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Distribution and Abundance of Microbial Biomass in Rocky Mountain Spring Snowpacks

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ABSTRACT

Snowpacks in both Colorado and Wyoming were sampled on 15 dates for total microbial biomass, ratio of bacteria to fungi, and major inorganic ions. Levels of viable microbial biomass remained low throughout the period, peaking at 0.05 micrograms carbon/ml. Microscopic analyses indicated this biomass was composed primarily of bacteria. Fungi were not detected in samples taken at or above treeline. With the exception of one date in early May at the Colorado site, bacteria were confined to a band within the snowpack approximately 20 cm above the snow/soil interface. Laboratory incubations using two Gram negative, motile rods isolated from this layer indicated these organisms were capable of growth at 0° +/- 0.5^{o} C but had optimum growth temperatures between 20° and 37° C. Based on observed population sizes and growth rates it is unlikely these organisms were capable of significantly affecting trace gas emissions or altering the chemical composition of snowmelt.

INTRODUCTION

Alpine environments in temperate latitudes experience a relatively short (3 - 4 months) growing season coupled with a long (8 - 9 month) snow covered period. A significant fraction of total system nutrient inputs are deposited either through wet or dry deposition during this snow covered period (Lewis 1980, Bowman 1992). Physical processes within the snowpack serve to concentrate these nutrients on the surfaces of ice crystal over the course of the winter. The initiation of spring snowmelt serves to flush nutrients from the snowpack in a pulse observable early in the spring (Reddy and Caine 1990, Williams and Melack 1991). This pattern of deposition effectively isolates the period of greatest nutrient availability (early snowmelt) from the period of greatest biological activity (the summer growing season). Microbiological activity during this snow covered period may have the potential to significantly affect both terrestrial and aquatic system nutrient inputs.

Recent work in the Wyoming alpine suggests the presence of significant biological activity both under and within winter/spring snowpacks (Sommerfeld et al. 1993). While elevated CO2 concentration under snowpacks led Kelley et al. (1968) to suggest the presence of significant biological activity under winter snowpacks, little work has been done to quantify the effect of this activity on biogeochemical cycles. ^{15}N Labeled nitrogen applied to snow has been recovered in organic matter following snowmelt further suggesting biological activity within or under snow (Preston et al. 1990). Winter decomposition rates have been estimated in forests (Moore 1983, Coxson and Parkinson 1987, Taylor and Jones 1990), and wetlands (Moore 1989) yet these studies did not directly address microbial activity at low temperatures. Many studies (Kol 1964, Hoham 1976, Hoham 1980) have addressed the distribution, abundance, and ecology of photosynthetic organisms (e.g. *Chlamydomonas nivalis*) within snowpacks, fewer, if any, studies have directly addressed heterotrophic microbial activity within snow and potential affects on snow chemistry.

The physical structure of the snowpack provides a unique mixture of physical and chemical environments. Temperatures within the snowpack

are well buffered in comparison to external air temperatures, remaining at or near 0° C during much of the spring. Physical processes within the snowpack tend to concentrate inorganic nutrients deposited in precipitation on the surfaces of ice crystals (Colbeck 1980). Heterotrophic organisms within the snow are isolated from the soil and litter and therefore dependent on carbon and nutrient pools within the snow itself. This combination of temperature and carbon limitation provides a simple system in which to investigate the effects of heterotrophic microbial activity on snow chemistry. This study addresses both spatial and temporal variations in microbial distribution within Wyoming and Colorado snowpacks in the context of four questions. 1) How are microbial populations within the snow related to CO2 fluxes from the snowpack? 2) Is the distribution of organisms related to the chemical composition of the surrounding snow? 3) What is the relative importance of bacteria vs. fungi within the snow? and 4) Are microorganisms within the snow a possible sink for inorganic nitrogen deposited within the snowpack over winter?

METHODS

Study Sites. Snow samples for this study were taken from the Glacial Lakes Ecosystem Experiments Site (GLEES) and Niwot Ridge International Biosphere Reserve (Niwot). GLEES is located in the Snowy Range of southeastern Wyoming, west of Laramie at 41° 20' N, 106° 20' W. Niwot Ridge is located in the Front Range of Colorado west northwest of Boulder at 40° 40' N 105° 30' W. GLEES samples were taken from snowpits dug adjacent to trace gas sampling plots located on the alpine/subalpine ecotone at an elevation of 3286m. The location is on an exposed ridgetop with poor soil development, sparse alpine ground cover, and widely scattered trees. Niwot samples were taken from pits dug in the seasonal snowpack at an elevation of 3525m. The site is completely above treeline with herbaceous ground cover and moderate soil development.

Sample collection. Samples for both microbiological and chemical analyses were collected from both sites beginning the first week of May 1992 until sites were free of snow (mid June for GLEES, late June for Niwot). Both sites were sampled at intervals of approximately one week. Snow chemistry samples were analyzed for 11 major inorganic ions, pH, ANC, and conductivity. The snowpack was sampled at up to seven regularly spaced depths beginning five centimeters above the snow/soil interface and ending five centimeters below the snow/air surface.

Sterile fifty ml centrifuge tubes were used for biological sampling in the field. A minimum of five replicate snow samples were collected at each depth. Three were preserved with formalin in the field for enumeration of bacteria and fungi. Two were unamended and used for the isolation of microorganisms.

Gas collectors were the same as used by Sommerfeld et al. (1993). Samples were taken at 15 cm below ground surface, at the surface, and within the snowpack at 5, 20, 50, and 100 cm above soil surface. Carbon dioxide samples were drawn with 20ml nylon syringes fitted with two way stopcocks. Samples were analyzed by gas chromatography within five hours of collection.

Laboratory Analyses. Viable biomass within the snowpack was estimated using a modification of the Total Extractable Lipid Phosphate (TELP) method of Brinch-Ivarson and King (1990). Ten ml aliquots of melted snow were filtered onto sterile 0.2 micron polycarbonate filters which were subsequently extracted in dichlormethane: methanol (2:1). Following addition of an aqueous phase the organic fraction was dried under nitrogen and digested in perchloric acid. Liberated phosphate was measured spectrophotometrically at 610 nm following reaction with malachite green and ammonium molybdate in polyvinyl alcohol.

The importance of bacterial relative to fungal biomass was determined microscopically following the filtering of melted snow onto 0.2 micron filters. The relative contribution of bacteria to total biomass was estimated using the epiflouresence method of Stamatiadis et al. (1990). Cells were resuspended, stained with acridine orange, and counted at 1250x magnification using a Leitz Dialux phase contrast microscope adapted for epiflouresence with a 50 V mercury light source. Total fungal biomass was estimated using methylene blue stain followed by enumeration under 687x magnification.

Laboratory incubations were conducted at the University of Colorado, Boulder Colorado. Organisms were isolated from 100 microliter aliquots of freshly melted snow on spread plates containing 5 percent Difco tryptic soy broth (0.85 gl^{-1} Bacto Tryptone, 0.15 gl^{-1} Bacto Soytone, 0.125 gl^{-1} Bacto Dextrose, 0.25 gl^{-1} Sodium chloride, 0.125 gl^{-1} Potassium diphosphate) in Difco Bacto-Agar. Plates were incubated at 0° +/- 0.5^{o} and examined

for growth at 12 hour intervals. Colonies were streaked on plates and incubated at 0^{0} until pure cultures were obtained. Growth vs. temperature experiments for pure cultures were conducted in triplicate at 0, 10, 25, and 37 degrees on plates inoculated with 100 microliters of a 10^{-6} dilution of a broth culture. Number and size of colonies were recorded at 12 hour intervals.

RESULTS AND DISCUSSION

Figure 1 illustrates the temporal variability of CO2 concentrations at GLEES during the spring of 1991. While the majority of gas production is presumed to be from soil, the cross ridge present at the 50 centimeter level suggests a source within the snowpack. This ridge was present to some degree at all four sampling locations, being most pronounced

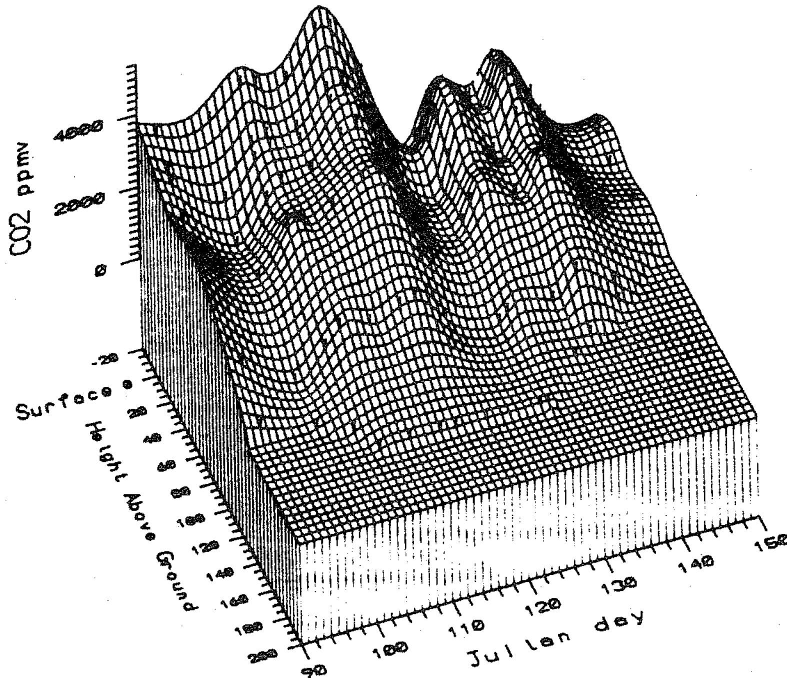

Figure 1. Three dimensional plot illustrating the temporal variability of CO2 concentrations at one of the GLEES sites in 1991.

on day 103 (Figure 2) and gradually decreasing through the end of the season. Although the three dimensional plot indicates great temporal variability in production at the soil surface, the short time lag for diffusion through the snowpack strongly suggests this is not the cause of the cross ridge. Using the formula of Sommerfeld et al. (1993), this lag time is estimated at less than 25 minutes. Neither could such a profile be due to a low porosity layer in the snow. In the extreme, a completely impermeable ice lense would result in a vertical profile below it. Porosity variations would provide a plausible explanation for the profile seen on day 127 (Figure 3).

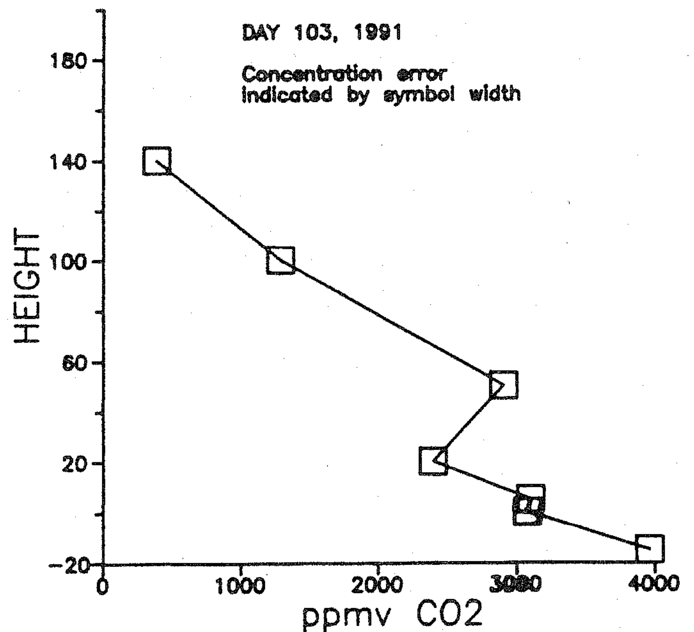

Figure 2. Vertical CO2 concentration profile for April 13th, 1991.

It is possible to estimate the contribution of a source within the snow to the total CO2 flux by assuming the main part of the flux is driven by the concentration gradient between the soil surface and the snow/air interface and that these fluxes are additive. Using these conditions maximum flux from within the snowpack peaks on day 103 at 360 mg C m^{-2} day^{-1} and decreases to 320 mg C m^{-2} day^{-1} by day 127. If the concentration profile on day 127 is assumed to be completely due to porosity variations then the minimum flux from the snowpack would be the difference; approximately 40 mg C m^{-2} day^{-1}. Over the 49 day sampling period this flux would require a carbon source within the snow pack of 1.96 g m^{-2}. Precipitation records indicate a two week dry period in the beginning of February (corresponding to an accumulated snow depth of approximately 50 cm) during which a detritus layer of this magnitude could have developed.

Measured CO2 fluxes from the GLEES site during 1992 were of a similar magnitude and

demonstrated a comparable degree of spatial and temporal heterogeneity as the data from 1991 (Sommerfeld et al. 1993). However, profiles of CO2 concentrations through the snowpack failed to identify a source within the snow. Neither do meteorolgical data indicate an extended period without precipitation when a significant litter layer could have developed within the snowpack.

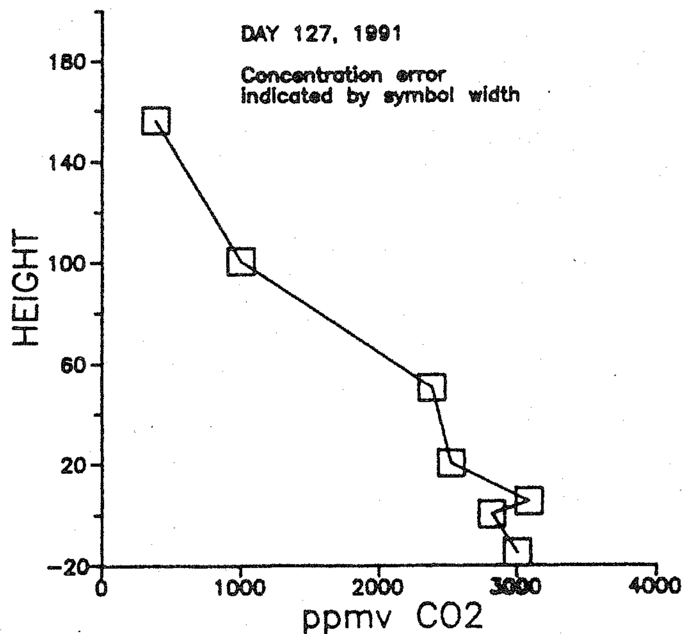

Figure 3. Vertical CO2 concentration profile for May 7th, 1991

Estimates of total microbial biomass were consistently small throughout the season at both sites. Based on microscopic cell volume estimates the highest recorded value, 0.05 micrograms biomass C ml^{-1} is approximately equivalent to 10^{-4} cells per ml. These values can be placed in context through a comparison with the observed carbon dioxide fluxes from 1991. Respiration rates for soil microbial communities *in situ* (Gray and Williams 1971) can be used to provide an estimate of CO2 flux from this population. Assuming a production layer 10 cm thick, a snow density of 0.33, and applying a conservative Q10 value of 2.0 the maximum CO2 production from these organisms would be 653 ug C m^{-2} day^{-1}. This is approximately two orders of magnitude lower the minimum flux calculated from the data presented in Figure 2.

In contrast to a single sample taken in 1991 (Sommerfeld et al. 1993), microscopic analyses during the 1992 season indicated the snow microbial community was dominated by bacteria. Very few samples contained fungal biomass, none at levels which were statistically quantifiable. Although blooms of the snow algae *Chalmydamonas nivalis* occurred during the later portion of sampling, they did not co-occurr with bacterial populations. The distribution of total viable microbial biomass throughout the snowpacks during the spring of 1992 is presented in Figures 4 and 5. Niwot shows a pattern of even

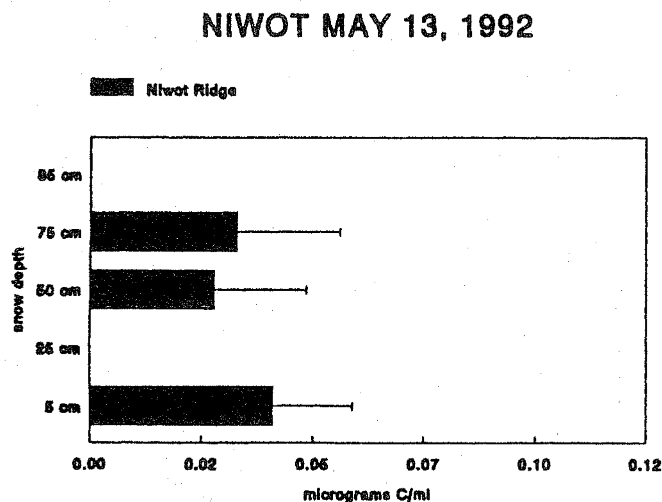

Figure 4. Distribution of viable microbial biomass, as estimated by total extractable lipid phosphate, on May 13th in the Niwot snowpack.

distribution throughout the snowpack in early May, followed by several weeks where viable biomass is limited to approximately 20 cm above the snow/soil interface. The early season distribution is absent at GLEES, although the vertical distribution of microorganisms shows a similar peak at 20 cm. The observed distribution of bacteria within the snowpack in 1992 is probably due to a balance between migration from the soil surface and elution from the snowpack by meltwater. The even distribution throughout the snowpack at Niwot in early May corresponds to the week just prior to snowmelt. Linear regressions using snow chemistry data (17 chemical variables including pH, nitrate,

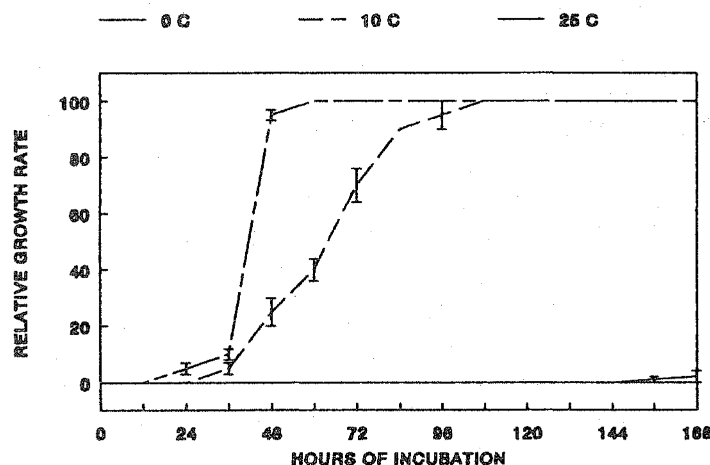

Figure 5. Relative growth rates for "OY" a gram negative, motile rod isolated form the Niwot snowpack. The organism was not capable of growth at 37° C, with extremely slow growth at 0° C.

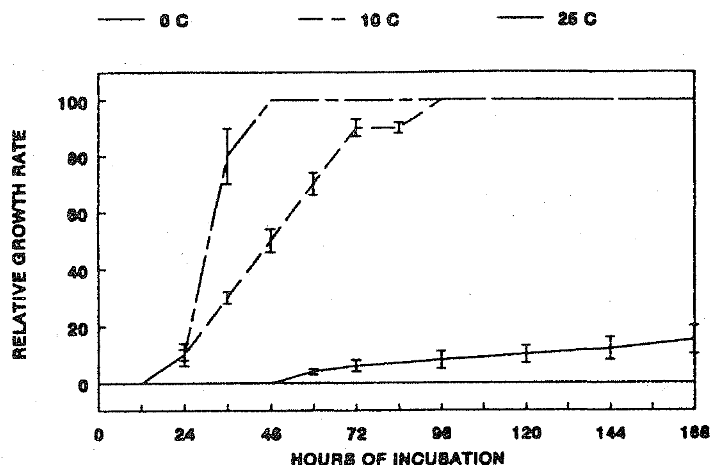

Figure 6. Relative growth rates for "DCW" a gram negative, motile rod isolated form the Niwot snowpack. The organism was not capable of growth at 37° C, and only slow growth at 0° C.

ammonium, and phosphorus) from both Niwot and GLEES against microbial biomass yielded no significant correlations.

A series of laboratory incubations were undertaken in an attempt to identify the potential of the bacterial biomass identified in 1992 to alter trace gas production and snowpack chemistry in the presence of a labile carbon source. Two microorganisms ("DCW" and "OY") isolated from the Niwot snowpack were both Gram negative, motile rods. Growth vs. temperature experiments suggest these organisms were psycrotrophs ("cold tolerant", but with growth optimum > 15° C) rather than true psycrophiles ("cold loving", growth optimum < 15° C). Neither organism was capable of growth at 37° C. Although both were capable of growth at 0°, both organisms had optimum growth temperatures near 20 degrees (Figure 6 and 7). Based on these growth rates it is unlikely these organisms had a significant effect on the chemical composition of snowmelt in 1992. It is similarly unlikely that the bacteria isolated from the snowpack in 1992 are the same organisms responsible for the fluxes observed in 1991. Carbon dioxide production observed in the 1991 snowpack presumably was due to the accumulation and subsequent decomposition of a significant litter layer during an extended snow-free period. It is likely that freeze/thaw cycles served to release a pulse of labile carbon and nutrients from this litter providing a substrate for microbial colonization as the snowpack warmed.

CONCLUSION

Psycrophilic and psycrotrophic organisms have been well studied in permanently cold environments such as Arctic ice flows (e.g. Gradinger et al. 1992) and the terrestrial Antarctic (e.g.
Davey 1989, Davey 1991). Significant work also has been done on snow algae found in temperate snowpacks (e.g. Kol 1964, Hoham 1980, Hoham 1990), yet relatively little work has been done on cold tolerant heterotrophic microorganisms within or under temperate snowpacks. While this study indicates heterotrophic microorganisms within snowpacks have limited effects on snowmelt chemistry, the observed gas fluxes in both 1991 and 1992 suggest cold tolerant organisms in soil may have significant impacts on biogeochemical cycles in seasonally snow covered environments. Although presumably somewhat limited by temperature, organisms capable of activity under snowpacks may face a relative lack of competition for nutrients. The importance of these organisms in biogeochemical cycling may be quite high in areas with short growing seasons and long periods of snow cover such as alpine tundra.

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