Final Report

Controls Over Nutrient Flow Through Plants and Microbes in Arctic Tundra

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Overall Introduction

Ecosystem productivity in the Arctic is strongly controlled by N availability to plants (Schimel et al. 1994). Thus, disturbances to the Arctic system are likely to have their greatest impacts by altering the supply of nutrients to plants. Thus, to understand the dynamics of Arctic tundra, a complete understanding of the controls on N cycling in tundra soils is necessary. This project focused on understanding nutrient dynamics in arctic tussock tundra, specifically evaluating the role of microbial uptake and competition for nutrients as a control on plant N-uptake. The project consisted of several major components:

- I. Short- and long-term partitioning of NH_4^+ in tussock tundra (1990-1991)
- II. Measurement of NH_4^+ uptake rates by *Eriophorum vaginatum* and by soil microbes (1991-1992).
- III. Determination of microbial NH_4^+ and NO_3^- uptake kinetics (1992).
- IV. Determination of the partitioning of NH_4^+ and amino acids between *E. vaginatum* and soil microbes (1992-1993).

Section I. ^{15}N Labeling of *Eriophorum vaginatum* tussocks

In this experiment, individual *E. vaginatum* tussocks were labeled with $^{15}\text{NH}_4^+$ and sections were harvested after 1 day, 1 month, and 1 year. Injections were carried out in June and July 1990. To ensure a uniform injection, we used a template that provided 36 holes and solution was injected into tussocks at several depths in each of them. Tussocks were then harvested by cutting a wedge out of them and separating the tussock section into the following fractions:

E. vaginatum shoots
E. vaginatum rhizomes
E. vaginatum roots
Live material from other plants
Dead shoots
Soil

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These materials were then dried at 60°C and then were analyzed for N and ^{15}N content using a Carlo-Erba C, N analyzer linked to a Mass Spectrometer.

RESULTS

By the end of one day there was no detectable N in soil solution, indicating that all the added $^{15}\text{NH}_4^+$ had been taken up by plants or microbes. The distribution of ^{15}N in the different compartments is shown in Table 1.2. The recovery of ^{15}N in live plant tissues was initially very low, most of the ^{15}N was found in the soil. The ^{15}N in dead plant material probably resulted from the labeling procedure; there is a thick mass of dead tillers and some label was inadvertently injected into this layer. These data indicate that in tussock tundra that soil microbes are N-limited and compete strongly for NH_4^+ with plants. Microbial uptake limits availability of NH_4^+ to plants. The proportion of recovered ^{15}N in plant tissue increased with time, indicating that during the growing season there was some N mineralization and plant uptake of the released N.

Table 1.1 Distribution of ^{15}N (% of recovered ^{15}N in each pool)

	Harvest		
	1 Day	1 Month	1 Year
<i>June Injection</i>			
Live plants	3.8	5.2	9.8
Dead plants	23.8	25.0	22.3
Soil	72.4	69.8	67.9
<i>July Injection</i>			
Live plants	5.0	6.2	8.1
Dead plants	6.8	22.7	11.5
Soil	88.1	71.1	72.9

The increase in the plant pool over time allows estimation of the turnover time of the soil N pool. If we assume that the partitioning factor measured over 1 day represents the proportion of the N mineralized that is taken up by plants rather than reimmobilized then the daily turnover of N equals:

$$\text{Turnover} = ((\% \text{ in plants at 1 month} - \% \text{ in plants at 1 day}) / 30 \text{ days}) / (\% \text{ in plants at 1 day} / 100)$$

This calculation gives an turnover of the labile N pool of approximately 1% per day. Since the growing season is about 100 days this suggests that the labile soil N pool turns over about once a growing season.

The distribution of ^{15}N within the plant pool over time is shown in Table 1.2. In each case, the proportion of ^{15}N in *E. vaginatum* roots starts high and decreases, while the ^{15}N in shoots increases over time, indicating uptake and translocation of N over time. The ^{15}N in rhizomes is somewhat variable but is higher in the June injection treatment than in the July treatment. In both cases, other plants (shrubs and mosses) initially take up a very small proportion of the plant ^{15}N , but this increases significantly over time. This suggests that while *E. vaginatum* is effective at competing for pulses of available N, it is less effective than other plants at competing for the very

low concentrations of available NH_4^+ that would be present normally. While we only added "tracer" levels of ^{15}N (approximately $2 \mu\text{g N/g soil}$) this was still a relatively concentrated pulse of available N. These results are consistent with earlier work that shows greatly increased productivity of *E. vaginatum* in water tracks where nutrient availability is elevated. Substantial amounts of ^{15}N are found in *E. vaginatum* roots after the winter. *E. vaginatum* roots are annual, and it is still unclear whether the ^{15}N in roots a year after the labeling was translocated into the rhizomes at senescence and back into new roots the year after or whether it was released to the soil microbial biomass as roots died and taken up afresh the next spring.

Table 1.2. Proportion of ^{15}N in plants that is in each plant component.

	Harvest		
	1 Day	1 Month	1 Year
June Injection			
<i>E. vag.</i> shoots	12.6	25.8	25.8
<i>E. vag.</i> rhizomes	30.3	19.8	31.9
<i>E. vag.</i> roots	37.5	29.5	13.5
Other plants	19.6	24.9	28.8
July Injection			
<i>E. vag.</i> shoots	5.4	7.1	10.1
<i>E. vag.</i> rhizomes	29.2	12.8	13.7
<i>E. vag.</i> roots	58.4	28.8	15.2
Other plants	7.0	51.4	61.0

This study shows that N availability to plants is strongly limited by microbial immobilization. This intense immobilization capacity will limit the ability of any disturbance to affect plant productivity. Until microbial N uptake capacity is saturated, little N extra N will be available for plant uptake. This may be particularly important in the context of climate change, where it has been hypothesized that increasing temperature will increase decomposition and mineralization rates and that the released N will be available for plant uptake. Our results suggest that much of this N will be immobilized, producing a lag between increased decomposition rates (gross mineralization) and increased plant N available N (net mineralization).

Effects of disturbance on N-partitioning

We determined the effects of road dust (a major development-related disturbance in the Arctic) on ^{15}N partitioning by labeling tussocks as described above in the "dust plots" established by Skip and Marilyn Walker. Tussocks were selected 20 m (heavily dust-affected) and 400 m (control) from the Haul Road. These tussocks were incubated for one month and then harvested as described above. The data are still in analysis.

Section II. Measurement of Short-term plant and microbial NH_4^+ uptake

While the technique used for the long term tussock labeling was extremely effective for the purpose of determining long-term partitioning of N through the system, it could not be used to measure short-term uptake *rates*, and to determine the total partitioning of ^{15}N as we could only partition the % of *recovered* N. Since we subsectioned the cores we couldn't determine % of *added* N. To address this issue, we used a modified technique to measure short-term uptake rates in intact cores taken from tussocks.

We took 5 cm diameter cores from the center of *E. vaginatum* tussocks, removed them from the ground and cut off the bottom to provide a 15 cm deep core and placed in a plastic bag to ensure that the isotope was fully contained. The cores were then injected with $^{15}\text{NH}_4^+$ solution, and replaced in the hole that it was taken from. It was incubated *in situ* for 24 hours, removed and harvested into the same components as in the whole tussock labeling experiment. The data from these experiments are still in analysis.

Section III. Measurement of Microbial N uptake kinetics

There are several possible explanations for the low nutrient supply to plants. The first is low decomposition and mineralization rates, which could result from a combination of poor substrate quality, low temperature, and wet soils. The second is that microbial N-uptake could outcompete plants for mineralized N. Such competition has been shown to be important in other ecosystem types. In tussock tundra several studies have shown that the majority of inorganic N added to the soil is immobilized into soil organic matter. This doesn't however prove that microbes are actually N-limited. This requires examining microbial uptake kinetics, which is what we did in this portion of the project.

We collected four soil types from three plant communities: tussock tundra (tussock and between tussock soils), shrub tundra, and wet meadow tundra. Soils were collected the day prior to experimentation to minimize sampling and storage effects. The experimental protocol was similar to that used by Schimel and Firestone (1989). Soils were hand mixed and samples (5 g wet material) were slurried in solutions containing varying concentrations of $(^{15}\text{NH}_4)_2\text{SO}_4$ or KNO_3 . The concentrations of N solutions were 20, 200, and 2,000 μM $(^{15}\text{NH}_4)_2\text{SO}_4$ and 200 μM K^{15}NO_3 . Slurries were incubated on an orbital for periods of 5 minutes, 3-4 hours and 6-7 hours. The 5 minute treatment was to provide a control for isotope that would be rapidly adsorbed into the material (ion exchange etc.) that was not washed out in sample processing. The samples were then dried, ground, and analyzed for ^{15}N content. The rate of N incorporation was calculated from the rate of increase in ^{15}N content over time. Kinetic uptake constants were calculated from the concentration data by fitting the data to the Michaelis Menton equation. This provided estimates of the maximum incorporation rate (V_{max}) and the NH_4^+ concentration at which the incorporation rate was half its maximum value (half-saturation constant or K_m).

Kinetic parameters for the different soils are shown in Figs. 3.1 and 3.2. Fig. 3.1 shows the V_{max} plotted against %N in the soil, while Fig. 3.2 shows K_m . There was a strong negative relationship between V_{max} and N in the soil, indicating that the soils with low N contents (tussock and intertussock) had a large N uptake capacity, while the shrub soil took up N slower, and the wet meadow soils took up NH_4^+ very slowly. None of the soils took up NO_3^- effectively. The patterns of K_m are slightly different, and extremely variable. The lowest K_m was in the wet meadow soil, while the highest K_m was in the shrub soil, which is generally the most fertile. Tussock and intertussock soils had intermediate K_m values. These data fit the observed patterns of soil fertility and support the idea that in tussock tundra soils microbial uptake limits plant N, having moderate K_m and very high uptake rates. In shrub tundra soils microbial competition for nutrients would be very slight- there is plenty of organic N available, the organisms have high K_m and low V_{max} .

Microbial NH_4^+ Uptake Kinetics in Tundra Soils

V_{max}

Microbial NH_4^+ Uptake Kinetics in Tundra Soils

Section IV. Plant Microbial Competition for amino acids vs. NH_4^+

It has long been thought that plants can take up only inorganic nitrogen, and that microbes must mineralize organic N before it becomes available to plants. The only exception to this has been if mycorrhizae are viewed as part of the root system, as they can take up organic N and supply it directly to their host plant. A recent study by Chapin et al. (1993), however, showed that tundra plants can directly take up amino acids from culture solution.

Chapin et al. (1993) finding was potentially extremely important in our understanding of tundra nutrient cycles. N is generally severely limiting plant growth in moist tundra, the soils generally show net N immobilization during the growing season (Giblin et al. 1991), and estimates of annual net mineralization are often lower than estimates of plant uptake. While NH_4^+ concentrations in tundra soils are often low, amino acid concentrations can be very high, water extracts often have higher amino acid than NH_4^+ concentrations (Kielland 1990). From these data it might be concluded that tundra plants actively take up amino acids in the field and that these could comprise an important component of their N-supply. However, it might be expected that heterotrophic soil microbes, which have a readily available supply of C, would outcompete plants for organic N sources. It has remained unclear to what extent tundra plants use organic vs. inorganic N sources in the field. Determining this is important to understanding tundra N cycles. In this paper we addressed this issue by measuring plant/soil partitioning of $^{13}\text{C}/^{15}\text{N}$ labeled glycine and aspartate relative to that of $^{15}\text{NH}_4^+$. We found that plants do in fact compete effectively for amino acids.

METHODS

The methods were similar to those used in short term measurements of NH_4^+ uptake in *E. vaginatum* tussock cores (described in Section 3)

RESULTS AND DISCUSSION

^{13}C RESULTS

We were unable to detect any enrichment of ^{13}C in any plant tissues. The NH_4^+ labeled plants were used as the control for background ^{13}C enrichments. The ^{13}C enrichment of these samples varied by up to ± 1.5 . Given the ^{15}N enrichment of the plant tissues, and assuming that each ^{13}C should be associated with each ^{15}N atom, it was possible to calculate the expected ^{13}C enrichment of each plant tissue. For roots the average expected enrichment was approximately 10 ppt, while shoots and rhizomes should have shown an enrichment of 3 ppt. These are well outside of the background variation and indicate that we should have detected the ^{13}C signal if the ^{13}C was in the plants. These data leave two possible explanations: either the amino acids were deaminated by soil microbes and the plants took up the ^{15}N as $^{15}\text{NH}_4^+$, or the plants took up the amino acids and respired the C.

RELATIVE UPTAKE OF AMINO ACIDS AND NH_4^+

Table 4.1 Amino acid uptake by *Eriophorum vaginatum*.

Sum of ¹⁵ N in shoots, rhizomes, and tillers (mg ¹⁵ N)						
	1 Day		5 Day		1 Month	
	Ave.	SE	Ave.	SE	Ave.	SE
Asp	21.22	2.57	Not done		Not done	
Gly	31.80	9.97	62.67	18.94	66.60	18.97
NH	12.88	3.83	19.48	5.48	22.52	9.57

Individual T-tests at 1 day comparing Aspartate and Glycine to NH_4^+ using two-tailed tests with pooled variance show significant difference at $p=0.108$ and 0.115 respectively.

We compared the total amount of ^{15}N taken up by *E. vaginatum* from the different N-form treatments. After 1 day, *E. vaginatum* had taken up roughly 2.5 times as much ^{15}N from glycine as from NH_4^+ and by 1 month this had increased to a factor of 3. This increase was significant at $p<0.002$. While the amount of $^{15}\text{NH}_4^+$ in *E. vaginatum* increases slightly from 1 day to 5 days, glycine doubled in plant tissues. This suggests that microbes take up NH_4^+ more rapidly than they take up amino acids, and therefore glycine remains in solution for several days, allowing plants to continue taking it up over time. Once the N is in plant material, it is not lost over one year as shown by the tussock labeling experiment. Approximately 50% more aspartate was taken up than NH_4^+ , but about 50% less than glycine over one day. Because we only had enough aspartate for a single labeling we did not do the longer time points.

Table 4.2 Recovered ^{15}N in soil

Total ^{15}N in unextracted soil (mg)			
	1 Day	5 Day	1 Month
NH_4^+			
Av=	2022.1	1678.8	2460.4
SE=	362.1	169.0	270.7
Glycine			
Av=	1603.8	1118.6	1471.2
SE=	214.6	159.1	183.3

There was significantly more ^{15}N from recovered in the soil from NH_4^+ than from glycine ($p=0.002$). This supports the conclusions that soil microorganisms immobilize significantly more NH_4^+ than amino acids.

Chapin et al. determined that *E. vaginatum* has much greater V_{max} for uptake of NH_4^+ than for either glycine or aspartate. The K_m values for NH_4^+ and aspartate are similar (242 and 293 mM respectively), while the value for glycine is much lower (12 mM). Based on these estimates, they calculated that *E. vaginatum* should take up approximately 60% of its N as amino acids. In our experiment, with quite high concentrations of all three N sources, NH_4^+ uptake should be favored

due to its high V_{max} . We found, however, that short-term uptake of glycine and aspartate were approximately 2.5 and 1.5 times as great as NH_4^+ uptake. Over longer periods *E. vaginatum* acquired an even greater amount of the glycine-N. These results suggest that at lower concentrations, glycine uptake should have been even higher relative to NH_4^+ . The higher specificity for amino acids in the field than predicted by Chapin et al. (1993) suggests that amino acids are more accessible to plant roots than is NH_4^+ . Amino acids should be more mobile than NH_4^+ and this could favor uptake by plant roots over microbes. Microbes are relatively uniformly distributed through the soil and so should compete more effectively than plants for an immobile substrate. Apparently soil microbes compete more effectively for NH_4^+ than for amino acids, and this may explain why plants adapted to the highly N-limited environment of tussock tundra would adapt to be able to use amino acids as an N source. This work supports the conclusions that amino acids comprise a substantial component of the tundra N-cycle.

Section V. References

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- Schimel, J.P. and M.K. Firestone. 1989. Inorganic nitrogen incorporation by coniferous forest floor material. *Soil Biol. Biochem.* 21: 41-46.

Products of the Research

Papers published or in press

Schimel, J.P., K. Kielland, and F.S. Chapin, III. 1994. Nutrient availability and uptake by tundra plants. In *Landscape Function: Implications for ecosystem response to disturbance; A case study in arctic tundra*. J.F. Reynolds and J.D. Tenhunen (Eds.). Springer-Verlag. In Press.

Papers in preparation

Schimel, J.P. and F.S. Chapin, III. Plant uptake of amino acids in Arctic tundra ecosystems.

Schimel, J.P. and F.S. Chapin, III. Partitioning of $^{15}\text{NH}_4^+$ in *Eriophorum vaginatum* dominated tussocks in the Arctic tundra.

Schimel, J.P. Kinetics of microbial uptake of NH_4^+ and NO_3^- in Arctic tundra soils.

Schimel, J. P. Short-term uptake and partitioning of $^{15}\text{NH}_4^+$ in Arctic tussock tundra soils.

Clein, J.S. and J.P. Schimel. Microbial activity in frozen soils.

Clein, J.S. and J.P. Schimel. Effect of freeze/thaw cycles on microbial processes in Arctic tundra soils.

Invited Presentations Resulting From this Work

Interactions between soil microbes and plant communities in high latitude ecosystems. ESA Symposium, Annual meeting. 1994.

Ecosystem consequences of microbial diversity and community structure. SCOPE workshop on the role of biodiversity in tundra ecosystems. Kongsvold, Norway. 1993.

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