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# Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events

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**Abstract** Amino acids have been shown to be a potentially significant N source for the alpine sedge, *Kobresia myosuroides*. We hypothesised that freeze-thaw and dry-rewet events allow this plant species increased access to amino acids by disrupting microbial cells, which decreases the size of competing microbial populations, but increases soil amino acid concentrations. To test this hypothesis, we characterized freeze-thaw and dry-rewet events in the field and simulated them in laboratory experiments on plant-soil microcosms. In one experiment, $^{15}N$,$^{13}C$-[2]-glycine was added to microcosms that had previously been subjected to a freeze-thaw or dry-rewet event, and isotopic concentrations in the plant and microbial fractions were compared to non-stressed controls. Microbial biomass and uptake of the labeled glycine were unaffected by the freezing and drying treatments, but microbial uptake of $^{15}N$ was lower in the two warmer treatments (dry-rewet and summer control) then in the two colder treatments (freeze-thaw and fall control). Plant uptake of glycine-$^{15}N$ was decreased by climatic disturbance, and uptake in plants that had been frozen appeared to be dependent on the severity of the freeze. The fact that intact glycine was absorbed by the plants was confirmed by near equal enrichment of plant tissues in $^{13}C$ and $^{15}N$. Plants under optimal conditions recovered 3.5% of the added $^{15}N$ and microbes recovered 5.0%. The majority of the $^{13}C$ and $^{15}N$ label remained in a non-extractable fraction in the bulk soil. To better understand the isolated influences of environmental perturbations on soil amino acid pools and population sizes of amino-acid utilizing microbes, separate experiments were performed in which soils, alone, were subjected to drying and rewetting or freezing and thawing. Potential respiration of glycine and glutamate (substrate-induced respiration; SIR) by the soil microbial communities was unaffected by a single freeze-thaw event. Glycine SIR was decreased slightly (~10%) by the most extreme drying treatment, but glutamate SIR was not significantly affected. Freezing lowered the concentration of water-extractable amino acids while drying increased their concentration. We interpret the surprising former result as either a decrease in proteolytic activity in frozen soils relative to amino acid uptake, or a stimulation in microbial uptake by physical nutrient release from the soil. We conclude that climatic disturbance does not provide opportunities for increased amino acid uptake by *K. myosuroides*, but that this plant competes well for amino acid N under non-stressed conditions, especially when soils are warm. We also note that this alpine tundra microbial community's high resistance to freeze-thaw and dry-rewet events is novel and contrasts with studies in other ecosystems.

**Key words** *Kobresia myosuroides* · Alpine tundra · Freezing · Drying · Nitrogen cycle

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## Introduction

The importance of organic nitrogen uptake by plants in some ecosystems (Abuzinadah and Read 1989; Chapin et al. 1993; Jones and Darrah 1994; Kielland 1994; Raab et al. 1996) raises the issue of plant-microbe competition. Although plants and microbes may compete directly for inorganic nitrogen in some instances (e.g., Jackson et al. 1989), inorganic nitrogen pools exceed microbial growth requirements during net mineralization. In essence, plants survive on the excess inorganic N discarded by microbes. Amino acids contain both C and N, are good substrates for microbial growth (Alef and Kleiner 1988), and should rarely, therefore, exist in excess. Plants must always compete directly with soil microbes if amino acids are to be an important N-source. Plants have traditionally been considered poor direct competitors with soil microbes for nutrient uptake (Chapin et al.

1986; Jackson et al. 1989; Rosswall 1982). Schimel and Chapin (1996) found that when $^{15}N$-labeled glycine was added to arctic soils, plants took up only 1–12% of the $^{15}N$ while 41–68% was recovered in the microbial biomass.

Amino acids may be an important N-source for plants in the Colorado alpine zone, particularly the dry-meadow dominant, *Kobresia myosuroides* (Vill.) Paol. and Fiori (Raab et al. 1996). *K. myosuroides*-dominated communities of the alpine are especially subject to climatic stresses. This species grows in wind-blown sites with a relatively shallow snow depth of 26–50 cm at maximum (Walker et al. 1993). Thus, this community type is highly susceptible to freeze-thaw events in the spring and fall when the soil is exposed, skies are clear, and night-time radiational cooling is frequent. The low snowpack also leads to drier conditions in the summer than in other alpine communities (Taylor and Seastedt 1994), causing a close coupling of the soil moisture regime to stochastic rain events. Many investigators have found that freeze-thaw or drying-rewetting events can disrupt microbial biomass (Kieft et al. 1987; Skogland et al. 1988; Soulides and Allison 1961; Stark and Firestone 1995; Van Gestel et al. 1993) and increase amino acid concentrations in soils (DeLuca et al. 1992; Ivarson and Sowden 1966). Amino acids are abundant in the cytoplasm of soil bacteria, especially during osmotic stress (Measures 1975). We hypothesized that plant-microbe competition for amino acids would be diminished by these climatic stresses through an increase of soil amino acid concentrations and a decrease of microbial population size. Freeze-thaw and drying-rewetting events would provide plants with windows of increased resources and decreased competition.

To test this hypothesis, experiments were performed on plant and soil microcosms, in which realistic freezing and drying conditions were simulated. We investigated the effect of these stresses on the uptake of glycine, a common soil amino acid, by *K. myosuroides* and soil microbes. To better understand the effects of these events on soil amino acid pools and on the microbial populations that can utilize them as respiratory substrates and thus compete with plants for their uptake, we also performed freeze-thaw and dry-rewet experiments on soils in the absence of plants.

## Materials and methods

### Site descriptions and field measurements

The study sites, hereafter called "W" and "B," are located at the Niwot Ridge LTER site in the Front Range of the Colorado Rocky Mountains. Both sites are dominated by *Kobresia myosuroides* (Vill.) Paol. and Fiori, and the soils are classified as pergellic cryumbrepts. Site W has been described earlier as the "West Knoll site" and site B is similar to and is located near the "East Knoll site" (Raab et al. 1996). The soil at site W is a drier, lower organic-matter soil than the soil at site B.

Hourly temperature measurements were made at the B site during the fall of 1995 and the spring of 1996 using copper-constantan thermocouples and a LiCor L1000 (Lincoln, Neb.) datalogger. Soil moisture was measured gravimetrically on triplicate soil cores (5 cm diameter, 10 cm deep) throughout the 1996 snow-free season.

### Plant-soil microcosm experiment

Whole tussocks of *K. myosuroides* with intact soil to a depth of 15 cm were collected near the B site. The tussocks were split into smaller pieces and were placed in containers constructed from polyvinylchloride (PVC) pipe (5 cm diameter). Care was taken to preserve the natural soil structure during transplantation. The microcosms were kept in one of two environmental chambers (Conviron, model E-15, Asheville, N.C.). One chamber was programmed to mimic a spring or fall regime (temperature range: 5–10 °C, 12 h photoperiod), and the other, a summer regime (temperature range: 10–15°C, 16 h photoperiod). Six microcosms were harvested to determine natural abundance levels of $^{13}C$ and $^{15}N$. The remaining 20 microcosms were randomly assigned to one of four treatments. The summer control and dry-rewet microcosms were kept in the summer regime and the fall control and freeze-thaw microcosms were kept at the fall regime. All cores were kept near 60% moisture content, except those in the dry-rewet treatment, which were allowed to dry. Moisture content was estimated periodically by weighing the microcosms. At the end of the drying period, the microcosms were watered and labeled glycine was injected, as described below. The freeze-thaw treatment was performed by placing the cores in coiled copper tubing attached to a temperature-controlled circulating bath of 20% glycerol. The apparatus was insulated with polyethylene and placed in a 5°C refrigerated room. This method directly froze only the belowground portions of the microcosms. The microcosms were frozen at the end of a simulated day. They were allowed to thaw the following morning in the environmental chamber. Temperature in the center of the cores was measured with thermocouples and recorded with a datalogger, as described above. When the cores reached ambient temperature, labeled glycine was injected.

All microcosms were injected with 40 ml of 2000 μM $^{15}N$, $^{13}C$-[2]-glycine (98 atom% $^{15}N$, 99 atom% $^{13}C$-2) (Sigma Biochemical Co., St. Louis, Mo.) to yield a final concentration of approximately 800 μM in the soil water, or approximately 6.0 and 5.2 μg $g^{-1}$ of $^{15}N$ and $^{13}C$, respectively. This concentration is 3 times higher than the maximum seasonal concentration of total amino acids at this site (D.A. Lipson, unpublished work), so pulses of amino acids from the treatments would not greatly dilute the label. The injection was made with a 50-$cm^3$ syringe attached to a 20-cm length of stainless steel tubing (2 mm outer diameter), which allowed the glycine to be injected evenly along the soil profile, by gradual withdrawal of the tube.

After 3 days of incubation with the label, cores were harvested. Rocks and dead plant material were sieved from the soil and discarded. Live plant tissue was separated into roots, stems (the fleshy white base of the tiller) and leaves (the green extended portion of the tiller). These tissues were rinsed in deionized water to remove any residual label, and were dried to constant weight in a 55°C drying oven. Subsamples of the soil were used for the measurement of microbial biomass by the fumigation-extraction method (Brookes et al. 1985). As pointed out by Schimel and Chapin (1996), this method uses a factor ($K_n$ = 0.54) to convert chloroform-labile N to total microbial biomass N, but freshly assimilated N may be more extractable than the total N. This method may lead to an over-estimate of $^{15}N$ uptake by microbial biomass, but allows direct comparison with the previously mentioned study. This factor was used only in the calculation of percent of total label recovered in microbes, as all other comparisons involving microbial biomass were made relative to each other and so scaling would be arbitrary. The method was modified to use water rather than $K_2SO_4$ as an extractant, so that solvent from the samples could be evaporated without leaving a salty residue, which would interfere with packaging for stable isotope analysis. Previous microbial biomass N measurements on this soil, read as nitrate after conversion with

persulfate (Fisk and Schmidt 1995), showed the two extractants produced identical results (both showed a mean of 15.7 μg N $g^{-1}$). Soil was weighed and dried for gravimetric water content and for stable isotope analysis.

Dried plant tissues were weighed and ground to a fine powder using a Wiley mill for roots and a mortar and pestle for stems and leaves. Plant tissues and bulk soil were weighed on a microbalance (Sartorious, Westbury, N.Y.), packaged in tin capsules and analyzed for $^{13}C$ and $^{15}N$ by Dumas combustion (Roboprep CN Biological Sample Converter) and mass spectometry (Europa Scientific, Crewe, UK) at the Stable Isotope Facility of the University of California at Berkeley. Extracts of chloroform-fumigated and non-fumigated soils were reduced in volume using a rotoevaporator (model RE 111, Büchi, Switzerland) (condenser temperature: 3°C, water bath temperature: 75°C), and were blotted onto glass fiber disks (Whatman GF/D). The disks were dried and analyzed as described above. The pH of these soil water extracts is 5.2, and so loss of ammonium through volatization would be minimal. The chloroform-labile N that was measured using this method agreed well with measurements of the non-labeled cores using persulfate digestion, as described above. Microbial biomass C measured using this technique was comparable to measurements on similar soils using the method of Bartlett and Ross (1988). Furthermore, levels of $^{13}C$ and $^{15}N$ measured in unlabeled microbial biomass using this method were plausible ($\delta^{13}C = -22.13‰$, $\delta^{15}N = -8.39‰$).

### Soil drying and freezing experiments

In the soil drying experiment, 33 g dry weight equivalent of soil from sites B and W were placed in 120-$cm^3$ plastic cups and brought to 55–60% of field capacity with deionized water. The cups were left at 22°C during the experiment. Control cups were watered every 1–2 days to a constant moisture content. Control samples and those in the slow dry treatment were covered with several layers of cheesecloth, while those in the fast dry treatment remained uncovered. The cups were weighed periodically for moisture content. At the end of the treatment, all samples were brought to 60% of field capacity and stirred with a microspatula.

In the two soil freezing experiments, soil was placed in cups and was subjected to a freezing event using the apparatus described for the microcosm experiment. Control cups remained at 5°C. Frozen soils were allowed to thaw in the 5°C cold room. Freeze-thaw experiment I used soil from site B and freeze-thaw experiment II used soil from both sites.

After the respective treatments in the soil drying and freezing experiments, soils were measured for glycine and glutamate substrate-induced respiration (SIR). For the SIR measurements, 10 g dry mass equivalent of each soil was placed in a biometer flask with 1 $cm^3$ NaOH (1 M) in the side arm to trap $CO_2$ (Schmidt 1992). Amounts of substrate previously determined to induce maximal respiration in these soils (2 mg glutamate-C $g^{-1}$ or 0.4 mg glycine-C $g^{-1}$ for site B, half these quantities for site W) were added to the flasks, with enough uniformly labeled $^{14}C$-amino acid (Sigma Biochemical Co.) to yield 150,000 dpm per flask. The base trap was removed and replaced at various time points and radioactivity was measured by liquid scintillation after mixing with Scintiverse II scintillation cocktail. Freeze-thaw experiments I and II were carried out at 5 and 3°C, respectively.

Soils were extracted with deionized water (5 $cm^3$ water per g soil), shaken for 30 min, and filtered on Whatman #3 filter paper. Extracts were frozen until analysis. Extracts were concentrated in a rotoevaporator, as described above, and total amino acids plus $NH_4^+$ were analyzed using ninhydrin (Rosen 1957). Ammonium was analyzed by the phenolate-hypochlorite method (United States Environmental Protection Agency 1983). Absorbance for both assays was read on a plate reader (Molecular Devices Corporation, Milpitas, Calif.) at 562 and 650 nm, respectively. Ammonium was subtracted from the ninhydrin value to yield total amino acid concentration.

### Statistical analysis and calculations

The first freeze-thaw experiment was analyzed with a one way analysis of variance (ANOVA). The dry-rewet experiment and the second freeze-thaw experiment were analyzed with two-way ANOVA, using drying and soil type as factors. The plant-soil microcosm experiment was also analyzed as a two-way ANOVA with growth chamber conditions (summer or fall) and climatic stress (stressed or not stressed) as categorical variables. Each plant tissue was analyzed separately, except for percent recovery of $^{15}N$ in the whole plant. The concentrations of isotopes in labeled samples were compared to those in unlabeled samples using one-way ANOVA. (All statistics were performed using SAS (SAS Institute 1990)). Probability values greater than 0.05 were considered non-significant, except in one post hoc ANOVA, in which the Bonferroni inequality was applied. All values in figures are means and standard errors. Delta notation for $^{15}N$ content was calculated relative to the atmospheric standard of 0.3663%. The percentage of total added isotope recovered in a pool was calculated by first subtracting the mean isotopic concentration of the unlabeled microcosms.

## Results

Freeze-thaw events were observed at the study site during the late spring and early fall. Some of the freeze events affected soil to a depth of at least 10 cm (Fig. 1). Temperature variation was more extreme near the soil surface. Soil moisture was high immediately after snowmelt, dropped sharply in mid-summer, and partially recovered with rain events in the late summer and early fall (Fig. 2). The maximum rate of moisture loss was 1.1% per day. The conditions of the drying and freezing experiments reasonably duplicated these observed phenomena (Fig. 3). The simulated rates of drying (0.9–3.25% per day) bracketed the observed field value. Because the microcosms were placed in series along the cooling apparatus, a range of freezing treatments was produced, with those closest to the bath outlet being more severe (Fig. 3A). The curves of soil drying in the laboratory shown in Figure 3B followed linear or negative exponential decreases, while the drying curve of the moisture content in the field was concave downward. This is likely the effect of increased temperatures as the summer progressed.

### Plant-soil microcosm experiment

In the plant-soil microcosm experiment, $^{13}C$ from the labeled glycine was recovered in plant roots, microbial biomass, and in bulk soil. Plant roots in all treatments were significantly enriched in $^{13}C$ ($P < 0.001$), but aboveground plant tissues and the water-extractable soil pool were not significantly enriched relative to unlabeled controls. Variation was high in the $^{13}C$-content of plant roots, and differences between treatments were not discernible. In contrast, the levels of $^{15}N$ were significantly enriched in both aboveground and belowground plant tissues ($P = 0.003$), and significant treatment effects were observed. The uptake of $^{15}N$ from labeled glycine into all plant tissues, expressed per unit tissue nitrogen

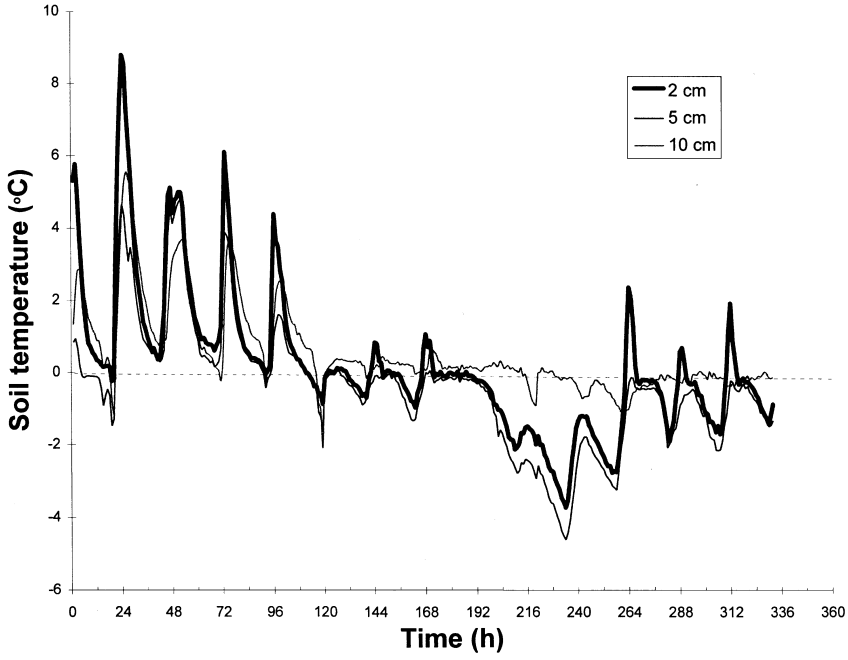


**Fig. 1** Hourly soil temperature at three soil depths at the B site over the period of 26 September to 10 October 1995. Values for 2 and 5 cm are means of two thermocouple probes. Values for 10 cm are from a single probe

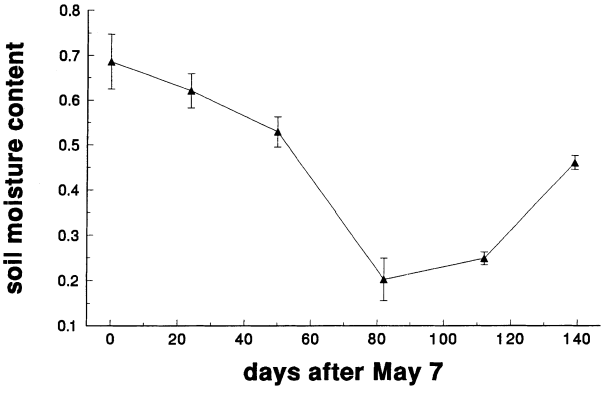


**Fig. 2** Soil moisture content of site B over the snow-free season of 1996. Values are means and standard errors of three replicates

(delta notation) to control for variation in plant size, was higher in the summer treatments than in the fall treatments (leaves and stems: $P < 0.001$, roots: $P = 0.013$), and lower in the stressed treatments than in the controls (leaves and stems: $P < 0.001$, roots: $P = 0.013$) (Fig. 4). The effect of freezing and thawing was significantly less than the effect of drying and rewetting (leaves and stems: $P < 0.001$, roots: $P = 0.020$). Analyzed separately, values were not significantly lower in the freeze-thaw treatment than in controls. However, the severity of the freeze appeared to influence plant root uptake of $^{13}C$ from labeled glycine (Fig. 5). The $^{13}C$ and $^{15}N$ contents of plants were comparable: on average, 82 ± 19% of the $^{15}N$ predicted from the $^{13}C$ content was found in the plant. Average percent recovery in plants across all treatments was 2.1 ± 0.5% for $^{15}N$, and 2.3 ± 0.2% for $^{13}C$. Recovery of $^{15}N$ in plant and microbial biomass is shown for individual treatments in Table 1. Expressed in this way,

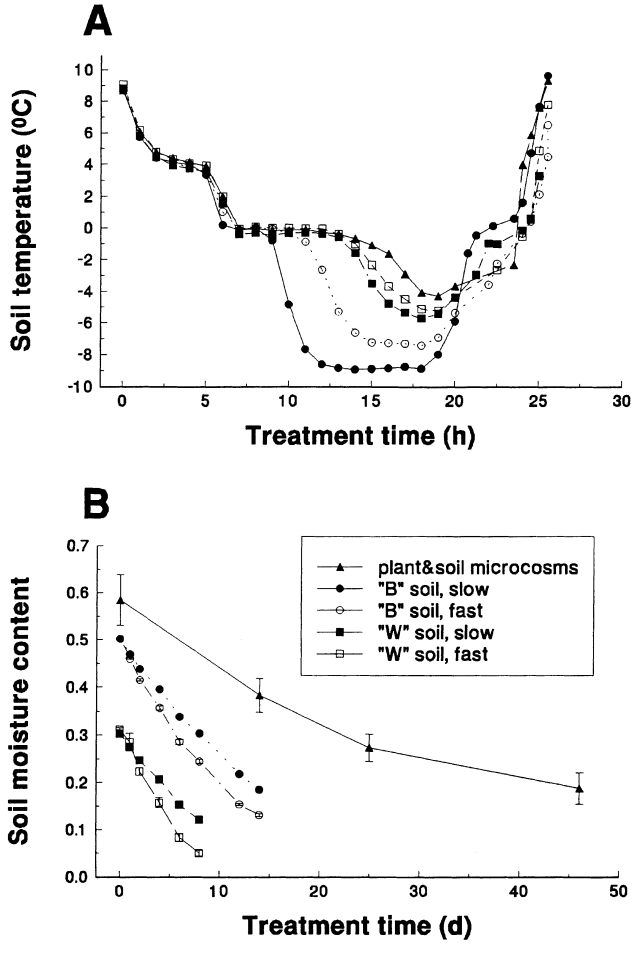


**Fig. 3** Climatic stresses simulated in laboratory experiments: **A** soil temperatures of 5 replicate plant and soil microcosms during a freeze-thaw experiment. The replicates were placed in a series along the cooling aparatus, thus producing a range of cooling rates and severities. **B** Soil moisture contents in dry-rewet experiments

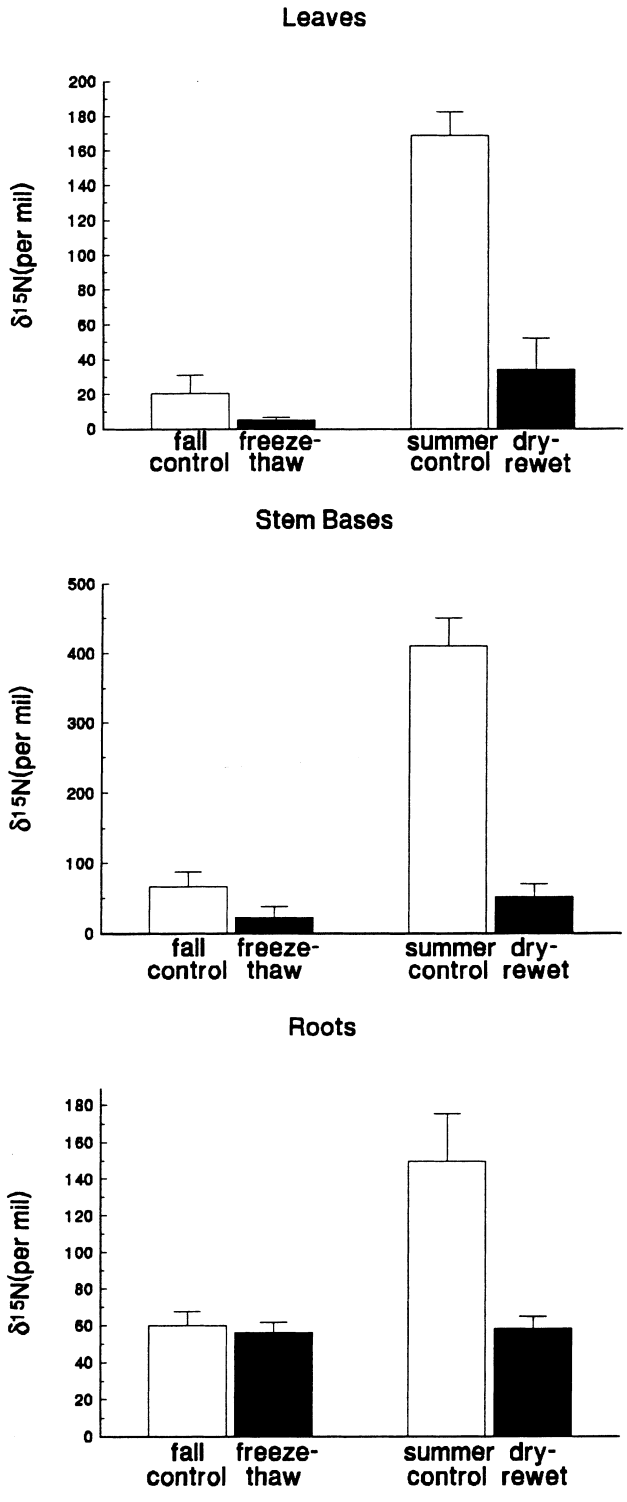


**Fig. 4** Uptake of $^{15}N$ from labeled glycine into plant leaves, stem bases and roots for four treatments. Values are expressed as $\delta^{15}N$ (per mil)

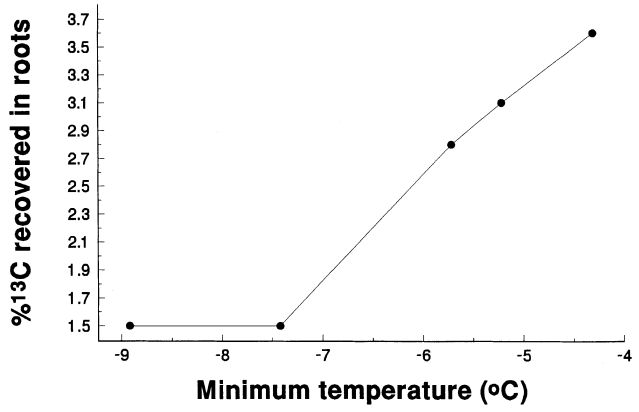


**Fig. 5** Recovery of $^{13}C$ from labeled glycine in plant roots in the freeze-thaw treatment as a function of minimum temperature reached in the treatment

**Table 1** Percent of $^{15}N$ from labeled glycine recovered in plants and soil microbial biomass. Microbial values are calculated from chloroform-labile $^{15}N$, using the correction factor, $K_n = 0.54$. Values are means and standard errors

| | Plant | Microbial biomass |
|---|---|---|
| Fall control | 0.8 ± 0.1 | 9.1 ± 0.7 |
| Freeze-thaw | 1.0 ± 0.2 | 10.5 ± 2.8 |
| Summer control | 3.5 ± 1.1 | 5.0 ± 1.0 |
| Dry-rewet | 0.9 ± 0.2 | 6.5 ± 1.1 |

treatment effects were not significant in the two-way ANOVA for plants, although the summer control group was higher than the other groups at a probability level of $P = 0.02$ in a post hoc ANOVA. This is marginally significant when the Bonferroni inequality is applied ($\alpha = 0.017$). Significantly less $^{15}N$ was recovered in microbial biomass from the warmer treatments (summer control and dry-rewet) than in the colder ones (fall control and freeze-thaw) ($P = 0.023$).

Microbial biomass (chloroform-labile) C, $^{13}C$, N and $^{15}N$ were not significantly affected by the simulated stresses (Fig. 6). However, uptake into microbial biomass of $^{15}N$ was significantly higher in the colder treatments compared to the warmer treatments ($P = 0.014$), and the similar trend for $^{13}C$ was marginally significant ($P = 0.06$). Water-extracts from warm treatments contained about twice as much C as cold treatments ($P = 0.005$), but a similar trend for N was not significant ($P = 0.123$). Recovery in bulk soil of $^{15}N$ and $^{13}C$ was 43.4 ± 2.8% and 89.7 ± 15.4% of total, respectively.

## Soil freezing and drying experiments

In the drying-rewetting experiment on two soils (without plants), small but significant decreases were detected in glycine substrate-induced respiration (SIR) in the drying treatments ($P = 0.001$) (Table 2). The glycine SIR in slow and fast drying treatments were also significantly different from each other in both soils ($P = 0.01$). No differences were detected in glutamate SIR.

**Table 2** Substrate-induced respiration (μg $CO_2$-C $g^{-1}$ $h^{-1}$) of glycine and glutamate measured in a drying-rewetting experiment using two soils at two experimental drying rates. Moisture loss was approximately linear during the experiment. Drying rates are listed for each treatment (g $H_2O$ 100 g $soil^{-1}$ $day^{-1}$). Values are means and standard errors for 3 replicates

| | Dry-rewet, W soil | | | Dry-rewet, B soil | | |
|---|---|---|---|---|---|---|
| | Control | Slow | Fast | Control | Slow | Fast |
| Drying rate | – | 2.25 | 3.25 | – | 2.0 | 2.5 |
| Glycine | 0.452 ± 0.003 | 0.447 ± 0.003 | 0.410 ± 0.012 | 1.81 ± 0.02 | 1.72 ± 0.01 | 1.63 ± 0.02 |
| Glutamate | 1.56 ± 0.06 | 1.58 ± 0.02 | 1.52 ± 0.04 | 7.64 ± 0.33 | 7.35 ± 0.40 | 6.95 ± 0.60 |

**Table 3** Substrate-induced respiration (μg $CO_2$-C $g^{-1}$ $h^{-1}$) of glycine and glutamate measured in two freeze-thaw experiments. Values are means and standard errors. For experiment I, $n = 3$ for control and $n = 4$ for freeze. For experiment II, $n = 2$ for all treatments

| | Freeze-thaw I | | Freeze-thaw II | | | |
|---|---|---|---|---|---|---|
| | (B soil) | | (W soil) | | (B soil) | |
| | Control | Freeze | Control | Freeze | Control | Freeze |
| Gly | 0.855 ± 0.008 | 0.900 ± 0.018 | 0.200 ± 0.015 | 0.179 ± 0.001 | 0.279 ± 0.003 | 0.264 ± 0.012 |
| Glu | 3.41 ± 0.18 | 3.14 ± 0.15 | 0.755 ± 0.032 | 0.831 ± 0.020 | 1.07 ± 0.01 | 1.10 ± 0.01 |

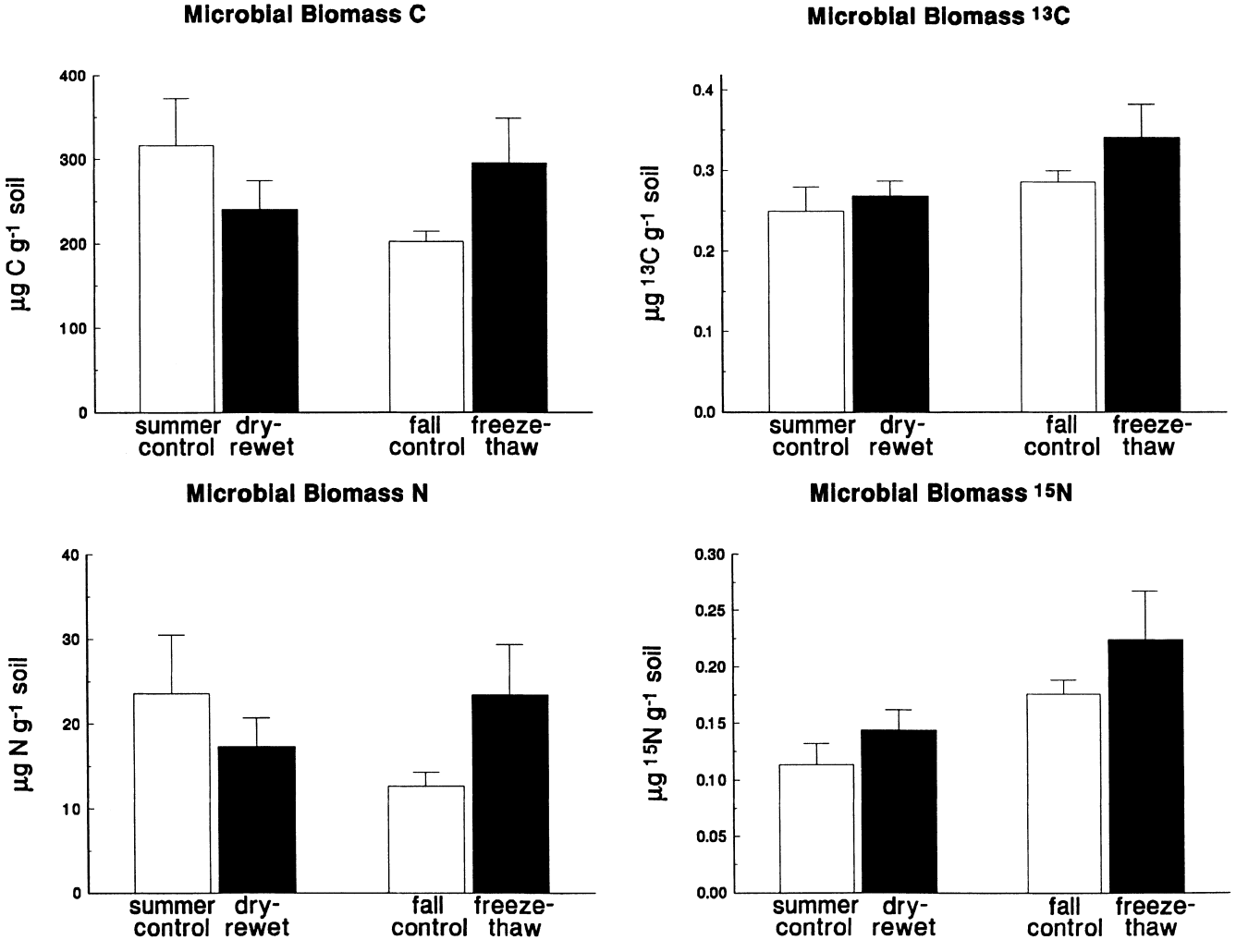


**Fig. 6** Microbial biomass (chloroform-labile) C, $^{13}C$, N, and $^{15}N$ in soils from the microcosm experiment. Natural abundance levels were subtracted from the stable isotope values. These values were not adjusted by $K_n$

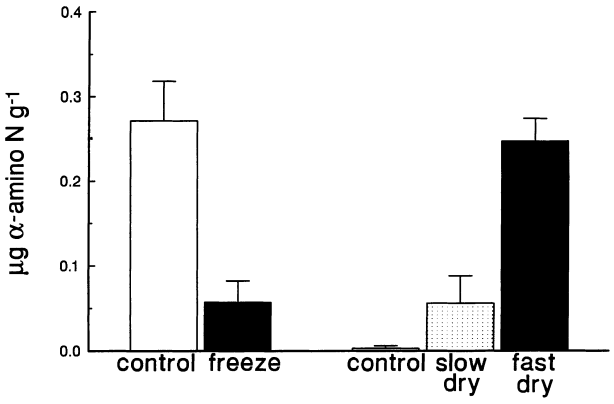


**Fig. 7** Water-extractable amino acids in soils from the freezing and drying experiments

In the two freeze-thaw experiments, microbial respiration of glycine and glutamate were not significantly affected by the treatment (Table 3).

Water-extractable amino acids were significantly affected both by freezing, in which the levels decreased ($P = 0.023$), and by drying, in which the levels increased ($P = 0.002$), depending on the rate and extent of drying ($P = 0.002$) (Fig. 7).

## Discussion

As hypothesized, alpine dry meadow soils are regularly subject to freeze-thaw and dry-rewet events. However, contrary to our original hypothesis, these events did not provide competitive advantages for plants over microbes with respect to glycine uptake. Of those amino acids that are prevalent in alpine soils, glycine is the one most readily absorbed by plants (Raab et al. 1996). We conclude that these climatic stresses are unlikely to allow plants increased amino acid uptake. Microbial biomass and glycine uptake were unaffected by these disturbances, and the potential rate at which the soil microbial community respired amino acids was unaffected or was only slightly depressed (10% or less). Apparently, plant roots are more susceptible to these environmental extremes, as evidenced by reduced glycine uptake after climatic stress, and the striking dependence of glycine uptake by plants in the freeze-thaw treatment on the severity of the freeze (Fig. 5). The plateau in $^{13}C$ recovery in plants that had been cooled to below −7°C could indicate that only passive absorption of glycine onto root surfaces or into the Donnan free space occurred in these more extreme treatments. *K. myosuroides*, being the dominant species of alpine dry meadows, can clearly tolerate climatic stress, but this species' success does not appear to be linked to the opportunistic exploitation of these disturbances. This stress-tolerant strategy (*sensu* Grime 1977) is consistent with the generally conservative patterns of growth and nutrient acquisition in alpine plants (Körner and Menendez-Riedl 1989; Lipson et al. 1996).

The frost- and drought-resistance of the microbial communities in these alpine soils is novel, and contrasts with the conclusions of several reports that a single freeze-thaw or dry-rewet event can kill up to half of the microbial population (Kieft et al. 1987, Skogland et al. 1988, Soulides and Allison 1961). Many others have concluded that flushes of respiration or mineralization after such events is largely due to biological disruption (Clein and Schimel 1994; DeLuca et al. 1992; Schimel and Clein 1996; Van Gestel et al. 1993; West et al. 1989). The resistence of the community in the present study could be due to unique properties of the alpine microbial community, such as cell-wall structure and cytoplasmic solutes. The difference between this and previous studies might also have resulted because the above-mentioned authors used more extreme freezing events [e.g., −11°C in Soulides and Allison (1961)], while freeze events in the present study simulated conditions of alpine late spring or early fall, where air temperatures rarely drop below −10°C. Microbial cytoplasm may not actually freeze under these conditions. Care was also taken not to exceed a realistic rate of freezing. Further comparison with soils from other ecosystems under identical experimental conditions is warranted.

Freezing of the soil in this study actually decreased water-extractable amino acids, contrary to the results of Ivarson and Sowden (1966). This unexpected result could be caused by a decrease in proteolytic activity in frozen soils. Diffusion of protein could be restricted by the narrowing of the liquid water film in frozen soils (Anderson 1970). Alternatively, the decrease could have resulted from stimulation of microbial amino acid uptake by physical nutrient release from soil during freezing and thawing (Edwards and Cresser 1992). Drying and rewetting increased soil amino acid concentrations, but given the minimal effects on microbial amino acid respiration and the large negative effects of the treatment on plant uptake, this flush is unlikely to benefit plants. In contrast to *K. myosuroides*, the microbial populations that respire amino acids may capitalize on these perturbations. Amino acid pools are small compared to their maximal rate of respiration, and so, presumably, turnover is very rapid. In the plant-soil microcosms, C and N concentrations in water extracts made 3 days after the disturbances were not significantly different from controls. Any pulse of amino acids due to a single stressful event would be short lived.

The partitioning of amino acid-N between plants and soil microbes was much more equitable in the present study, compared to a similar study performed in the arctic tundra (Schimel and Chapin 1996). These investigators found that after a 5 day incubation, microbial biomass contained 41.3–68.1% of the $^{15}N$ from a labeled pulse of glycine, while plants contained 1.0–12.1%. In contrast, the values in this study (after a 3-day incubation) were 5.0 and 3.5% for microbes and plants grown in non-perturbed, summer-like conditions

(Table 1). The slightly shorter incubation time in the present study should conservatively affect this comparison, as microbial biomass N turns over more rapidly than plant N unpublished work (M. Fisk, T. Seastedt, S. Schmidt) and plants should compete better for N over longer time periods (Jackson et al. 1989). The inconsistencies between these two studies highlight the large difference in N immobilization potential between the two ecosystems. Fisk and Schmidt (1996) found that N fertilization of alpine tundra did not affect the soil microbial N pool during the growing season. However, arctic tundra strongly immobilizes N during midsummer and has lower N mineralization rates than does the alpine ecosystem (Fisk and Schmidt 1995; Nadelhoffer et al. 1992). Furthermore, Schimel and Chapin (1996) detected no $^{13}C$ from the dual labeled glycine in plant tissues. One possible reason for the difference in $^{13}C$ recovery between these two studies is that Schimel and Chapin (1996) used $^{13}C$-[1]-glycine, whereas $^{13}C$-[2]-glycine was used in the present study. The carboxyl carbon of the glycine molecule may be more readily respired and lost from plant tissues than the methylene carbon. The near-equal enrichments of $^{13}C$ and $^{15}N$ in plant tissues in the present study verify that glycine was taken up intact by *K. myosuroides*, and thus, direct competition with soil microbes for amino acids is possible. Refixation of respired $^{13}CO_2$ by photosynthesis was a possible source of contamination for plants. However, the recovery of label in roots but not in leaves, and the comparable $^{13}C$ and $^{15}N$ enrichments argue that the majority of $^{13}C$ was absorbed directly by the roots.

The majority of $^{13}C$ and $^{15}N$ from the added glycine was found in the bulk soil, whereas neither isotope was recovered in the water-extractable fraction and only a fraction of the $^{13}C$ and $^{15}N$ were recovered in the microbial biomass. These results imply that glycine was rapidly converted to complex insoluble organic matter. This may indicate a rapid turnover of microbial cell walls. The $^{15}N$ found in the non-soluble, non-biomass fraction of the soil could be either in the form of strongly-bound ammonium or as humified organic matter. Thus, physical immobilization of N could be more significant than immobilization into microbial biomass in this soil.

In conclusion, alpine soil microbes are resistant to freezing and drying stresses, but are a relatively weak sink for amino acid-N. *K. myosuroides* does not utilize climatic disturbances as opportunities for amino acid acquisition, but competes well with soil microbes for this resource under non-stressed conditions, particularly when soils are warm.

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