Microbial population dynamics in an extreme environment: controlling factors in talus soils at 3750 m in the Colorado Rocky Mountains

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Abstract. High elevation talus soils are extreme environments for life. They are pertinent to the hydrology and biochemical cycling of high elevation ecosystems that supply drinking water to major metropolitan areas, and are undergoing change as a result of N deposition. As biological systems, they are virtually unstudied. In order to gain a basic understanding of these important systems, we studied the seasonal timing of C inputs and microclimate, and how they corresponded to microbial biomass dynamics, in vegetated and unvegetated soils from a high altitude talus slope at 3750 m in the Colorado Front Range. The soil microclimate was described by soil moisture and temperature measurements; C inputs were estimated with measures of eolian dust inputs and photosynthetically active radiation (PAR). The biomass of different microbial functional groups (glutamate and salicylate mineralizers adapted to different temperatures) was estimated seasonally over 3 years. We found that the soil microclimate can be divided into three distinct seasons: Winter, with free water and temperature between −2.9 and 0 °C; spring, characterized by wet isothermal soils (0 °C); and summer, characterized by hot (mean 10.3 °C; range 0–29.3 °C) dry soils occasionally wetted by precipitation. The highest OM inputs to unvegetated soils occurred in spring; PAR only reached soils in the summer. In unvegetated soils, the biomass of glutamate mineralizers (GM) was highest in spring and summer. In contrast, the GM of vegetated soils had highest biomass in the winter when plants were senesced. In both unvegetated and vegetated soils, salicylate mineralizers (SM) had greater biomass levels in the summer when temperatures were highest.

Introduction

The soils of high elevation talus slopes are extreme environments for life. They are oligotrophic cold soils with very little microbial biomass, similar in this respect to high latitude polar soils. These types of soils are used as models for soils that may support or have supported life on Mars (Gilichinsky et al. 1993; Soina et al. 1995). Polar soils have been studied extensively, however analogous high altitude soils are virtually unstudied. These soils are interesting in their own right as one of Earth's most extensive extreme environments: high mountains are present on six of the seven continents. These kinds of soils are also key components of water catchment areas that supply drinking water to

major metropolitan areas world-wide. This study is part of a larger effort to characterize the microbial community dynamics in this important system.

Unvegetated soils at our sites are coarse textured, with very little organic matter, low water retention capacity, low levels of nutrients and, consequently, low levels of microbial biomass (Williams et al. 1997; Bieber et al. 1998; Ley et al. 2001). In these respects they are similar to polar soils (Virginia and Wall 1999), but unlike polar soils they are accessible year-round and therefore are useful for understanding the processes that control microbial biomass in oligotrophic cold soils. Yet like polar soils, most research to date has occurred during summer months (e.g., Bieber et al. 1998; Ley et al. 2001). This seasonal bias in research is problematic because microbial population dynamics are almost certainly seasonal. Indeed, we recently showed that fungi dominate C turnover in unvegetated soils under the snowpack during the cold months of spring, whereas bacteria are more metabolically active in the summer months (Ley and Schmidt 2002). We know, therefore, that the microbial community is dynamic on a seasonal basis, but we do not know the overall seasonal pattern, nor what drives it.

A year-round picture of the biomass dynamics in high elevation cold soils is not only interesting for understanding life in extreme environments, it also bears considerably on water quality issues. High elevation catchments provide water for many major metropolitan areas, such as the Denver metropolitan area in Colorado. These high elevation drainage areas are undergoing change attributed to increased N deposition from anthropogenic sources. N deposition in the Colorado Rocky Mountains is causing NO_3^- levels to rise in streams draining high elevation water catchments in the spring months when the snowpack is melting (Campbell et al. 1995; Williams et al. 1996). The majority of the snow containing this N falls on areas consisting of rock talus slopes with little or no vegetation cover (Caine and Thurman 1990). The unvegetated soils in these slopes are thought to process the N from snow, by nitrifying the NH_4^+ to NO_3^-, and by cycling the N through biomass (Williams et al. 1997). The degree to which the soil microbial biomass can process N deposition will depend on the size of the biomass during the critical snowmelt period, and will affect the timing as well as the form of N released to streams. At present nothing is known about either the seasonality of the microbial biomass, or the controls of microbial biomass in unvegetated talus soils. As a result the long-term response of these oligotrophic systems to increased N loading remains very unclear.

Mountain talus slopes are composed of unconsolidated boulders of various sizes that form a block-like structure where fine material (e.g., sand and clays) can accumulate within the matrix (Williams et al. 1997). Climatic conditions are extreme, with mean annual temperatures below zero, strong winds, high levels of solar radiation, and snow cover much of the year. The duration of the snowpack and the position of water channels on the slopes are important in determining whether plants can establish (Caine 1995) and substantial expanses of soil are unvegetated. While plants supply carbon to the vegetated soils, carbon inputs to the unvegetated patches include eolian deposition and soil particles (Litaor 1987), and possibly CO_2 fixation by soil algae and lichens.

If the seasonality of these sources of C differ, so might the population dynamics of the microbial organisms that depend on them.

We can use the properties of vegetated alpine soils to understand the properties of unvegetated soils thanks to the broad and extensive research conducted on alpine vegetated soils on a seasonal basis. We know, for example, that in alpine vegetated soils, microbial biomass is highest in spring months because it grows all winter on C released by plants senescence (Fisk and Schmidt 1996; Brooks et al. 1998; Lipson et al. 1999, 2000). In late spring a sudden decline in microbial biomass makes nutrients available for plant uptake at the onset of the growing season (Lipson et al. 2000). In unvegetated soils, there are no plants to regulate C flow into the microbial biomass. If the microbial biomass in unvegetated soils responds to the same environmental factors as in vegetated soils, we can expect the highest biomass levels to occur during the period of highest C input. However, because of the extremely cold conditions that prevail at high elevation in winter, we predicted lowest biomass levels to occur in winter when soils were expected to be frozen.

To characterize the microclimate in talus soils, we monitored soil water content and soil temperature. To gauge the potential for CO_2 fixation via photosynthesis, light was measured at the soil surface. Eolian C inputs were determined by measuring the amount of organic matter deposited as dust by the snowpack compared to the amount deposited by wind in summer. The origin of SOM was evaluated by a survey of C isotope ratios in soils and eolian dust.

To describe the seasonal trends in microbial biomass, we characterized the biomass of two microbial functional groups using the substrate-induced growth response (SIGR) technique (Schmidt 1992; Colores et al. 1996; Colores and Schmidt 1999) over the seasons characterized by our soil microclimate data. We estimated the biomass of glutamate mineralizers (GM) and a more specialized group, salicylate mineralizers (SM). The GM are general heterotrophs (Ley 2001) that are the largest functional group in tundra soil and whose biomass correlates well with other measures of total soil biomass such as chloroform-labile biomass (Lipson 1999). The SM are a more specialized group composed of fungi in unvegetated soils (Ley and Schmidt 2002) that are thought to degrade soil SOM (Ley et al. 2001). Thus we chose the GM and the SM to represent the general biomass and a more specialized group respectively. These two groups were further divided into warm (22 °C), cold (3 °C) and subzero (−1 °C) adapted groups to determine if the biomass of temperature-specialized groups tracked soil temperature.

Methods

Site

We conducted this research in Green Lakes Valley, included within the City of Boulder, CO, USA, watershed in the Front Range of the Colorado Rocky

Mountains (40 °03′N, 105 °35′W). Green Lakes Valley drains the eastern slope of the Continental Divide and the southern aspect of the Niwot Ridge Long Term Ecological Research/UNESCO Biosphere Reserve and is closed to the public. Because of the remoteness of the talus slopes and the difficulty of the terrain, soils were sampled from a 1 ha area of south-facing talus that extends from the floor of Green Lakes Valley to just under the ridge top of Niwot Ridge, from approximately 3600 to 3750 m. The physical characteristics of the site are described by Williams et al. (1997).

Unvegetated soils were poorly developed sandy gravel soils containing 0.6–2% OM. Vegetated soils were loam cryocepts containing 8–16% OM (Ley et al. 2001). The parent material for both soils was granite. Unvegetated soils were collected from distinct randomly located patches within a 250 m^2 area that receives a very thick snowpack ($\sim$10 m) annually that persists until late August most years. We sampled vegetated soil from three distinct patches adjacent to the unvegetated area, ranging in size from 5 to 50 m^2, and dominated by sedges (*Carex ebenea*, *Carex misandra*) or mixed graminoids and forbs (such as *Acomastylis rosii*).

Soils were collected in 1997–1999. Collections were made monthly from June to November, and about every 3 months from December to June as avalanche conditions permitted. The biomass values reported for winter and spring are for soils collected under snow (0.5–1 m) unless otherwise specified, and the summer values are for exposed soils. The soil isotope analyses were performed on soils collected in July and August 1997. For all soil collections, we collected three randomly located soil cores (50 ml volume) per patch to a depth of 10 cm. Soils were kept on ice and immediately returned to the laboratory the same day of collection. All soils were stored at 3 °C and processed within 48 h of sampling. Replicate cores for each soil were homogenized and sieved to 8 mm.

Soil moisture and temperature

Soil gravimetric water content and temperatures were determined in a vegetated soil and an unvegetated soil using a datalogger (model CR10X, Campbell Scientific, Inc., Logan, UT) equipped with one water content reflectometry probe (CS 615 Campbell Scientific, Inc., Logan, UT) and two thermistor probes (107, Campbell Scientific, Inc., Logan, UT) for each soil type. The probes were placed parallel to the surface of the soil at a depth of 5 cm. The water content reflectometry probes measure the period of an electromagnetic wave, which was converted to gravimetric water content by empirically derived calibration equations. To derive these equations, soils in the laboratory were equilibrated to a range of gravimetric water contents and the wave period measured over a range of temperatures (−50 to 20 °C). Briefly, homogenized soils were placed in replicate (2) steel cylindrical tubes (10 cm diameter × 40 cm length) with water content and thermistor probes. The soil tubes were frozen with liquid nitrogen and allowed to thaw slowly over 2 days, and the wave

period and the temperature were recorded every 10 min. The procedure was repeated for known soil gravimetric water content (GWC) ranging from dry to saturated in 1% increments for unvegetated soils and 5% increments for vegetated soil. The dataset was modeled with polynomial equations which were tested against independent data. The equation that fit the independent data best over a range of temperatures was applied to the wave period measurements recorded by the field datalogger. The calibration equation used for the unvegetated soil was:

$$GWC = 5316 - 21{,}107\theta + 31{,}015\theta^2 - 19{,}985\theta^3 + 4770\theta^4, \quad r^2 = 0.98$$

where GWC is the gravimetric water content, and θ is the wave period. The calibration used for the vegetated soils was:

$$GWC = 322 - 807\theta + 509\theta^2, \quad r^2 = 0.95.$$

Once the calibration equations were generated, we ran soil thawing experiments in the same manner as described above in order to determine the amount of free water available at below-freezing temperatures in these soils.

Soil surface PAR measurements

We measured photosynthetically active radiation (PAR, 400–700 nm) at the unvegetated soil surface from 1 January to 15 September 2000. We placed a radiation sensor (quantum sensor model LI-190SA, LI-COR, Inc., Lincoln, NE) with a millivolt adapter on the surface of the unvegetated site and recorded a millivolt output hourly with the datalogger. The millivolt output was converted to $\mu mol\ s^{-1}\ m^{-2}$ using the calibration constant supplied by the manufacturer. The sensitivity of this instrument was $\pm 1\ \mu mol\ s^{-1}\ m^{-2}$, with a lower detection limit of 4 $\mu mol\ s^{-1}\ m^{-2}$.

Dust and soil C analyses

Two types of dust traps were used in this study: initially these consisted of simple marble traps, later the marble traps included a lower chamber filled with glass wool (described below). The initial dust traps were deployed in the unvegetated study area during the winter-spring periods of 1997–1998, and in the summer of 1998. These traps consisted of 10 cm square pans 7 cm deep filled with glass marbles and placed flush with the soil surface. The winter-spring traps ($n = 11$) were placed just before the onset of permanent snow-cover (October 1997) and once exposed (July 1998), the summer traps ($n = 10$) were installed to collect dust during the exposed months, from July 1998 to October 1998. After collection, the marbles were rinsed with deionized water and the rinse water filtered through pre-combusted glass fiber (Whatman GF/F 7 cm) filters. Organic matter content of the dust was determined by loss on

combustion. Filters were weighed after drying at 100 °C, then weighed after 24 h of combustion at 500 °C in a muffle furnace.

The second set of improved dust traps ($n = 12$) were deployed for the summer months in early July 1999 and collected in October 1999. These traps consisted of 15 cm diameter × 20 cm depth PVC tubes containing a layer of glass wool overlain by marbles. The glass wool was added because it was more effective in trapping dust than the marbles alone. The fibers were separated from the soil surface by 2 mm plastic mesh and the marbles were suspended above the fiber glass also by mesh. After collection, the dust was removed from the fibers in the laboratory. The marbles were also inspected for dust but found to not retain measurable quantities. Dust trapped in snow was collected by scooping snow into 50 ml sterile tubes ($n = 10$) on two dates in late spring, and melting and evaporating the snow in the laboratory at 60 °C. The dust trapped in snow was assumed to be aeolian in origin and not from lateral flow because it was frozen in place in the snow matrix.

To determine the carbon isotope ratios of soil, subsamples of soil collected for the biomass estimates were dried at 60 °C and ground to a powder (<250 μm particle size) by tumbling. Depending on the %C of the sample, between 2 and 40 mg of material for vegetated soils, and 20–146 mg of material for unvegetated soils was analyzed, such that on average the amount of C analyzed per sample was 0.45 mg C. Subsamples were analyzed for %C and δ^{13}C by continuous flow mass spectrometry at the Stable Isotope Research Facility for Environmental Research (SIRFER) at Utah State University. Dust was separated from the glass fibers and packed into tin capsules and analyzed for %C and δ^{13}C at the Stable Isotope Facility at the University of California at Davis. Isotope ratios are reported as δ^{13}C (‰) $= 1000 \times (R_{sa} - R_{std})/R_{std}$, where R_{sa} and R_{std} are the ^{13}C/^{12}C isotope ratios of the sample and the standard (Pee Dee Belemnite).

Biomass estimates

In order to track seasonal changes in microbial biomass, we estimated the biomass of GM and SM using the SIGR method (Colores et al. 1996; Ley et al. 2001; Ley and Schmidt 2002). GM-SIGRs give biomass estimates that correlate highly with total microbial biomass as measured by the chloroform fumigation method (Lipson et al. 1999), whereas SM-SIGRs measure a smaller, specialized population of fungi in talus soils (Ley and Schmidt 2002). We further sub-divided the GM and SM functional groups into biomass that responded at warm (22 °C) and cold (3 °C) temperatures. We also performed SIGR assays at -1 °C on some dates, but because growth did not occur in every case at this temperature, we report substrate-induced respiration (SIR) rates (after 4 h) as an index of the sub-zero adapted microbial biomass.

At each sampling date, glutamic acid (2 mg C g^{-1} veg soil, 0.1 mg C g^{-1} for unveg soil) or salicylic acid (0.1 mg C g^{-1} veg and 0.05 mg C g^{-1} unveg soil) was added to replicate soil samples ($n = 2$ per treatment) of 10 g for vegetated

soils and 20 g for unvegetated soils in sterilized biometer flasks. A total of 320 SIGR assays were conducted over the course of the study. The substrates added were spiked with uniformly ^{14}C -labeled glutamic acid and salicylic acid (Sigma Biochemical Co.) so that the final radioactivity was 150,000 DPM and 250,000 DPM per flask, respectively. Incubations were performed at room temperature ($22\,°C \pm 2\,°C$) for the warm-adapted group, 3 and $-1\,°C$ for the cold-adapted group and sub-zero adapted groups in environmental chambers. The CO_2 evolved during the assay was trapped in 1 ml of 0.5 M NaOH in the sidearm of the biometer flask. Every 2–3 h, the NaOH was removed, mixed with 2.5 ml of scintillation fluid (Scintiverse^{TM}) and the DPM counted. Fresh NaOH was added back to the sidearm after each sampling. The exponential growth phase was used in the derivation of the biomass estimates was approximately the first third of the full respiration curve. Respiration data were analyzed with Kaleidograph^{TM} software using equations derived by Colores et al. (1996). To convert the units of µg C-CO_2 g^{-1} to µg C-biomass g^{-1}, empirically derived yields (Y_c) of 0.11 for salicylic acid and 0.50 for glutamic acid were used in the equation $X_a = X_1(Y_c/(1-Y_c))$, where X_a is the actual biomass in µg C-biomass g^{-1}, and X_1 is the biomass in units of µg C-CO_2 g^{-1} (Colores et al. 1996; Lipson et al. 1999).

Statistical analyses

Comparisons between mean values were made by analysis of variance using a model comparison approach (Judd and McLelland 1989). Data were log-transformed to fit normal distributions prior to analysis. Differences between means are not statistically significant ($p < 0.05$) unless reported in the text.

Results

Soil microclimate

The air temperatures measured at the Niwot Ridge D1 station provide a dramatic background to the soil temperatures. The lowest air temperature recorded during this study was $-31\,°C$ in 1998. Air temperatures are below $0\,°C$ for more than half the year at this elevation. The insulation of the snowpack against these temperatures was remarkable: the lowest soil temperature recorded over 3 years was $-2.9\,°C$ in unvegetated soils. The soils are essentially isothermal for most of the year, at temperatures that allow water to stay free, and therefore microbial activity to persist.

Three distinct periods (winter, spring and summer) characterized the microclimate in both the unvegetated and vegetated soils of high elevation talus slopes. Winter, the longest period, lasted typically from early October to May and was characterized by very stable temperatures and stable levels of free water. Over the winters of 1997–1998, 1998–1999 and 1999–2000, the soils never froze

completely. The spring period was shorter than the winter period and corresponded to the timing of snowmelt infiltration through the soils, with soil temperatures close to zero and water-saturated soils. As soon as the snowpack had completely melted and the soils were exposed, summer produced soil temperatures that fluctuated diurnally and drier soils that were periodically wetted by rain. We describe these three seasons in more detail below, emphasizing the 1998 record, the most complete and representative of the other years.

Winter. In winter, soils were covered by thick snow and soil temperatures remained below zero. In both vegetated and unvegetated soils, this period lasted from the onset of permanent snow cover in October to the beginning of snowmelt in May when water from the melting snowpack percolated down into soil. In 1998, the average temperature during this period was $-0.89\,°C$ in unvegetated soils (Figure 1(A)) and $-0.80\,°C$ in vegetated soils (Figure 1(B)). The soils were not entirely frozen in the winter: they had free water contents just around 4% (Figure 1(C) and (D)). Thus, the snowpack insulated the soils well against mean air temperatures that approached $-30\,°C$ in 1998 (Figure 1(E)). Snowmelt began to percolate to a depth of 5 cm mid-May in vegetated soils and one week later in unvegetated soils (Figure 1(C) and (D)).

Spring. Spring was the period of meltwater influence on talus soils, when the snowpack still covered the soils. This period was brief. In 1998, it lasted from May 20 to August 6 in unvegetated soils and from May 14 to July 10 in vegetated soils (Figure 1). During this period, soil water content was high, averaging 10% in unvegetated soils (roughly field capacity) and 38% in vegetated soils (Figure 1(C) and (D)). The sustained high water content in the unvegetated soils suggests that during this period water was draining out of the talus slopes. Temperatures were very close to zero in both soil types, averaging $0.85\,°C$ in the unvegetated soil, and $-0.10\,°C$ in the vegetated soil (Figure (A) and (B)).

Summer. Summer was a period of desiccation and high temperatures, relieved by periodic rain events. Soil temperatures began to fluctuate on a diurnal basis as soon as the soils were snow-free. In 1998, summer temperatures averaged $10.3\,°C$ in unvegetated soil, and $6.6\,°C$ in the vegetated soil (Figure 1(A) and (B)), and never dipped below zero. Vegetated soil had lower maximum temperatures and higher moisture content than the unvegetated soil. The gravimetric water content averaged 0.95% in unvegetated soil, and 46.2% in vegetated soil (Figure 1(C) and (D)). Generally, temperatures soared at midday, particularly in unvegetated soils, where they reached the unexpected high of $29.3\,°C$. Soil water had transient increases that correspond to precipitation events (Figure 1(F)). Summer ended each year with a large snowfall event, such as the one that occurred October 1 in 1998 (Figure 1(F)).

Thawing dynamics of soil

Our laboratory thawing experiments indicated that ice likely formed in both soils during the winter, yet free water was available in both soils year-round.
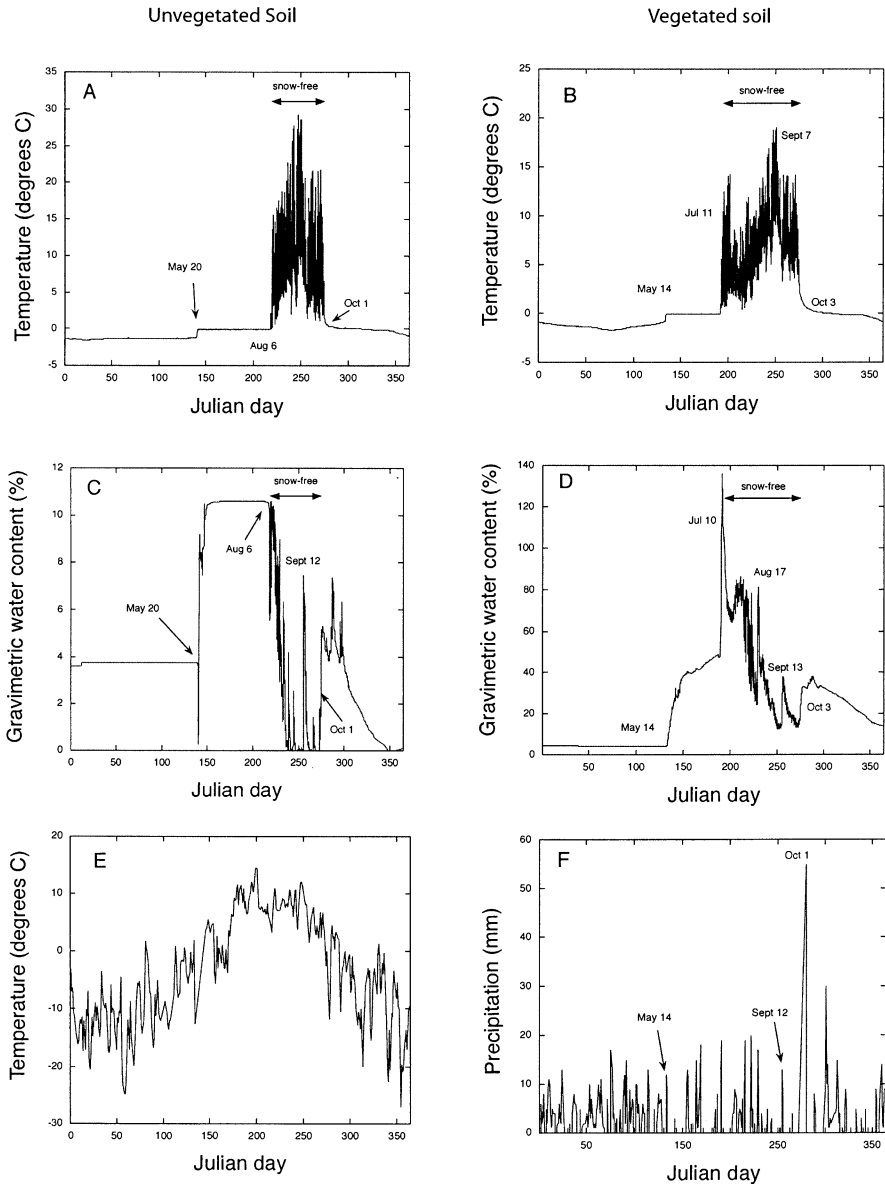
Unvegetated Soil   Vegetated soil

Figure 1. Soil microclimate and meteorological data. (A) Temperature, unvegetated soil. (B) Temperature, vegetated soil. (C) Soil free water content, unvegetated soil. (D) Soil free water content, vegetated soil. (E) Air temperature for the Niwot Ridge D1 climate station. (F) Precipitation at the Niwot Ridge D1 climate station. The soil temperatures are mean of $n = 2$.

At $-2.9\,°C$, the minimum soil temperature recorded in the field, a mix of ice and water was observed in both soil types (Figure 2(A) and (B)). Unvegetated soils at 4% moisture did not begin to thaw until temperatures reached $-5\,°C$ and above (Figure 2(A)). In vegetated soils with higher moisture contents, all water was frozen at around $-15\,°C$, and a mix of ice and water occurred until temperatures reached $0\,°C$ (Figure 2(B)). For both soil types, the probes gave a positive value for the amount of liquid water in fully frozen soils at $-50\,°C$. This could be due to the liquid layer present at that temperature (Anderson 1967). Ice is not detected because the measurements of soil liquid water content are based on dielectric constants, and the dielectric constant of ice (3.2) is closer to that of mineral soil (3.9) than that of liquid water (80) (Dasberg and Hopmans 1992). We applied the same calibration equation, developed for soils above $0\,°C$, to measurements taken at below freezing temperatures, so the field water measurements in the winter and spring months are probably over-estimated by 0.4% in unvegetated soils and 2% in vegetated soils. However, it is clear that water was present in both soils during the winter.

Soil surface PAR

We recorded PAR at the surface of the unvegetated soil hourly from 1 January to 15 September 2000 (Figure 3). No PAR was detected at the soil surface until July 11 when the snowpack was less than 30 cm deep (field observation). During this period, and during the night in snow-free periods, the millivolt output of the light sensor was negative due to instrument noise. Intermediate light levels, indicating penetration through snow to the surface, were only recorded for 4 days just before full sunlight was measured and the snowpack had exposed the sensor.

Dust and soil C

The amount of dust trapped over the winter and spring of 1997–1998 was 43 kg ha^{-1}. The organic matter content, determined by loss on combustion, was 8.75% (SE$=2.9$). Based on the corrected bulk density estimate of 1780 kg m^{-3} reported by Williams et al. (1997) for unvegetated soils, and an average soil depth of 10 cm, the amount of OM deposited by the snowpack was 2.1 μg OM g^{-1} soil. In the summer months, 7.7 kg dust ha^{-1} fell on the soils, which amounts to 0.38 μg OM g^{-1}.

The carbon isotope ratios of the unvegetated soils ($-27.1‰, \pm 3.0, n=11$) showed the soils were slightly more depleted in ^{13}C than the vegetated soils ($-26.1‰, \pm 0.1, n=6$) (Figure 4(A)). The range in values observed in the unvegetated soils (-24.8 to $-29.3‰$) was far greater than that of the vegetated soils (-25.8 to $-26.7‰$). The soils with greater C:N were more depleted in ^{13}C (Figure 4(A)) ($F_{17,1}=43, p < 0.001$). The average C:N ratio of the unvegetated
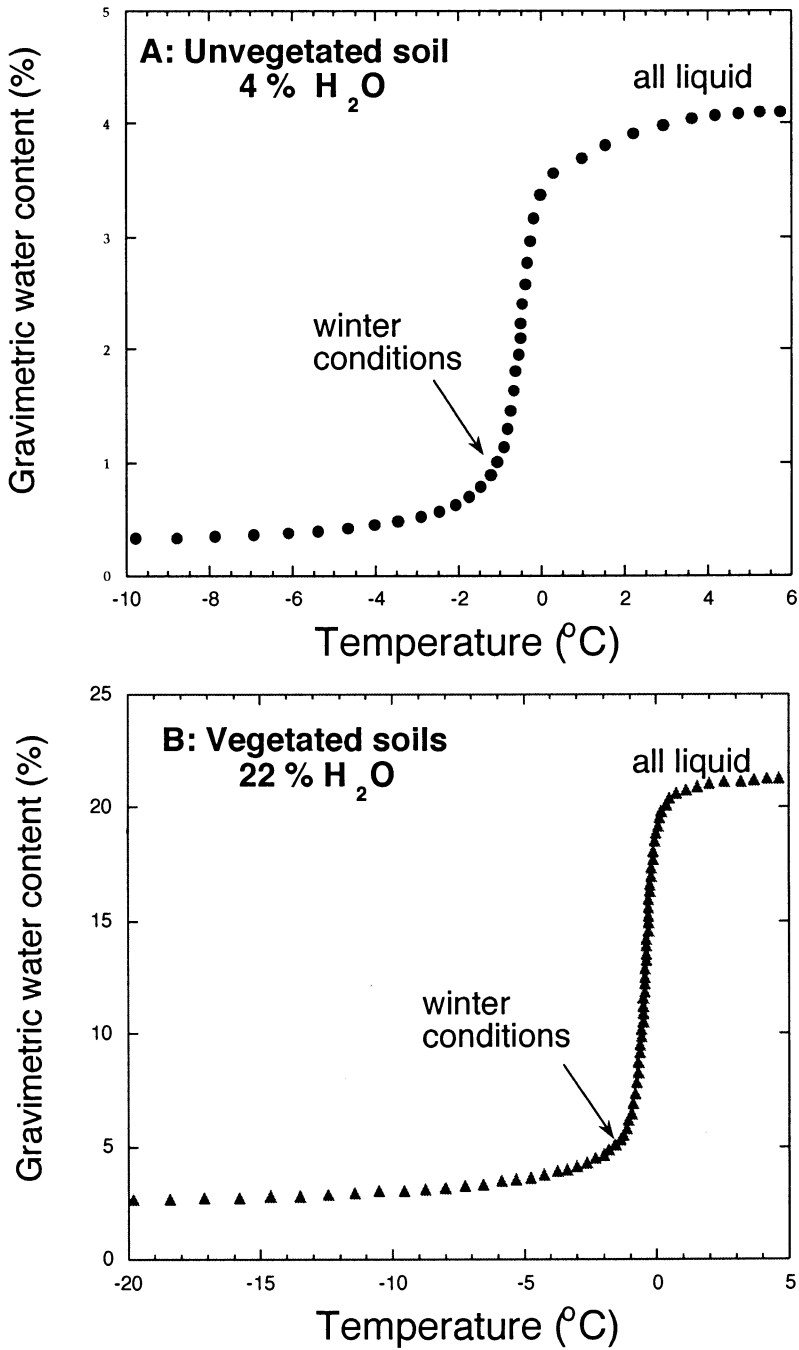
Figure 2. Free water measurements in thawing soil. (A) Unvegetated, 4% GWC. (B) Vegetated, 22% GWC.
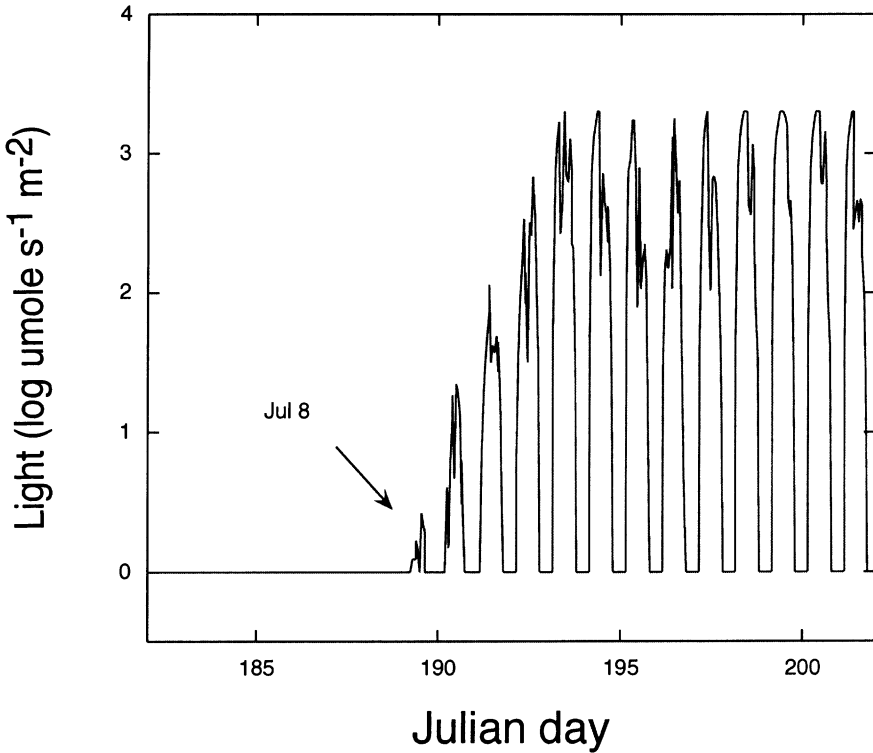
Figure 3. PAR at the surface of the unvegetated soil, during the melt-out of the snowpack.

soil (24 ± 4) was not significantly different from the average C:N ratio of vegetated soils (15.3 ± 1.3), but the range was considerably greater (10.1–58 and 12.8–20.1 for unvegetated and vegetated soils respectively). The eolian dust that fell on unvegetated soils was isotopically heavier than the soil SOM (Figure 4(B)). Dust collected from snow was isotopically heavier than dust collected in the summer.

Seasonal trends in microbial biomass

GM in unvegetated soils had higher biomass in spring and summer months than in winter. The highest biomass recorded (5.4 µg C g^{-1}) was of the cold-adapted GM in the spring. The cold-adapted GM biomass was much larger than the warm-adapted GM biomass (0.6 µg C g^{-1}) (Figure 5(A)) in the spring, suggesting the dominance of a cold-adapted guild. The summer biomass (2.5 µg C g^{-1} for both warm and cold adapted) was higher than the winter biomass (0.6 and 0.6 µg C g^{-1} for warm and cold adapted, respectively) although the difference was not statistically significant.

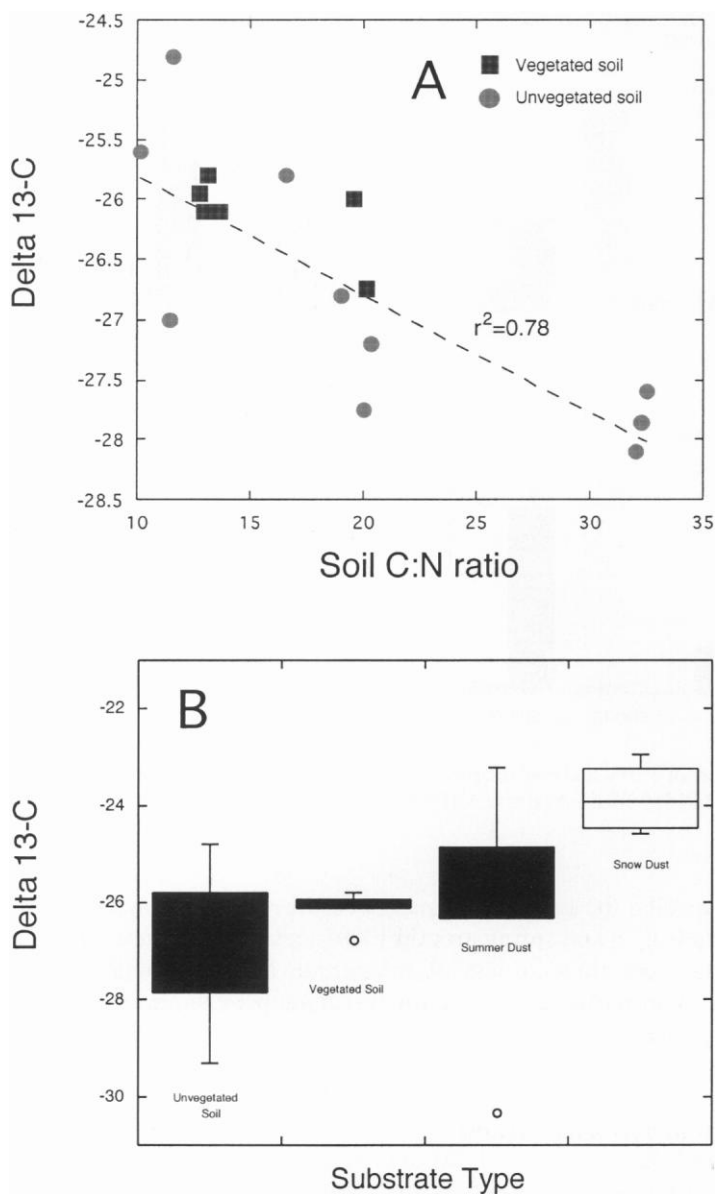*Figure 4.* (A) $\delta^{13}C$ of unvegetated and vegetated soils as a property of the C:N ratio of the soils. (B) Box plots of $\delta^{13}C$ ranges for unvegetated soil, vegetated soil, dust deposited in summer and dust deposited in the winter from snow. The outline of the box = 1 SD from the mean, the bars = 2 SD. The bar inside the box = the mean. The circles are outliers.
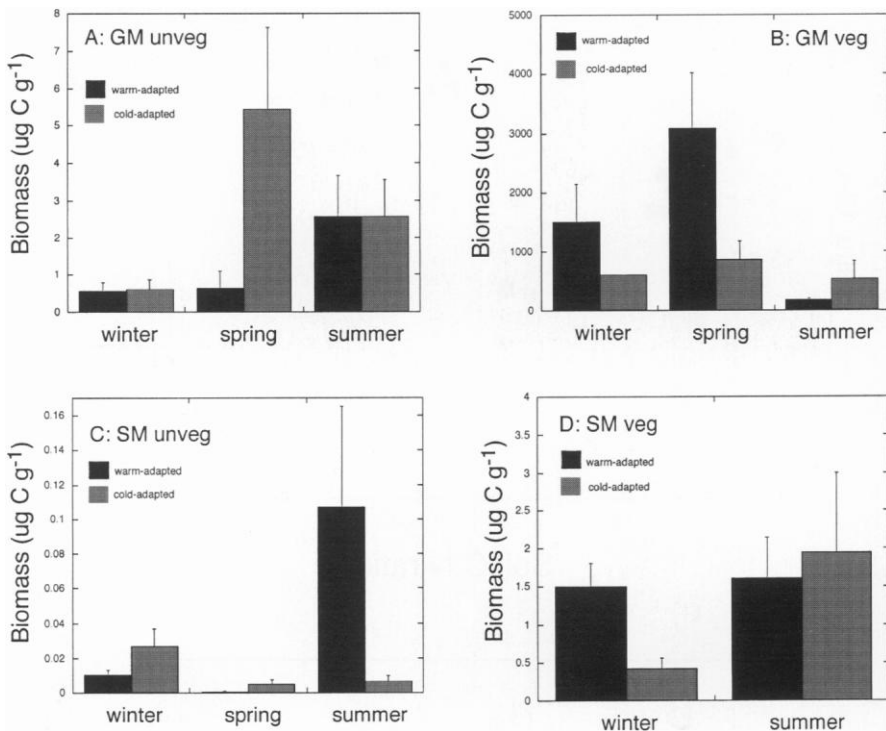
Figure 5. Biomass of warm- and cold-adapted GM and SM. (A) GM in unvegetated soil. (B) GM in vegetated soil. (C) SM in unvegetated soil. (D) SM in vegetated soil. Bars are averages ± 1 SE of $n = 2$–12.

In vegetated soils, the greatest biomass was the warm adapted GM in the winter (1500 μg C g^{-1}) and spring months (3088 μg C g^{-1}) (Figure 5(B)). These biomass values were three orders of magnitude higher than those of un-vegetated soils. During these seasons, the warm adapted biomass in vegetated soils was far greater than the cold adapted biomass, which averaged 602 and 862 μg C g^{-1} in winter and spring, respectively. In summer, the GM had comparatively low warm and cold adapted biomass averages of 526 and 179 μg C g^{-1}.

The warm and the cold-adapted SM in unvegetated soils had seasonal trends in biomass that mirrored the seasons in a more intuitive way (Figure 5(C)). The warm adapted SM had the highest biomass (0.1 μg C g^{-1}) in summer, which was significantly higher than the warm adapted SM in spring and winter ($F_{21,1} = 6.9$, $p = 0.02$). The cold adapted SM biomass peaked in winter (0.027 μg C g^{-1}) and was significantly lower in spring (0.0050 μg C g^{-1}) and summer (0.0063 μg C g^{-1}) ($F_{10,1} = 11$, $p = 0.01$).

Due to the difficulty in obtaining samples from vegetated soils under snow on an avalanche slope, winter and spring SM biomass values were pooled.
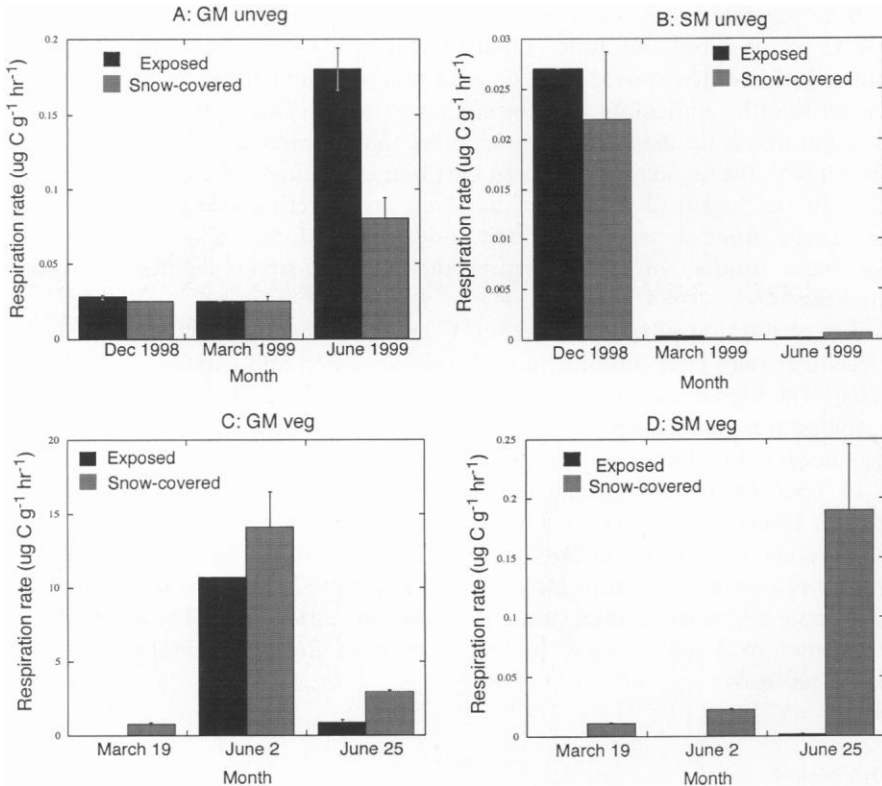
Figure 6. Respiration rates at $-1\,°C$ of glutamate or salicylate added to soil collected in exposed patches or under snow cover. (A) Glutamate respiration in unvegetated soil. (B) Salicylate respiration in unvegetated soil. (C) Glutamate respiration in vegetated soil. (D) Salicylate respiration in vegetated soil. Bars are average ± 1 SE of $n = 2$–8.

Overall, there is no clear seasonal pattern for the SM biomass in vegetated soil (Figure 5(D)). The warm adapted SM was approximately the same in the winter (1.5 μg C g^{-1}) as it was in the summer (1.6 μg C g^{-1}).

Effect of snow cover on respiration at $-1\,°C$

We performed SIGR biomass assays at $-1\,°C$ (close to the average winter soil temperature) in soils collected in winter and spring of 1999. For these measurements, we contrasted exposed and snow-covered soils for both vegetated and unvegetated soils. Growth did not occur at all dates, but respiration always occurred, therefore we report substrate-induced respiration (SIR) as an index of biomass.

In unvegetated soils, snow cover only affected the respiration of glutamate at
$-1\,°C$ in the month of June (Figure 6(A)), when respiration rates were de-
pressed relative to exposed soils. Overall, respiration in June was higher than in
earlier months, indicating that the change in season from winter to spring had
brought about an increase in the biomass that respires at $-1\,°C$. This is con-
sistent with the higher cold-adapted GM biomass values observed in the spring.
Due to the logistical difficulties involved in collecting these samples on an
avalanche-prone slope during winter and spring months, we have an uneven 'n'
for these results, and we interpret the obvious trends without statistical
analysis.

The respiration of salicylate at $-1\,°C$ in unvegetated soils was far greater in
December than later months, in both snow-covered and exposed soils (Figure
6(B)). The higher winter respiration suggests that the SM that respired at low
temperatures had higher biomass in December, which is consistent with the
decline in cold adapted ($3\,°C$) SM seen from winter to spring.

In contrast to unvegetated soils, the snow-covered vegetated soils had
greater levels of glutamate respiration and salicylate respiration than the ex-
posed soils at all three dates (Figure 6(C)). Overall, the salicylate respiration
increased significantly from December to late June (Figure 6(D)). The highest
glutamate respiration rates were recorded in early June. These trends are
consistent with the seasonal biomass trends of the cold adapted groups in
vegetated soils.

Discussion

This study constitutes the first seasonal analysis of microbial groups in an
extreme oligotrophic soil. This is also the first record of soil microclimate, C
inputs and PAR in a high elevation cold unvegetated talus soil. The un-
vegetated soils of talus slopes are an extreme environment for microbial life,
and extreme environments are seldom studied on a year-round basis. Our
results show that the seasonal trends of the microbial biomass are different in
unvegetated and vegetated soils, because although the highest microbial bio-
mass levels occur when C inputs are highest in both soil types, the seasonality
of C inputs differ between soil types.

Microclimate

Remarkably, free water was available and temperatures were moderate in
unvegetated and vegetated soils during the winter. The heavy snowpack
insulated soils against air temperatures as low as $-31\,°C$ in 1998, such that the
lowest temperature recorded was $-2.7\,°C$ in vegetated soil and $-2.9\,°C$ in
unvegetated soil (year 2000). This is very warm compared to the temperatures
at which microorganisms have been found to be metabolically active: $-17\,°C$ in

snow (Carpenter et al. 2000) and $-20\,°C$ in soil (Rivkina et al. 2000). The activity of microbes at below freezing temperatures is allowed by free water in the soil. The thickness of the water layer around an ice crystal has been calculated to be about 50 nm at a temperature of $-10\,°C$ (Anderson 1967). Soil solutes further depress the freezing point, as does the presence of fine particles such as clays (Neresova and Tsytovich 1966; Frolov and Gusev 1973). Our soil moisture measurements showed that free water was always available in talus soils in the winter despite very low air temperatures. Therefore, it is very unlikely that cold temperatures ever completely inhibit microbial activity during the winter in talus soils.

Winter in soil was milder than expected, and surprisingly similar to the spring snowmelt period with a couple of notable exceptions. Both winter and spring periods had free water available and stable, moderately low temperatures. The important difference between winter and spring periods was not the mean temperature as we had expected, but the draining of the water from the snowpack through the soils in the spring and the resulting release of carbon from dust in the snowpack. These factors most likely account for the high microbial populations observed in the spring (discussed below).

In contrast to the prolonged isothermal and moist conditions of winter and spring, the summer period was short, variable and probably the hardest period for the microbes to survive. In unvegetated talus soils, the soils dried down, and soil temperatures rose as high as $29.3\,°C$ in the summer and fluctuated widely. Thus the summer was the only period when soil microbes had to resist desiccation. We had expected the winter to be the period when microbes had to survive desiccation because of soil freezing. These microclimate data contrasting winter, spring and summer have thus altered our view of the talus soils as a microbial environment. It is clearly an extreme environment, but not so much because of winter conditions as we had thought, rather because of dry summer conditions.

Sources of C in unvegetated talus soils

Photosynthesis is not the dominant source of C for unvegetated talus soil. Although potential photosynthesis was not measured directly, our PAR measurements show it was restricted to a short window in time due to darkness imposed by the persistent snowpack in spring. Curl et al. (1972) report that only 1% of incident light penetrates 18–110 cm into snow, although most of this is in the 400–600 nm range, within the range of PAR. Snow at our unvegetated site accumulates to a depth of approximately 10 m, precluding photosynthesis for most of winter and spring. If any PAR reached the surface during spring, it was below the detection limit of our sensor (4 μmol s^{-1} m^{-2}). Some algae require little light, having light compensation points of 0.18 μmol s^{-1} m^{-2} (Cota 1985). Thus some photosynthesis may have taken place under the snowcover, particularly in spring since more light penetrates melting snow

than frozen snow (Wiscomble and Warren 1980; Gerlans et al. 1999). Nevertheless the amount of CO_2 fixed would have been small under such low levels of PAR. In all likelihood, photosynthetic C inputs from soil algae and cyanobacteria are not the dominant source of C for unvegetated soil in spring.

Dust inputs on the other hand are probably the dominant source of C for the soil microbial biomass. Far more dust was released by the spring snowmelt (42.2 kg ha^{-1}) than in the summer by wind (7.7 kg ha^{-1}): snow contains all of the dust accumulated by wind over the winter and spring seasons. The dust consisted of roughly 10% organic material and thus may represent a substantial energy source in this oligotrophic system. Our estimate of the OM content of dust is lower than the 17% C measured by Litaor (1987) in Arikaree Cirque, a little further up Green Lakes Valley from our site. The discrepancy can be attributed to the design of the dust traps: Litaor used pans on 1 m stands, ours were placed flush with the ground surface and probably caught more saltating sand, lowering the %C. The isotopic composition of dust did not point to a clear origin, but its depletion in ^{13}C relative to SOM argues against the possibility that local SOM was blown into the dust traps. The different ranges obtained for dust in winter and summer suggests different dust sources at different times of the year.

The source of SOM in unvegetated soils is probably plant-derived organic matter blown in as dust. The photosynthetic inputs of soil algae and bacteria are not distinguishable from C3 plant signatures, so we cannot partition the SOM into endogenous vs. eolian inputs. The unvegetated talus soils had a lower mean δ^{13}C than the vegetated soils, whose signatures reflected those of C3 plants closely (Craig 1953, 1954). The range of δ^{13}C values in the unvegetated soils was similar to the -29.8 to -24.3‰ range measured by Balesdent et al. (1993) in forest soils. The lowest values measured in the unvegetated soils were close to the lowest values measured in alpine tundra plant species (Miller 2002).

Although its source is unclear, the soil C isotope ratios suggest that SOM has been highly processed in unvegetated soils by the microbial biomass. Carbon isotope ratios decrease with greater decomposition of organic matter in soil (Agren et al. 1996). Unvegetated soils had the lowest %C and the most depleted C isotope ratios, which suggest high levels of SOM processing by microbes. This high degree of SOM processing could in part be due to the higher proportion of SM in the microbial biomass of unvegetated soils (Ley et al. 2001). Microbes that degrade salicylate in soil are representative of those that degrade the polyphenol components of humic acids in soil, and therefore the greater proportion of this functional group in the unvegetated soils is consistent with higher levels of SOM degradation. We know that the SM in unvegetated soils are exclusively fungi (Ley and Schmidt 2002). It is interesting to note that fungi can fractionate carbon sources (Henn and Chapela 2000), and that the difference in carbon isotope ratios in SOM could be a property of the microbial community that processed it. In the case of unvegetated soils, the activity of fungi may contribute to the ^{13}C depletion of the organic matter.

Seasonal trends in microbial biomass

C inputs drive peaks in GM biomass in both unvegetated and vegetated soils: the highest biomass levels occur when C inputs are highest to the system. The GM were representative of soil organisms that grow on labile C and are a much larger functional group than the SM. In vegetated soils from nearby tundra the source of labile C is primarily plant turnover, with highest inputs occurring in the fall, stimulating microbial biomass increases that continue into the spring (Fisk et al. 1996; Lipson et al. 1999, 2000). We found that the vegetated soils of the talus slope were like tundra soils in that microbial biomass increased in winter and spring when C availability was high. In contrast, the largest C inputs to unvegetated soils occurred not in fall but during the spring snowmelt when dust was released from the snowpack. In accordance with this timing of C inputs, the GM biomass reached its highest levels in the unvegetated soils in the spring. Therefore the GM respond to C inputs in unvegetated soils as they do in vegetated soils, although the source of the C is different.

The high GM biomass in unvegetated soils in spring could also be attributed partially to soil warming. Soil temperature increased on average by $1.7\,°C$ during the transition from winter to spring, which, although a small increase, could have a strong effect on microbial metabolism at such low temperatures. Indeed, respiration at $-1\,°C$ was far higher in June than March or December in unvegetated soils even though free water was available in all months. Clein and Schimel (1995) reported Q_{10} values for microbial activity in arctic soils as high as 23.4 for temperature increases around the freezing point ($-2\,°C$ to $5\,°C$). Warmer temperatures may also explain why the GM biomass was higher in summer than in winter despite occasional soil drying. It is likely then that both warmer temperatures and greater C availability together promote the spring increase in GM biomass.

Temperature certainly seems to regulate the biomass of the SM. The seasonal trends of the SM biomass suggest that it is temperature rather than C availability that controls the biomass of this particular functional group. The SM are a smaller group than the GM and is composed of fungi in unvegetated soils (Ley and Schmidt 2002). The warm-adapted SM had highest biomass in the summer, and the cold-adapted SM biomass was highest in the winter: the warm and cold-adapted subgroups could be made up of different kinds of fungi that grow best at different temperatures. Fungi such as these, with low and high optimal growth temperatures, have been isolated from high latitude cold soils (Kerry 1990).

Talus soils as models for Mars soils

Mars appears to meet the environmental conditions necessary to support life deep below the surface (hundreds of meters to several kilometers) where temperatures are high enough to allow liquid water (Boston et al. 1992).

Although these regions are not readily accessible to exploration, large areas of the regolith near the polar caps are, and these areas appear to contain more water than previously thought (Boynton et al. in press). These areas are characterized by soil mixed with ice, and in this regard they are very similar to cold unvegetated talus soils on Earth. Our study has shown that liquid water can exist at below-freezing temperatures in such soils, and that microbial activity occurs in partially frozen soil at low temperatures. Temperatures on Mars may be high enough during periods of high obliquity (when the axis of the planet is tilted towards the sun, every 10E8 years) to allow the presence of liquid water in regolith soils (Costard et al. 2002). Although there is no liquid water today that could support organisms, it is possible that the regolith or polar ice cap could hold dormant organisms or spores that are active at periods of high obliquity (Jakosky et al. in press).

Impact of anthropogenic N deposition to talus soils

Our observations of microbial activity throughout the winter in talus soils have broad implications for the processing of anthropogenic N deposition by soil. N deposition rates have increased in recent years in the Colorado Front Range, causing water draining high elevation catchments to contain measurable amounts of nitrate (NO_3^-) during snowmelt (Campbell et al. 1995). The source of this NO_3^- remains uncertain. Because it occurs at the same time seasonally as the release of inorganic N from storage in the snowpack, it has been attributed to release of NO_3^- from snow (Caine and Thurman 1990; Williams et al. 1993; Reuss et al. 1995). Soil nitrification was deemed an unlikely source of NO_3^- because most of the soils in high elevation catchment areas are unvegetated and were considered abiotic (Caine and Thurman 1990). Recently however, Bieber et al. (1998) and Ley (2001) have measured positive rates of nitrification in unvegetated soils of talus slopes, which confirms these soils as plausible sources of NO_3^-. Consistent with these observations, NO_3^- in streams is isotopically lighter than NO_3^- in snow (Kendall et al. 1995) which suggests that NO_3^- in streams is a mix of snow NO_3^- and NO_3^- produced by nitrification (Amberger and Schmidt 1987). Williams et al. (1997) hypothesized that nitrification in unvegetated soils over the winter feeds the NO_3^- pools that are flushed by snowmelt, contributing to the increase in NO_3^- in surface runoff in spring. Our findings that microbial populations are active all winter allows the possibility of over-winter nitrification. Furthermore, the addition of NH_4^+ from the melting snowpack to soil during the spring coincides also with high microbial biomass and therefore NH_4^+ is likely to be assimilated and/or nitrified by talus soil microbes.

Conclusions

This is the first seasonal analysis of microbial biomass trends and environmental factors in a unique extreme environment: unvegetated soils of high

elevation talus slopes. The trends observed did not support our initial hypothesis that microbial biomass would be lowest when soils were frozen. Contrary to expectations, the soils were always thawed, as soil temperatures never reached below $-2.9\,°C$. Temperature plays an unexpected role in talus soils: it is not low enough to freeze soils completely in winter, but it is high enough to desiccate soils in summer. The dominant C inputs to unvegetated soils are eolian deposits of dust rather than surface photosynthesis. As a result, the seasonal microbial biomass dynamics track the seasonal inputs of dust, much as the seasonal biomass levels in vegetated soils track the phenology of plants. However, the complexity of the microbial community is made apparent by the seasonal dynamics of the more specialized SM, which respond to temperature rather than dust inputs. Therefore these oligotrophic soils constitute a distinct extreme environment with ecosystem processes that cannot be assumed to mirror those of vegetated systems.

Our results have broad implications: they offer insight into the biology of an extreme oligotrophic environment that may be analogous to Martian soils; they are relevant to N processing in an ecosystem that performs a critical ecosystem service, water delivery to urban areas. They also may have broad implications for global estimates of carbon cycling in cold soils: the level of microbial respiration under deep snowpacks is likely underestimated in current assessments of carbon fluxes from cold regions.

Acknowledgements

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