valley (GLV), part of the City of Boulder watershed (Site 1: 40°03'25"N, 105°37'27"W; Site 2: 40°03'07"N, 105°37'56"W), and from one site 1 km north of GLV in the South St. Vrain Valley (Lake Isabelle Valley) (Site 3: 40°04'18"N, 105°38'04"W), part of Longmont watershed. The Green Lakes Valley and the Lake Isabelle Valley respectively flank the south and north sides of Niwot Ridge, Boulder County, Colorado. Both valleys are on the eastern face of the North American continental divide in the Rocky Mountains and were last glaciated in the early Pleistocene (Outcalt and MacPhail, 1965). Henceforth these valleys will be collectively referred to as the Colorado sites. Snowmelt begins in these valleys in May; however, our sites remain snow-covered until late June or July. The late timing of snowmelt appears to prohibit vegetation in these soils, which are all classified as pergelic cryumbrepts. The bedrock differs between the three sites, however, the difference is mainly metamorphic and the general chemistry is similar (Lovering and Goddard, 1950). All previous microbial work has been restricted to the eastern GLV site (Ley et al., 2004). Sampling sites were arranged in a triangle of side length 1 km (Fig. 1).

### 2.4. Perú sites

In August of 2003, samples were collected from the talus and glacial till of the Puca Glacier Valley at an average elevation of 5000 m. The glacier lies in the Laguna Sibinococha Basin, in the Cordillera Vilcanota range in the Peruvian Andes (Site 1: 13°46'41"S, 71°04'57"W; Site 2: 13°46'17"S, 71°04'28"W; Site 3: 13°46'47"S, 71°04'17"W). Henceforth these sites will be referred to as the Perú Sites. The soils and bedrock of the Sibinococha Basin have not been classified, however, the soils contain high quartz and calcite content and are likely cryosols. The Sibinococha Basin, like the Niwot Valleys, is also glacial in origin, though the study area was still glaciated in the late 1800s. The forelands of the Puca glacier are largely unvegetated at distances up to 1000 m from the receding snout of the glacier. Past 1000 m there is a significant amount of vegetated soil. The only previous microbial work done at this site was conducted by Nemergut et al. (2007). Samples were collected at 2 sites on the western talus slope and 1 site on the eastern talus slope of the valley, also arranged in a triangle of side length 1 km (Fig. 1).

### 2.5. Soil parameter measurements

Soil water content, microbial biomass carbon, and dissolved organic carbon (DOC) were measured via the chloroform fumigation method (Cleveland et al., 2003) for a total of 117 samples from the Colorado sites and 21 samples from the Perú Sites. Total organic carbon (TOC) was measured via loss on ignition for the 117 Colorado samples but was not measured for the Peruvian soils. Microbial extracellular enzyme activity was measured for 89 of the 117 Colorado sites samples and for all 21 samples from the Perú Sites. Due to high autocorrelation at the 1 m sampling scale at the Colorado sites, 28 of the 81 samples taken in 1 m triangles were excluded from the enzyme analysis. Microbial biomass carbon is indicative of the total size of the microbial community, while extracellular enzyme (exoenzyme) activity measures the activity of enzymes metabolizing large, marginally soluble, organic compounds. Enzyme activity was assayed for *N*-acetylglucosaminidase,  $\beta$ -glucosidase,  $\alpha$ -glucosidase,  $\beta$ -xylase, leucine amino peptidase, cellobiosidase, and acid phosphatase activity using the method of Weintraub et al. (2007).

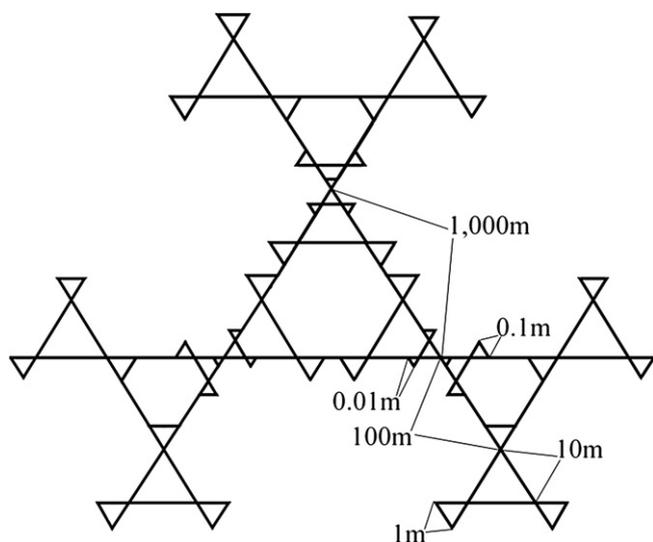


Fig. 1. A diagrammatic representation of the nested triangle sampling scheme employed in our study.

## 2.6. Soil respiration measurements

Soil respiration experiments were conducted to assess nutrient limitation in the Colorado soils. Treatments were sucrose, phosphate, sucrose and phosphate, and distilled water as a control. Sucrose was chosen to serve as a labile source of carbon and has been used successfully in past experiments to stimulate soil microbial respiration (Jonasson et al., 1999). Soil samples were collected from three spatially separated locations in the Colorado study area and analyzed separately. For each treatment, 35 g of soil (dry weight equivalent) from each sampling location was added to a separate 250-ml flask. Sucrose was added to obtain a concentration of 100  $\mu\text{g C/g}$  dry soil. Phosphate ( $\text{Na}_2\text{HPO}_4$ ) was added to obtain a concentration of 75  $\mu\text{g P/g}$  dry soil. The necessary amount of substrate was dissolved in enough distilled water so that the final soil moisture level was at 50% of field capacity, which was determined gravimetrically. Each flask was closed with rubber stoppers containing two valves. An EGM-4  $\text{CO}_2$  analyzer (PP Systems, Amesbury, MA) was attached to the valves before they were opened and the  $\text{CO}_2$  concentration in the headspace was non-destructively assayed for approximately 30 s. Measurements were taken on an approximately  $\log_2$  timescale (powers of 2, e.g. 1/2, 1, 2, 4, 8, 16, 32 h). Curves of  $\text{CO}_2$  accumulation were fit using the non-linear regression function of KaleidaGraph software (Synergy Software, Reading, PA, USA) and the integrated logistic equation (Schmidt et al., 2004).

## 2.7. Statistical analyses

Correlation analyses and *t*-tests were performed using MVPStats (MVP Programs, Vancouver, WA, USA) using type two error tolerance of 0.05. All tests were two tailed with the exception of the respiration experiment which was one tailed. The Colorado sites were separated into the nine sub-sites based on the 10 m triangle groups (Fig. 1) for further analysis. For each sub-site, microbial biomass C was plotted against soil water.

## 3. Results

Microbial biomass levels across the Colorado sites were higher than previously estimated (Ley et al., 2004), with a mean of 82  $\mu\text{g C/g}$  dry soil ( $N = 112$ ,  $SD = 45$ ). Microbial biomass was significantly higher ( $p < 0.001$ ) in Peruvian soils with a mean of 140  $\mu\text{g C/g}$  dry soil ( $N = 21$ ,  $SD = 52$ ). Upon further analysis, eight of the nine 10 m sites in Colorado display the same correlation between microbial biomass and soil water. However, Colorado site 2A (one of the three 10 m subsites at site 2) was significantly different from the other Colorado sites (Fig. 2). In addition to differences in the biomass to water correlation, site 2A has higher TOC and soil water content, but lower DOC than the rest of the Colorado talus soils ( $p < 0.001$ ). Summary statistics for the soil parameters measured for Perú, Colorado site 2A, and the remaining Colorado samples are listed in Table 1. Of the environmental variables measured in both Perú and Colorado environments, percent soil water most strongly correlated with biomass levels across both Perú and Colorado ( $r = 0.270$ ,  $p = 0.009$ ). Colorado site 2A, the remaining Colorado samples, and Perú when regressed separately yield strikingly better fit to the data (Fig. 2). In turn soil water levels correlated with total organic carbon in the Colorado soils including site 2A ( $r = 0.672$ ,  $p < 0.001$ ).

To complement previous work that had shown relatively high heterotrophic activity in Colorado talus soils (Ley et al., 2002), we measured a standard array of exoenzyme activities in both the Colorado and Perú soils. Most of the enzymes measured showed very low activities (Table 1), with the exception of peptidase and phosphatase in the Perú soils and  $\beta$ -glucosidase and phosphatase in the Colorado soils. In addition, Colorado site 2A had significantly lower exoenzyme activities than the remaining Colorado sites

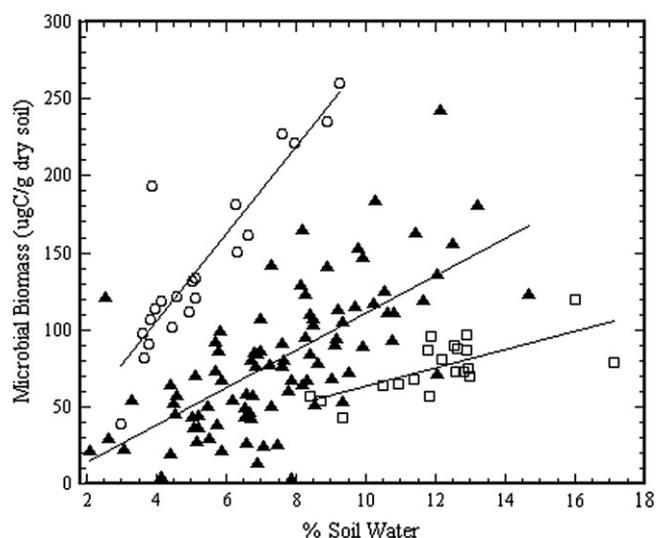


Fig. 2. Correlation between soil water content and microbial biomass regressed separately for Colorado site 2A (open squares,  $r = 0.673$ ,  $p < 0.001$ ), the remaining Colorado sites (filled triangles,  $r = 0.687$ ,  $p < 0.001$ ), and the Perú site (open circles,  $r = 0.899$ ,  $p < 0.001$ ).

across all enzymes measured ( $p < 0.001$ ). In Colorado,  $\beta$ -glucosidase showed correlation with microbial biomass C ( $r = 0.231$ ,  $p = 0.032$ ), while phosphatase showed correlation with DOC ( $r = 0.503$ ,  $p < 0.001$ ) and with microbial biomass C ( $r = 0.306$ ,  $p = 0.004$ ) but neither enzyme was significantly correlated with soil water content. In Perú, peptidase and phosphatase were not significantly correlated with any soil carbon measurement or with soil water content.

To determine if the relatively high phosphatase activity in these soils indicates phosphorus limitation of microbial activity, we carried out incubation studies on the Colorado soils to see if P stimulates microbial respiration. Addition of P alone significantly stimulated microbial respiration as did the addition of C (Fig. 3). However, the addition of C and P had the greatest effect on respiration (Fig. 3). In contrast nitrogen additions to these soils either decreased respiration or had no effect (data not shown).

## 4. Discussion

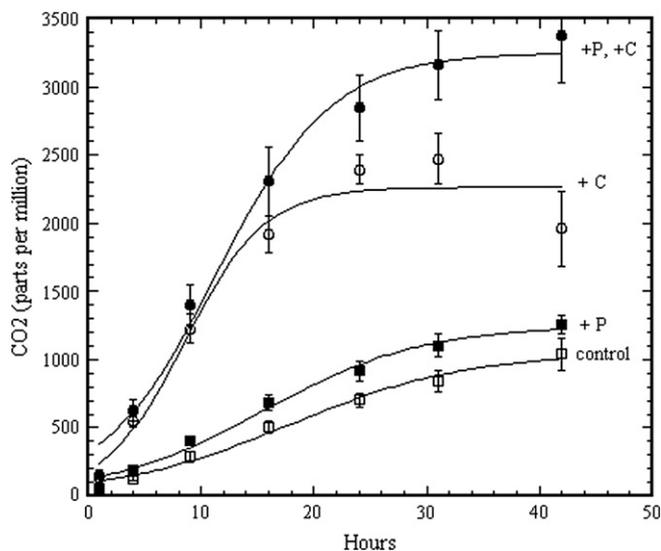
### 4.1. Microbial biomass levels

We found unexpectedly high levels of microbial biomass in unvegetated subnival zone soils in both Colorado and Perú. The

Table 1  
Soil parameter summary statistics for Colorado and Perú soils

Parameter	Perú sites		Colorado site 2A		Colorado sites w/o 2A	
	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
%H <sub>2</sub> O	5.3 (1.8)	21	12 (2.1)	21	7.4 (2.5)	95
DOC	17 (4.0)	21	24 (4.1)	20	51 (16)	95
%TOC	nd	nd	3.3 (0.58)	21	1.9 (0.68)	96
MBC	140 (57)	21	76 (18)	20	83 (49)	92
NAG	0.22 (0.21)	21	1.6 (0.96)	20	3.4	69
CBH	0.16 (0.17)	21	3.4 (1.7)	20	6.2 (4.7)	69
AG	0.36 (0.26)	21	0.22 (0.22)	20	0.64 (0.61)	69
BG	1.1 (1.1)	21	15 (6.6)	20	23 (13)	69
LAP	26 (11)	21	nd	nd	0.18 (0.37)	45
BXYL	0.18 (0.28)	21	0.61 (0.43)	20	1.9 (1.5)	69
PHOS	20 (9.1)	21	13 (4.5)	20	27 (14)	69

%H<sub>2</sub>O, soil water content ( $100 * \text{g H}_2\text{O/g}$  wet soil); DOC, dissolved organic carbon ( $\mu\text{gC/g}$  dry soil); %TOC, total organic carbon ( $100 * \text{g C/g}$  dry soil); MBC, microbial biomass carbon ( $\mu\text{gC/g}$  dry soil). Soil enzymes (nmol/h per g dry soil): AG,  $\alpha$ -glucosidase; BG,  $\beta$ -glucosidase; BXYL,  $\beta$ -xylosidase; CBH, cellulobiosidase; LAP, leucine amino peptidase; NAG, N-acetyl-glucosaminidase; PHOS, acid phosphatase.



**Fig. 3.** Effects of added P and C on the kinetics of CO<sub>2</sub> production in Talus soils from Colorado. Data points represent mean values of three replicates for all treatments ( $\pm$ SE). Curve fits are from the integrated logistic equation (Schmidt et al., 2004). *r* values for curve fits were 0.993, 0.994, 0.981, and 0.994 for control, +P, +C and +P, C, respectively. There was a significant difference between CP and C at 30 and 40 h (one-tailed *t*-test). There was a significant difference between P and control at 15, 20, and 30 h ( $p < 0.05$ , one-tailed *t*-test).

microbial biomass in the alpine talus from our study was 30 times that estimated by previous work on alpine talus (Ley et al., 2001, 2004). Despite being higher than anticipated, the values we obtained were similar to some of the harshest environments worldwide; for example, the average microbial biomass in Alpine and Antarctic glacial moraines is 60  $\mu\text{g C/g}$  soil and 100  $\mu\text{g C/g}$  soil respectively (Tschirko et al., 2003a,b) while both Arctic polar desert and temperate desert average 150  $\mu\text{g C/g}$  soil (Jones et al., 2000; Bailey et al., 2002). In comparison, the alpine tundra in close proximity to the Colorado sites displays biomass levels of 500–2000  $\mu\text{g C/g}$  soil (Lipson et al., 1999; Oline and Grant, 2002). Wardle's meta-analysis of ecosystems worldwide (Wardle, 1998) helps put our data in a global context. He estimates that microbial biomass is on average 800  $\mu\text{g C/g}$  dry soil for grasslands and 750  $\mu\text{g C/g}$  dry soil for forests. Clearly, the soils of harsh environments harbor significantly lower biomass than soils of more mesic plant dominated systems.

The consistently lower biomass levels observed in the wide variety of unvegetated soils described above lends strong credence to the idea that carbon inputs from plants determine the size and activity of soil microbial communities. It should be pointed out, however, that there are too few detailed studies of plant-free soils to make broad generalizations about the controls on microbial biomass levels in barren soils. Indeed, some barren soils are early successional and will eventually support plants (e.g. glacial chronosequences: Ohtonen et al., 1999; Tschirko et al., 2003a,b), whereas others experience conditions that are simply too extreme for plants to establish (e.g. some deserts: Bailey et al., 2002; Jones et al., 2000). While glacial recession chronosequences clearly demonstrate a buildup of higher microbial biomass associated with increasing soil organic matter and plant cover (Ohtonen et al., 1999; Tschirko et al., 2003a,b), permanently plant free soils exist in a semi-equilibrium state with microbial heterotrophic activity hypothetically balancing ecosystem carbon inputs. We contend that our sites are in the latter category based on the fact that they have been deglaciated for thousands of years (Ives, 1953), yet due to the extreme duration of the snowpack, still do not support vegetation.

The closest analog to our unvegetated subnival system is found in small snow-free patches of Antarctic and Arctic barren soils

(Gajananda, 2007; Jones et al., 2000). For example, the Antarctic soils studied by Gajananda (2007) receive substantial amounts of snow and, like our subnival zone soils, are not limited by precipitation or soil age, so much as by the long duration of the snowpack. Deep late melting snowpack is what excludes plants in both Antarctic and subnival barren soil environments, limiting carbon availability and, ultimately, microbial growth. However, in light of global warming trends, these systems may be pushed out of their equilibrium state onto a successional trajectory similar to classical glacial recession.

#### 4.2. Implications of biomass underestimation using heterotrophic stimulation

While unvegetated soils do indeed contain lower biomass than vegetated systems, the talus soil biomass levels are only lower than alpine tundra by a factor of 5 not 150 as suggested by Ley et al. (2004). This unexpectedly higher estimate of subnival zone microbial biomass stems from the use of the chloroform fumigation (CF) approach to estimate biomass levels. Previous workers at the Colorado site (Ley et al., 2001, 2004) used a substrate induced growth response (SIGR) method that had been developed to measure microbial biomass levels in tundra soils (Colores et al., 1996; Lipson et al., 1999) but had never been used for barren soils. The Ley et al. (2001,2004) results underestimated microbial biomass levels because they were calculated under the assumption that the established CF to SIGR ratio of 7:1 for tundra soils in the fall of the year (Lipson et al., 1999) was applicable to barren unvegetated soils. However, our new data indicate that the SIGR approach may greatly underestimate total microbial biomass in unvegetated alpine soils and that such a method should be recalibrated to better agree with chloroform fumigation measurements.

There are two possible reasons for why the SIGR approach so grossly underestimates microbial biomass in barren soils. The first reason is that the SIGR approach and related SIR methods are based on heterotrophic respiration of added carbon sources, whereas unvegetated soils likely have large populations of photoautotrophic and chemoautotrophic microbial communities. Indeed, preliminary community analyses of our talus soils in Colorado (Kristen Freeman, personal communication, 2007) and molecular phylogenetic analyses of unvegetated early successional soils in Perú (Nemergut et al., 2007) indicate that these soils have a significant number of autotrophic microbes that would not be assayed by the SIGR method.

An additional reason for this underestimation of the microbial biomass is that SIGR measures the microbes that are immediately responsive to substrate additions, while the chloroform fumigation method we employed measures both active and inactive biomass. Therefore, there may exist a population of dormant microorganisms that was only detectable via the CF method. These microbes may be relict from the spring peak of heterotrophic activity during snowmelt as observed by Ley et al. (2004) and have become dormant during the hot, dry summer conditions. Alternatively, there may be a population of warm temperature adapted microbes that are only active for short periods under sporadically favorable summertime conditions such as after rain events.

#### 4.3. Controls on microbial biomass in subnival soils

Whether high altitude talus soil biomass is dominated by autotrophs, ephemeral heterotrophs, or a combination of the two, it is intriguing that microbial biomass was significantly higher in the dry, high altitude, tropical sites of Perú than in the wetter, lower altitude, temperate sites of Colorado (Fig. 2). Moreover, while DOC was higher in Colorado than Perú (46 vs 17  $\mu\text{g C/g}$  soil), DOC plus microbial biomass is higher in Perú than Colorado (160 vs 127  $\mu\text{g C/}$

g soil). There are a number of factors that could potentially explain these differences but the present study provides only glimpses into the possible reasons. It may be that the Colorado community is under a higher predator pressure resulting in a lower standing biomass. We have ample evidence of active microphageous protozoa, mites, nematodes, and rotifers in the Colorado soils (Kristen Freeman and Michael Robeson, personal communication, 2007), but, thus far, attempts to amplify eukaryotic DNA out of the Peruvian samples have failed (Diana Nemergut, personal communication, 2007). It is also possible that the low pH of the Colorado soils could help explain their relatively low levels of biomass. The pH of the Colorado sites averages about 4.5 (Ley et al., 2001) compared to 7.5 for the Peruvian soils (Nemergut et al., 2007). Whatever the reason, the microorganisms of the Peruvian soils appear to incorporate carbon more efficiently than those of the Colorado soils, achieving a higher microbial biomass with lower DOC.

Although the Perú soils display a larger microbial biomass under significantly drier conditions, both the Perú and Colorado soils have a significant correlation between soil water and microbial biomass (Fig. 2). This water dynamic was somewhat expected given that the low water content of the Colorado summertime talus soils (Ley et al., 2004) suggested that microbial biomass levels might show a dependence on water availability. The correlation between microbial biomass and soil water was clearly demonstrated in the present study even when all samples from Perú and Colorado were analyzed together. On the other hand, the Colorado sites with similar conditions group to the exclusion of our wettest site (2A), and to the exclusion of the Perú samples (Fig. 2). Thus, while there is a general trend toward higher biomass with higher soil water, this trend is much stronger when the data are separated into these three groups.

What then is it that makes these three sites so different? Snow depth studies in the Colorado talus indicate that the position of site 2A at the base of a cliff face leads to a deeper snowpack than at the other Colorado sites (Erickson and Williams, 2005). The deeper snowpack may cause 2A to remain frozen later into the summer and, therefore, to have a shorter active period than the other sites. This idea is supported by the fact that 2A, in addition to having lower than expected microbial biomass, has the highest TOC and lowest DOC and exoenzyme levels of the Colorado sites, suggesting impeded breakdown of soil carbon. The Peruvian sites, on the other hand, appear to accumulate a much smaller snowpack, to the point that snowmelt does not result in the formation of any visible seeps. It may be that microbial biomass is correlated with the size of the late melting snowfield in all subnival barren soils, and that areas with similar size snowpack have similar trends in the correlation between soil water content and microbial biomass. Snowpack depth may in turn regulate the period of microbial activity and/or microbial predator abundance by either restricting the length of the growing season or by excluding predators in the driest, earliest melting environments. The persistence of the water to biomass correlation through the dry season appears to reflect not just a seasonal but a yearlong influence of water on site fertility. Although the dynamics of microbial biomass have not been extensively studied in unvegetated polar soils, it has been shown that microbial activity is related to soil water content and TOC (Gajananda, 2007; Tschirko, 2003a). Obviously more work is needed to better understand microbial population dynamics in snowpack structured systems, but our study provides an informative first look into this little understood world.

#### 4.4. Substrate limitation in barren soils

In an effort to characterize the types of substrates that may fuel microbial activity in talus soils we carried out a standard array of

enzyme assays. Most of these enzymes showed very low activities, but the few that showed significant activity (Table 1) provide a glimpse of the types of substrates that may be fueling and/or limiting microbial activity in the talus. The specific exoenzyme results suggest that cellulose and organic phosphate are important substrates in Colorado talus, while protein and organic phosphate are important in Perú. The level of activity of phosphatase was especially striking; on a unit biomass basis (activity divided by microbial biomass) the talus soils of Colorado showed phosphatase activity twice as high as those in well developed nearby forest soils (Weintraub et al., 2007). In order to determine if these high phosphatase levels indicate P limitation of microbial activity we carried out a substrate incubation experiment on the Colorado soils (Fig. 3). Interestingly, the microbial community appears to be limited primarily by labile carbon and then by phosphorus in the presence of excess carbon.

This carbon limitation and the higher microbial biomass than DOC suggest that by late summer the labile carbon sources are gone from the talus soil. While carbon limitation is not unexpected in barren soils, phosphorus limitation is normally predicted to occur only in very young or in old, highly weathered soils. In addition, microorganisms of most other barren soil types (temperate and polar deserts, glacial moraines) have been shown to have nitrogen limitation in addition to carbon limitation, but none of those environments have shown phosphorus limitation (Gallardo and Schlesinger, 1992; Hopkins et al., 2006; Yoshitake et al., 2007). Though many theories predict that phosphorus availability decreases over time due to leaching and to sorption to Al and Fe oxides (Walker and Syers, 1976; Lajtha and Schlesinger, 1988), the Late Pleistocene origin of the Colorado talus (Ives, 1953) implies that these soils are too young for this to be a feasible explanation for the observed microbial phosphorus limitation. We have not measured available phosphorus in these specific soils, but work by Hood et al. (2002) has shown strong ecosystem retention of phosphorus in waters draining the Colorado talus sites, with dissolved organic phosphorus levels averaging 4.5  $\mu\text{g P/l}$ . Thus, it is more likely that the observed phosphorus limitation is the result of either the parent rock having been originally poor in phosphorus or the soils having been insufficiently weathered despite their intermediate age. Subnival soils of Colorado appear to have a suite of nutrient limitations unique among barren soil systems.

#### 4.5. Broader implications

The Sibinococha Basin was created by the continuing recession of the Puca glacier, which has been linked to global warming (Seimon et al., 2007). Moreover, snowmelt in the Niwot Valleys has occurred earlier in recent years, and we have observed that the barren sites used in this study have shown an increase in plant colonization. If these trends continue, we expect many of these environments to lose the protection of the late melting snow and many of these interesting microbial communities will become plant dominated communities as seen in faster melting polar oases (Jones et al., 2000). However, the current altitude of the subnival zone is still lower than many of the mountaintops worldwide. Thus, these dynamic and intriguing barren soil microbial communities may soon become restricted to only the most extreme heights of the Earth.

## 5. Conclusions

The small amount of information known about the microbial communities in barren soil areas tells us that they are all carbon limited, largely due to the exclusion of plants. Barren alpine soils harbor higher than predicted levels of microbial biomass but these levels are still low in comparison to plant dominated systems.

Although only a small subset of the community in Colorado talus soils have been shown to be immediately responsive to substrate induced respiration (Ley et al., 2004), the remaining portion of the microbial community may be composed of autotrophic organisms or ephemerally active heterotrophs. In general, the abundance of microbes in barren subnival soils of both Colorado and Perú is correlated with soil water content. However, there are exceptions, which, in the case of the Perú soils, cause higher than expected microbial biomass in spite of drier soils. The size of the snowpack and timing of its meltout may explain such phenomena, and certainly helps explain the lower microbial biomass in the wettest Colorado site. Furthermore, the activity of the heterotrophic portion of the community appears to be substrate limited and exoenzyme activities are predictive of this limitation. The barren soils of the alpine subnival zone show surprising levels of microbial biomass and activity, but their dependence on snowpack melt rates makes them an endangered ecological system in the face of global climate change.

### Acknowledgements

We thank J. Rosen, A. Seimon and P. Sowell for field and logistical support and K. Freeman with laboratory assistance. This work was supported by a grant from the NSF Microbial Observatories Program. Travel and fieldwork were supported by a grant from the National Geographic Society Committee for Research and Exploration.

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