Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass

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Abstract

In Colorado alpine dry meadow soils, microbial biomass has been observed to increase during fall and winter and to rapidly decline after snowmelt in the spring. It has been shown that these microbial population dynamics are linked to N availability to alpine plants, but the underlying mechanisms have not been explained. We hypothesized that: (1) freeze-thaw events in the spring cause reduction of the microbial biomass, (2) the winter microbial community is sensitive to prolonged temperatures above $0°C$, and (3) the increase of biomass in fall and its decline in spring are due to changes in C availability. We performed laboratory experiments to test the effect of temperature regime on soil microbial biomass, respiration and C availability, and made seasonal measurements of C pools. Soil microbial biomass was unaffected by freeze-thaw events in which realistic rates of freezing and thawing were used. Some significant effects were observed at faster freezing rates. Despite this tolerance to temperature fluctuations, the winter microbial community showed sensitivity to prolonged temperatures above $0°C$. This effect may have been caused indirectly by an effect of temperature on substrate availability. Two week incubations at increased temperatures caused a reduction in the quantity of extractable organic C in the soil. The soil concentrations of cellulose and hot water-soluble organic C were the lowest in the summer and the highest in spring and autumn, mirroring previously measured patterns of microbial biomass. This suggests that C from litter inputs could be a strong control over microbial biomass. Respiration rates in soils collected before snowmelt were high at $0°C$, and did not respond immediately to addition of glutamate. At $22°C$, or after a two week incubation at $0°C$, respiration in these soils became substrate-limited. Respiration rates in soils collected during the summer were very low at $0°C$, but responded immediately to glutamate addition at both 0 and $22°C$. These results show that the C balance of the soil microbial biomass undergoes a critical shift between winter and summer due to an increase in temperature and a corresponding decrease in C availability. This shift could explain the decline in microbial biomass after snowmelt. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Previous studies in the Colorado alpine ecosystem have shown two predictable seasonal patterns in soil microbial biomass. Microbial biomass increases in the fall and winter (Brooks et al., 1996; Jaeger et al., 1999; Lipson et al., 1999) and decreases in the spring at the start of the plant growing season (Brooks et al., 1998; Lipson et al., 1999). The decline of microbial biomass in the spring has been linked to a pulse of N availability to plants in the alpine dry meadow (Lipson et al., 1999). However, the causes for these patterns have not been established. It has been speculated that the decline in microbial biomass in spring time is linked to freeze-thaw events (Brooks et al., 1998; Jaeger et al., 1999). In alpine dry meadows, the snowpack recedes in mid- to late-spring, leaving soils exposed at a time when night time air temperatures are regularly below

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0°C. Numerous studies have observed that freeze-thaw events cause a burst of microbial activity such as mineralization of C or N (DeLuca et al., 1992; Ross, 1972; Schimel and Clein, 1996; Skogland et al., 1988). This burst of activity has generally been attributed to increased levels of labile substrate in the soil resulting from damage to micro-organisms by freeze-thaw events. Such damage to soil microbes has been demonstrated (Morley et al., 1983; Soulides and Allison, 1961). However, previous work showed that the soil microbial biomass in alpine dry meadows was not affected by a single freeze-thaw event (Lipson and Monson, 1998). Also, as mentioned above, microbial biomass has been observed to increase during the fall when soils are also exposed to fluctuations around the freezing point (Lipson et al., 1999). The reason that previous experiments showed no effect of freeze-thaw events may have been that the experimental cooling and warming rates were carefully controlled to simulate soil temperatures during the alpine spring or autumn (Lipson and Monson, 1998). It has been shown that rates of freezing and thawing and depth of freeze can have large effects on survival of micro-organisms (Mazur, 1980; Meyer et al., 1975). However, these previous studies have been performed in pure culture, and have used a range of cooling/warming rates (12–60,000°C h^{-1}) that are much higher than those observed in the alpine during the spring or fall (see Section 3).

Herein, we test the hypothesis that freeze-thaw events explain the post-snowmelt decline in microbial biomass by subjecting soils to realistic simulations of vernal freeze-thaw events. As an alternative to this hypothesis, we propose that the input of carbon (C) from plant litter in the fall allows cold-adapted microbes to grow until snowmelt, then a decline in C availability and prolonged temperatures above 0°C cause a decline in the winter-adapted soil microbial biomass. If the winter soil microbial community is dominated by psychrophilic organisms, increased temperatures could directly affect the biomass. Also, higher metabolic maintenance requirements at warmer temperatures coupled with low C availability in the spring could lead to starvation and cell death.

Specifically, we tested the hypotheses that the winter microbial community is sensitive to temperatures above 0°C, and that the increase of biomass in fall and its decline in spring are due to changes in C availability. These hypotheses are tested by seasonal measurements of soil C pools, and by laboratory experiments that measure the effects of substrate addition and incubation temperature on respiration rates, microbial biomass C and C availability. Our ultimate aim was to explain the mechanisms of the microbial population dynamics that have proved to be central in the alpine N cycle.

2. Materials and methods

The study site is located at the Niwot Ridge Long Term Ecological Research (LTER) site in the front range of the Colorado rocky mountains (3545 m elevation, 40°03′ N, 105°35′ W). The dry meadow habitat that we studied is dominated by *Kobresia myosuroides* (Vill.) Paol. and Fiori, and the soils are classified as loamy-skeletal Pergelic Cryumbrepts (Burns, 1980; Birkeland et al., 1989). Physical properties of the soils have been described earlier (Raab et al., 1999). The dry meadow is generally snow-free from May/June to October/November. In 1996, the first snow-free day was May 7. For seasonal measurements of soil C pools, a minimum of three soil cores (10 cm deep, 5 cm diameter) were collected randomly across the landscape at each site. Soils were sealed in plastic bags and brought to the laboratory for analysis. Soil was coarsely sieved (4.75 mm) to remove rocks and large roots. Laboratory experiments were performed on soil composited from the spatial replicates. Soil microbial biomass was quantified using the substrate-induced growth response (SIGR) method (Colores et al., 1996; Schmidt, 1992). Previous work showed that glutamate is a good general substrate for microbial growth (Alef and Kleiner, 1986) and that glutamate SIGR is highly correlated to other measures of microbial biomass (Lipson et al., 1999). Potassium glutamate was added to soils in sufficient quantities to induce growth along with a ^{14}C-tracer, and respiration of the substrate was followed over time. The respiration data were fit with the SIGR model to yield a biomass estimate. The initial respiration rate provided the substrate-induced respiration rate (SIR). Glucose SIR was also used for one freeze-thaw experiment. Chloroform fumigation-extraction (CFE) measurements were performed as described by Brookes et al. (1985). Total organic C in (0.5 M) K_2SO_4 extracts of fumigated and unfumigated soil was measured using a Mn(III)-reduction assay (Bartlett and Ross, 1988). Most probable number determinations were performed as described earlier (Lipson et al., 1999; Schmidt and Gier, 1990). The growth media contained 0.14 g K_2HPO_4, 0.02 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 0.10 g NH_4NO_3, and 0.05 g $CaCl_2$, per l H_2O, to which was added 1 ml soil extract (10 : 1 v/w, sterile filtered), and 0.1 ml yeast extract (1% w/v, sterile filtered). Glutamate was added to this media to obtain a final concentration of 1 mM.

Soil was analyzed for two C fractions that contain the important plant litter components starch and cellulose. The hot water-soluble fraction was extracted from soils that had been stored frozen (−20°C). Soil was first shaken with cold water, centrifuged, and the supernatant was discarded. The residue was extracted in boiling water for 2 h and the tubes were again cen-

trifuged. The supernatant was analyzed for total organic C, as described above. This fraction contained starch and other compounds that are soluble in hot water, such as proteins and condensed tannins (Chapin et al., 1986). The cellulose-containing fraction was extracted from soils that had been oven dried (100°C) and stored at room temperature. Cellulose determinations were performed as described by Updegraff (1969), with the following modifications. Lignin, hemicellulose and xylosans were removed with 10 : 1 80% acetic acid : concentrated nitric acid, and the pellet was rinsed three times with water to remove residual acetic acid. The cellulose fraction was dissolved in 67% sulfuric acid and measured using the total organic C method mentioned above.

In the three freeze-thaw experiments, soil was placed in 120 ml specimen cups. In Experiment 1, soil was collected immediately after the snow had receded in spring. In Experiments 2 and 3, soil was collected in the fall. Controls were left at 3°C in all experiments. An additional 0°C control treatment was included in Experiment 2. Freeze-thaw cycles were produced by moving the containers between a room refrigerated to 3°C and a freezer set to −5°C. Each half of the cycle lasted for 2 days. The temperature changes were non-linear, showing a plateau near 0°C as ice formed or melted. For simplicity, the freezing and thawing rates are expressed as average linear rates (maximum temperature minus minimum temperature divided by the time interval required for the transition). In Experiment 1, and in the 'slow' treatments of Experiments 2 and 3, the containers were placed in a small sand bath. In the 'fast' treatments, containers were uninsulated. Temperature was monitored using copper-constantan thermocouples. Thawing and freezing rates were roughly symmetrical. After six cycles in Experiments 1 and 2, and seven cycles in Experiment 3, all samples were allowed to equilibrate in the 3°C refrigerated room. The measurements of microbial biomass described above were performed in each experiment.

Two respiration experiments were performed on soil collected during April (about one month before snowmelt) and during July. In the spring experiment, 10 g soil was placed in tightly stoppered biometer side arm flasks. The flasks were incubated at either 0°C (on ice in a 3°C refrigerator) or 22°C. The side arm contained 5 ml (1 M) NaOH. Control flasks contained, instead of soil, a volume of water equivalent to the water content of the soil samples. All treatments contained two duplicate flasks. Soil samples were amended with glutamate (1 mg C g^{-1} soil) or an equivalent volume of water as a control (1 ml per flask). The base trap was replaced regularly and CO_2 was measured using acid titration in $BaCl_2$ (Zibilske, 1994). After two weeks, the glutamate or water addition was repeated and the measurements continued for 4 days. In the summer ex-

periment, only one addition was performed and the measurements were carried out for 4 days.

In the two constant-temperature experiments, soils were collected during the spring before snowmelt and were subjected to two-week incubations at various temperatures. In the first experiment, the temperature treatments were −5, 0, and 10°C. Microbial biomass C and K_2SO_4-extractable organic C were measured using the CFE technique initially after collection, and after the incubation period. The second experiment consisted of three treatments: −2.5, 10, and 10°C with starch added as a dry powder at a concentration of 1 mg C g^{-1} soil. The starch amendment was included to test for interactions of C availability with temperature. Microbial biomass C and K_2SO_4-extractable organic C were measured after the two-week incubation period. In both experiments, the sub-zero treatments were produced using a slow freeze ($< 1°C\ h^{-1}$) by insulating the containers in a sand bath as described above.

Differences between treatments in the freeze-thaw and constant-temperature incubation experiments were analyzed with one-way analyses of variance
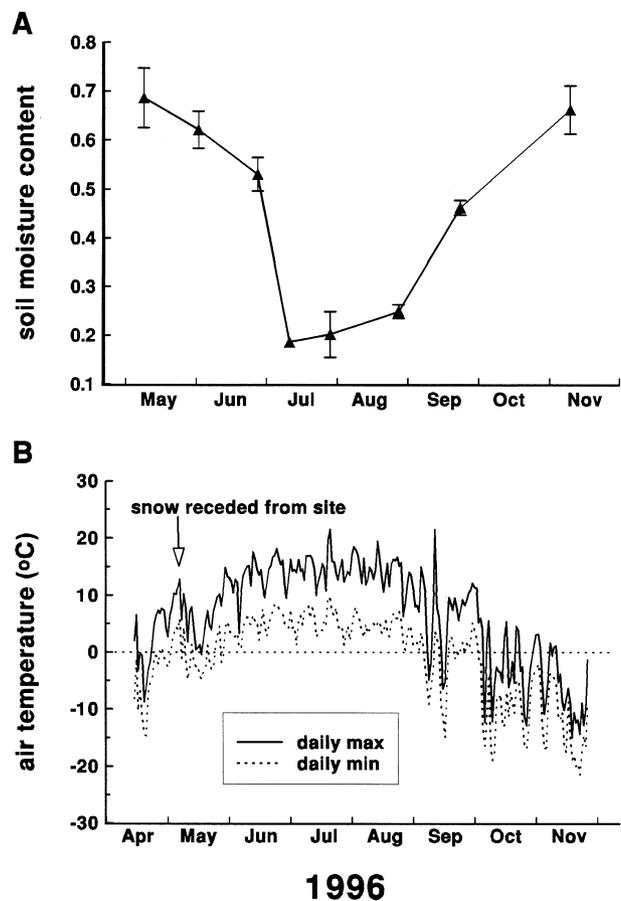
Fig. 1. (A) Soil gravimetric water content for the snow-free season of 1996. (B) Minimum and maximum daily air temperatures in the Saddle on Niwot Ridge in 1996. The dry meadow site was free of snow by May 7, 1996 (data provided by Niwot Ridge LTER).

Table 1

Results of three freezing experiments. Various measures of soil microbial biomass after freeze-thaw cycles or incubations at a constant temperature. Controls were kept at the temperature indicated. Freeze-thaw treatments (F-T) were cycled between 3 and $-5°C$, (average cooling rate shown in parentheses). In Experiment 1, glucose SIR was measured at $12°C$. In the remaining experiments SIR, SIGR, and maximum exponential growth rate (μ_{max}) were measured at $3°C$ using glutamate. Values are means and standard errors for three replicates, except for SIR in Experiment 1, which had two replicates (nd = not determined)

| | SIR (μg C g^{-1} h^{-1}) | SIGR | | MPN (10^7 cells g^{-1}) | CFE (mg C g^{-1}) |
		Biomass (mg C g^{-1})	μ_{max} ($h^{-1} \times 10^{-3}$)		
Experiment 1					
Control ($3°C$)	9.52 ± 0.25	nd	nd	nd	0.69 ± 0.02
F-T ($0.8°C$ h^{-1})	9.57 ± 0.24	nd	nd	nd	0.67 ± 0.03
Experiment 2					
Control ($3°C$)	1.22 ± 0.04	0.16 ± 0.01	8.0 ± 0.2	nd	nd
Control ($0°C$)	1.23 ± 0.03	0.17 ± 0.01	8.0 ± 0.2	nd	nd
Slow F-T ($1.1°C$ h^{-1})	1.13 ± 0.06	0.16 ± 0.01	8.0 ± 0.2	nd	nd
Fast F-T ($3.0°C$ h^{-1})	1.07 ± 0.05	0.12 ± 0.01[a]	10.0 ± 0.3[a]	nd	nd
Experiment 3					
Control ($3°C$)	1.86 ± 0.03	0.15 ± 0.005	9.3 ± 0.2	4.51 ± 0.26	nd
Slow F-T ($1.4°C$ h^{-1})	1.71 ± 0.07	0.13 ± 0.01[a]	9.7 ± 0.1[a]	5.19 ± 0.71	nd
Fast F-T ($2.0°C$ h^{-1})	1.71 ± 0.08	0.12 ± 0.01[a]	10.2 ± 0.2[a]	5.91 ± 1.69	nd

[a] A significant difference compared to controls.

(ANOVA). The Bonferroni inequality was applied to the critical P-value to adjust for multiple tests on the data set. In the respiration experiments, differences between C-addition treatments and controls were tested using analyses of covariance (ANCOVA), with time after addition as the continuous variable and treatment as a categorical variable. The time-treatment interaction term was only included when significant (this term was significant only in the spring time $22°C$ treatments).

3. Results

Over the first four weeks after snow receded from the site (the interval during which microbial biomass declines (Lipson et al., 1999)) soil moisture content did not change dramatically (Fig. 1A). Night time air temperatures at the field site regularly fell below $0°C$ during the first month after snowmelt, and during late summer and fall (Fig. 1B). On nights when air temperatures dropped below $0°C$ in the spring, the average air cooling rates ranged from 0.2 to $0.9°C$ h^{-1}. The slowest soil cooling rate produced in the laboratory simulations ($0.8°C$ h^{-1}) was similar to the fastest observed drop in air temperature during the snow-free season.

In the first freezing experiment, neither glucose SIR nor CFE-C was significantly affected by freeze-thaw cycles (Table 1). In the second freeze-thaw experiment, glutamate SIR was unaffected by slow and fast freeze-thaw cycles (Table 1). Glutamate SIGR was significantly lower than controls in soils exposed to fast ($3.0°C$ h^{-1}) freeze-thaw cycles, but not in the slow ($1.1°C$ h^{-1}) freeze-thaw treatment. Maximum exponential growth rate during the SIGR incubation was sig-
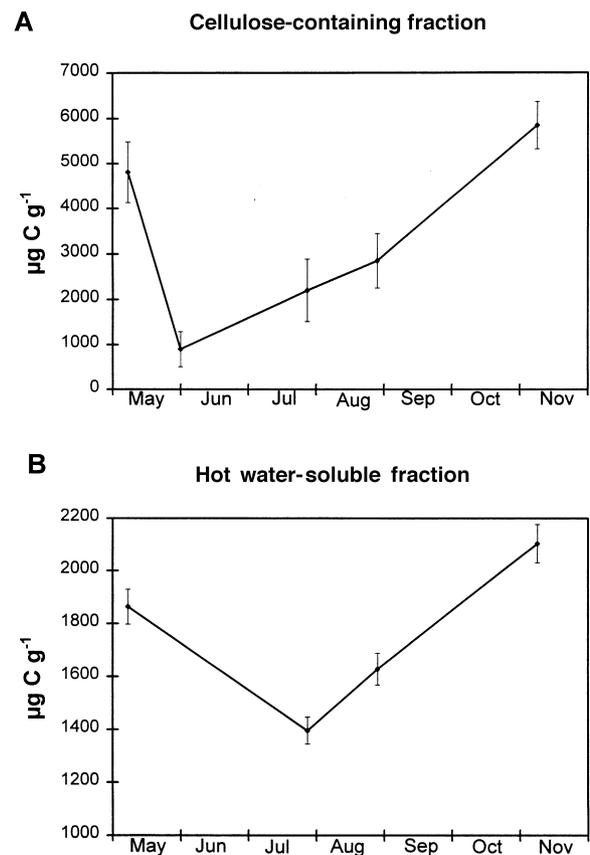
Fig. 2. Concentrations of the (A) cellulose-containing fraction and (B) hot water-soluble fraction in soils during the snow-free season of 1996.

nificantly higher in the fast freeze-thaw treatments. In the third freezing experiment, glutamate SIR and MPN were not affected by freezing (Table 1). Glutamate SIGR in the freeze-thaw (1.4 and 2.0°C h^{-1}) were significantly lower than controls, and the maximum exponential growth rate was higher in these treatments.

The cellulose-containing and hot water-soluble organic fractions of soil were high in late spring (immediately after snowmelt), decreased in summer and recovered by fall to slightly above the spring levels (Fig. 2). This pattern led to the hypothesis that microbes become C-limited after snowmelt, leading to a decline in microbial biomass. To test whether the C balance of soil micro-organisms changes between winter and summer, respiration experiments were performed. In soils collected before snowmelt, respiration proceeded well at 0°C, and was not increased by the addition of glutamate relative to controls ($P = 0.75$) (Fig. 3A). However, after a 360 h incubation at this
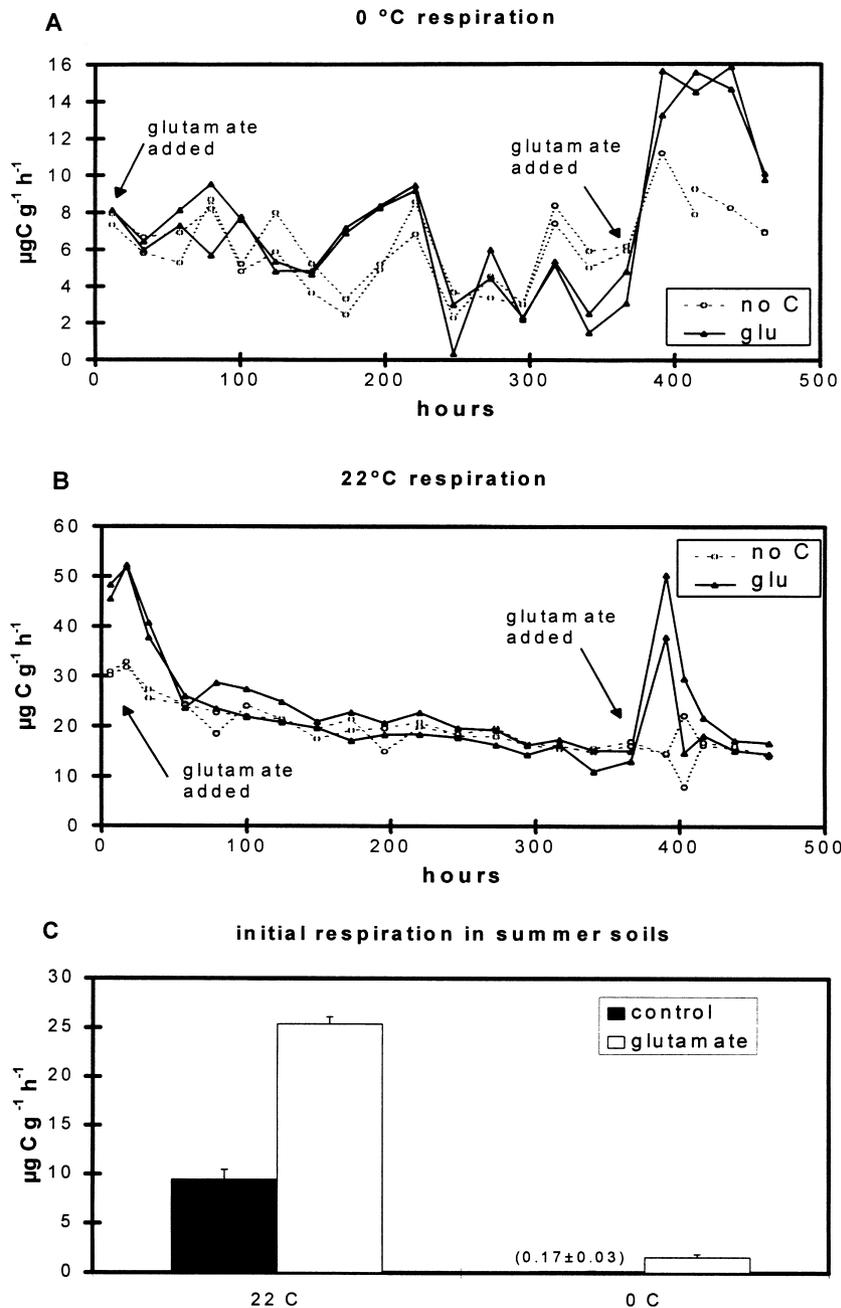
Fig. 3. Respiration rates in soils collected from under snowpack at 0°C (A), and 22°C (B), with either potassium glutamate (glu) or an equivalent amount of water added as a control. The times of glutamate or water addition are indicated by arrows. Data for both duplicate flasks are shown for each treatment. (C) The inital respiration rates in soils collected in the summer, at 0 and 22°C, with addition of glutamate or water (control).
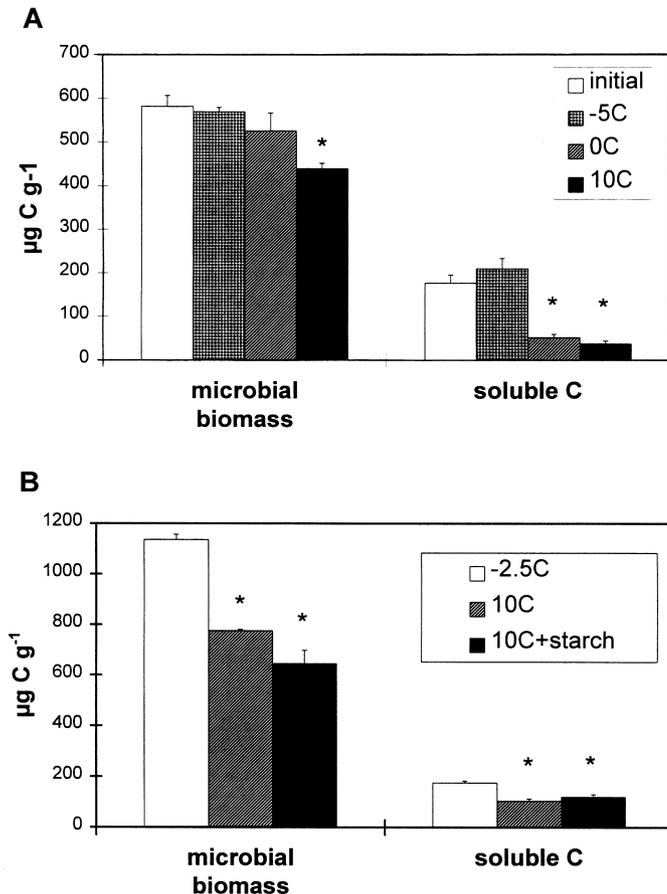
Fig. 4. Two experiments in which soils were collected under snowpack in the spring (April 10, 1997, soil temperature $-2°C$), and incubated for two weeks at various temperatures. (A) Microbial biomass C (CFE) and K_2SO_4-extractable organic C initially, and after the incubation. An asterisk (∗) indicates that the mean is significantly different from the initial value. (B) The effect of starch addition and temperature on microbial biomass C and K_2SO_4-extractable organic C. (No initial measurements were made in this experiment.) An asterisk (∗) indicates the mean is significantly different from the $-2.5°C$ value.

temperature, respiration rates did increase in response to a second addition of glutamate ($P < 0.001$). In the $0°C$ control treatment, there was also a stimulation due to the second water addition. At $22°C$, respiration rates were 3.75 and 5.75 times higher than at $0°C$ for control and glutamate treatments, respectively, and respiration responded to both glutamate additions ($P < 0.001$, $P = 0.002$ for first and second additions) (Fig. 3B). Respiration rates in the control flasks at $22°C$ declined over the 360 h period before the second additions of water and glutamate ($R^2 = 0.74$, $P < 0.001$, slope $= -0.037$). Respiration rates in the $0°C$ flasks also declined significantly over this interval, despite the higher relative variability, but with a lower slope ($R^2 = 0.21$, $P < 0.001$, slope $= -0.009$). Note that the ratio of the respiration rates between 22 and $0°C$ and the ratio of decline of respiration rates at 22

and $0°C$ are both about four. Thus, the decline in respiration rate is proportional to the rate of respiratory loss of C from the soil. This experiment was repeated on soil collected in the summer, although only initial rates were measured. In the summer, respiration rates were barely above detection limits at $0°C$, but respiration responded immediately to added glutamate at this temperature ($P = 0.009$) (Fig. 3C). At $22°C$ the summer soil respired 54.9 and 16.4 times faster than at $0°C$ for control and glutamate treatments, respectively, and the respiration rate responded strongly to the glutamate addition ($P = 0.006$).

To test the effects that prolonged temperatures above $0°C$ have on the microbial biomass and soil soluble organic C, we incubated soil collected prior to snowmelt at a range of temperatures. After two weeks at $-5°C$ (after a slow freezing procedure), microbial biomass C (CFE) and K_2SO_4-extractable organic C did not significantly change from their initial values, but at $10°C$, microbial biomass declined, and at both 0 and $10°C$, extractable organic C declined (Fig. 4A). To test whether the effects of increased temperature were a result of C limitation that could be mitigated by the addition of C, the experiment was repeated, but starch was added to one set of replicates at $10°C$. The declines in microbial biomass C and extractable organic C at $10°C$ were again significant, and the addition of starch did not alter the effects of temperature (Fig. 4B).

4. Discussion

Laboratory freeze-thaw experiments designed to realistically mimic the temperature regime in alpine dry meadow soils in spring and fall did not cause appreciable reduction in soil microbial biomass. We therefore reject our hypothesis that freeze-thaw events explain the previously observed decline of microbial biomass after snowmelt. The results of the present study show that careful control of the experimental freezing rate is important. Decreases in biomass were seen in the 1.4, 2.0, and $3.0°C$ h^{-1} treatments but not in the 0.8 or $1.1°C$ h^{-1} treatments. In previous studies that measured effects of freeze-thaw events on microbial counts in soils, the rates of freezing and thawing were not described (e.g. Soulides and Allison, 1961; Skogland et al., 1988). The difference between this and earlier studies could be due to the use of a more realistic freezing rate. Previous work has shown that rates of cooling and warming affect survival rates of microorganisms subjected to freeze-thaw events (Mazur, 1980; Meyer et al., 1975).

The microbial community of these alpine tundra dry meadow soils may be uniquely resistant to freeze-thaw events. It is not surprising that microorganisms in a

cold ecosystem can tolerate moderate freeze-thaw events. Such tolerance has been observed in Antarctic peat microbes (Wynn-Williams, 1982), and in pure cultures of cold-adapted bacteria (Panoff et al., 1995). Given reported internal solute concentrations of bacteria (Measures, 1975), intracellular ice would not form at the temperatures observed in these soils in the spring ($> -5°C$). Microbial activity has been detected in soils at $-5°C$ (Brooks et al., 1996; Clein and Schimel, 1995), and significant liquid water exists in soils at this temperature (Anderson, 1970; Brooks et al., 1997). Alpine soil microbes might be vulnerable to more extreme freezing events during the winter. Survival has been shown to decrease with an increased depth of freeze (Mazur, 1980). During the winter, minimum soil temperatures of $-11.0°C$ have been reported for a nearby dry meadow with similar snow accumulation (Brooks et al., 1998). It is possible that winter conditions select for a cold tolerant microbial community, thus explaining the resistance to freeze-thaw events in the spring.

The SIR and MPN were not affected by even the fastest freeze-thaw treatments, while the SIGR measurements were. This illustrates that the SIGR technique can be more sensitive than the SIR technique because the SIGR uses the growth rate in addition to the initial respiration rate to estimate biomass. The SIR technique requires that the initial respiration rate be calibrated with the level of respiration per unit biomass. The SIGR technique uses the maximum exponential growth rate to determine this conversion factor. In this study, the initial respiration rates did not vary significantly, but the maximum exponential growth rates during the SIGR incubations did, leading to significant differences in the SIGR biomass estimates. Only bacteria grew in the MPN plates, probably because the fungi are harder to extract from soil due to their complex filamentous growth form. The lack of effect in the MPN in the fast freeze-thaw treatments indicated that the sensitive biological component measured by SIGR, an in situ technique that does not require extraction and culturing, is either fungi or bacteria that cannot grow in the culture conditions used.

Although the soil microbial biomass was resistant to repeated fluctuations around $0°C$, the winter microbial community was sensitive to prolonged temperatures above $0°C$. The effects of temperature could be mediated by C availability. After two weeks at temperatures above zero, soluble organic C levels were greatly reduced. The fact that the addition of starch did not reverse the effects of temperature could indicate that there is a direct negative effect of temperature on winter microbial biomass. Sensitivity of psychrophilic bacteria to increased temperatures can be caused by thermal instability of enzymes (Feller et al., 1996). Alternatively, it may be simply that the starch treat-ment was not sufficient to reverse the depletion of substrate in the soil, and that other nutrients or substrates may be required. Any sensitivity of the winter microbial biomass to increased temperature would be exacerbated by a decline in C availability after snowmelt.

Moreover, in dry meadows with greater maximum snow accumulation than in the current study, the microbial biomass was observed to decline before the snow had fully retreated from the site, during which time soil temperatures remained near $0°C$ (Brooks et al., 1998). This indicates that C limitation may be the dominant mechanism by which the winter microbial biomass decreases. A critical shift in the C balance of soil microbes occurs between winter and summer. Under the snowpack, available C is initially sufficient to saturate respiratory activity near $0°C$, but after a period of time as C is lost through respiration, or when soils become warm and metabolism increases, the soil C reserves are no longer sufficient to maximize respiration. This C limitation could lead to starvation and hence, reduction of the microbial biomass. This interpretation is borne out in the time course of the soil organic C fractions. The cellulose and hot water-soluble organic pools decline after snowmelt and recover in the fall as plants senesce. The C inputs from plant litter and the low temperatures allow the winter microbial community to thrive until snow melts and temperatures warm. Our results are consistent with a study in arctic tundra that showed significant mass loss from litterbags during the winter (Hobbie and Chapin, 1996), and studies that found microbial activity in soils at subzero temperatures (Brooks et al., 1996; Clein and Schimel, 1995).

The summer and winter microbial biomass have different responses to temperature. Winter microbial biomass is sensitive to temperatures at which the summer community survives. Also, respiration proceeded rapidly at $0°C$ in winter soil, but was barely measurable at this temperature in summer soils. This suggests that a shift in species composition occurs between winter and summer, although more direct evidence is needed to verify this. The decline of biomass after snowmelt has been shown to release organic N that becomes available to plants (Lipson et al., 1999). Whether the different properties of winter and summer microbial biomass are due to a shift in species dominance or to a physiological acclimation of a constant set of microbial species, this linkage between specific physiological characteristics of the microbial biomass and a higher level ecosystem process is novel.

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