

**CHANGES IN MICROBIAL RESPIRATION  
AND NITROGEN CYCLING FOLLOWING  
SOIL FREEZING**

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## SUMMARY

Microbial respiration and nitrogen cycling activities have been shown to increase after soil freezing events. These events have also been correlated with canopy dieback in maple trees. The goal of the current study was to examine some of the factors which might influence the effect of soil freezing on microbial processing. Soil samples from three different soil horizons ( $O_e$ ,  $O_a$ , and  $A_1$ ), were taken from plots dominated by either sugar maple or yellow birch, at the Hubbard Brook Experimental Forest in New Hampshire. The samples were frozen in the laboratory at two different temperatures ( $-13^\circ\text{C}$  and  $-3^\circ\text{C}$ ) for 10 days. Soil respiration, nitrogen mineralization, and nitrification were measured for three weeks following freezing. Overall rates of microbial processes differed significantly among soil horizons, with higher rates in the  $O_e$  horizon and lower rates in the  $A_1$ . Freezing to  $-3^\circ\text{C}$  had no significant effect on any of the measured responses. The effects of freezing to  $-13^\circ\text{C}$  varied by both horizon and species. Freezing at  $-13^\circ\text{C}$  increased respiration in the  $O_a$  and  $A_1$  horizons of both species and mineralization in the maple plots. In the birch plots, freezing decreased mineralization in the  $O_e$  horizon. Nitrification exhibited little response to freezing in any of the horizons.

## INTRODUCTION

Snow insulates soil and protects it from freezing, but when there is no snow, the soil can freeze. Soil freezing due to thaw-freeze events, in which a warm period that melts the snow is followed by a cold period that freezes the soil, has been positively correlated with severe canopy dieback, especially among sugar maples (*Acer saccharum*), in the Northern Hardwood forests of the United States and Canada. Although the mechanism is not fully known, this correlation suggests that soil freezing was responsible, at least in part, for this damage (Auclair et al. 1996).

Soil freezing may become a more significant problem as global climate change advances. One of the potential indirect effects of global warming is a reduction in snowfall in the middle to high latitudes. Although it is not certain that this effect will occur, it is known that a slight increase in mean winter temperature can significantly reduce snowfall, even with constant or increasing overall precipitation (Moore and McKendry 1996). Less snowfall means more soil freezing; snow insulates the soil and protects it from the harsh temperatures of winter. In regions which receive significant snowfall, the soil does not usually freeze below a few centimeters in depth. However, a reduction in snow cover will cause the soil to freeze deeper, colder, and longer, and to undergo more drastic

temperature fluctuations resulting in more freeze/thaw cycles (Edwards and Cresser 1992, Boutin and Robitaille 1995).

Sudden freezing causes significant mortality among soil microbes and fine roots (Soulides and Allison 1961). This releases a great deal of labile organic matter into the soil, which is then consumed by the surviving soil microbes when the soil thaws, resulting in a flush of microbial activity. Respiration, nitrification, denitrification, and nitrogen mineralization have all been shown to increase after a soil freezing event (Edwards and Cresser 1992, Burton and Beauchamp 1994).

These microbial processes result in the release of  $N_2O$ , nitrogen oxides, and  $CO_2$ , all of which contribute to the greenhouse effect (Jarvis 1996). In addition, increased nitrification leads to increased nitrate leaching. Ammonia is retained well in soil, since it binds to negatively charged sites on soil particles, but once it is transformed into nitrate it is more easily leached out of soil (Atlas and Bartha 1986). This effect is intensified by the fact that the early spring, when the flush of post-freezing nitrification would occur, is a time of low nitrogen demand by plants, as they can make use of excess nitrogen they extracted from senescing leaves in the fall (Edwards and Cresser 1992). An increase in nitrate leaching has been correlated with soil freezing events in the past (Boutin and Robitaille 1995, Mitchell et al. 1996). Increased nitrate leaching leads to several problems. Nitrate pollutes drinking water (Jarvis 1996). Nitric acid formed during nitrification reduces the pH of the soil and nearby surface water (Ehrlich 1990,

Boutin and Robitaille 1995). Excess nitrate contributes to the eutrophication of coastal waters. Also, the leaching of nitrate, as a mobile anion, facilitates a corresponding loss of nutrient cations from the forest ecosystem (Mitchell et al. 1996).

Correlational research has been done on the relationship of soil freezing and microbial processes, but few, if any, manipulative experiments have been performed (Mitchell et al. 1996, Auclair et al. 1996, Pilon et al. 1994, Soulides and Allison 1961, Edwards and Cresser 1992). The goal of this study was to determine how soil microbial processes respond to a variety of freezing conditions. The study compares soil from three distinct organically rich soil horizons, from plots containing two different dominant tree species, frozen at two different temperatures. This allows an examination of how the factors of soil horizon, dominant tree species, and freezing temperature interact in their effects on microbial processes.

## **MATERIALS AND METHODS**

### *Site description*

The Hubbard Brook Experimental Forest (HBEF) is a Long-Term Ecological Research station in the White Mountains of central New Hampshire. This site is located in a region where soil freezing is currently rare due to heavy

snowfall, but which may be affected by reduced snowfall caused by global climate change. HBEF is a northern hardwood forest dominated by sugar maple (*Acer saccharum*), yellow birch (*Betula lutea*), and American beech (*Fagus grandiflora*). Elevations range from about 250 to 750m above mean sea level. The soil is relatively fine till derived from granite or acid metamorphic rock (Bormann and Likens, 1979). More specifically, the soils are acidic (pH 3.9) Typic Haplorthods. Three soil horizons were examined: the O<sub>e</sub>, which consists of partially decomposed organic material; the O<sub>a</sub>, which consists primarily of decomposed organic matter with some mineral soil; and the A<sub>1</sub>, which contains mineral soil mixed with some organic material. Four sampling areas were chosen, two dominated by sugar maple, and two dominated by yellow birch, and within each sampling area, two 10m x 10m plots were delineated.

#### *Sample collection and initial handling*

Samples were collected June 4<sup>th</sup> and 5<sup>th</sup>, 1997. In each plot, two small pits were dug using a trowel, and soil was collected from the three horizons, combining the samples from the two pits. Each sample was sorted by hand, and any debris which appeared upon visual inspection to be too large to fit through an approximately 1mm sieve was removed. All data are expressed on an oven dried soil basis (75°C). Seven grams of field moist soil from each sample were mixed with 30mL of 2N KCl solution for one hour and then filtered in order to extract ammonia and nitrate. The filtrate was analyzed with a flow injection

autoanalyzer (Perstorp 3000), using the salicylate/hypochlorite method for  $\text{NH}_4^+$  and cadmium reduction for  $\text{NO}_3^-$ .

### *Sample treatment*

A factorial design was employed using sites dominated by two different tree species (maple and birch), three soil horizons ( $\text{O}_e$ ,  $\text{O}_a$ , and  $\text{A}_1$ ), and two temperatures ( $-13^\circ\text{C}$  and laboratory temperature controls). In addition, a separate group of  $\text{O}_a$  horizon samples from both species was treated at  $3^\circ\text{C}$ . Each group contained 8 samples: 2 sample collection areas x 2 plots x 2 laboratory replicates.

Samples were weighed out as follows: 20g for the  $\text{O}_e$  horizon samples, 35g for  $\text{O}_a$ , and 50g for  $\text{A}_1$ ; and placed in 200mL-capacity incubation vessels (microlysimeters), filtration devices with an upper chamber where the soil is placed and a lower chamber into which filtrate is drawn, separated by glass fiber filters having a  $1.0\ \mu\text{m}$  nominal pore size. To each sample, 100mL of micronutrient solution (4.0mM  $\text{CaCl}_2$ , 2.0mM  $\text{KH}_2\text{PO}_4$ , 1.0mM  $\text{K}_2\text{SO}_4$  and  $\text{MgSO}_4$ ,  $2.5\ \mu\text{M}$   $\text{H}_3\text{BO}_3$ ,  $2.0\ \mu\text{M}$   $\text{MnSO}_4$  and  $\text{ZnSO}_4$ , and  $0.5\ \mu\text{M}$   $\text{CuSO}_4$  and  $\text{Na}_2\text{MoO}_4$ ) was added (Nadelhoffer, 1990). The solution was allowed to remain in contact with the soil for one hour and was then drained and analyzed for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  as described above. The microlysimeters were then placed in sealed quart-size mason jars for 24 hours, after which two 9mL samples of the headspace gas were taken with a syringe through a septum in the mason jar lid. The two

headspace samples were analyzed for CO<sub>2</sub> and N<sub>2</sub>O respectively, using a gas chromatograph with a thermal conductivity detector.

Next, the microlysimeters were covered with plastic wrap and placed under treatment conditions for 10 days. The frozen samples were placed in freezers with temperatures of approximately -13°C (range of -15°C to -11°C) and approximately -3°C (range of -5°C to -1°C) respectively, and control samples remained in the laboratory. Leaching and headspace measurements as described above were then repeated once a week for three weeks, starting immediately after treatment. Finally, a second KCl extraction as described above was performed on the treated samples.

Total respiration was determined by extrapolating the individual CO<sub>2</sub> evolution measurements to cover the entire length of the experiment. To calculate nitrification, all NO<sub>3</sub><sup>-</sup> that was either leached or extracted was aggregated, and to calculate nitrogen mineralization, total leached or extracted NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were aggregated.

#### *Statistical analysis*

For each of the four measured responses (CO<sub>2</sub>, N<sub>2</sub>O, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup>), a two-way analysis of variance was run for each sampling date, with species, treatment, and species x treatment interaction as the main effects. As there were

no significant interaction effects, to determine specific differences between the three treatments, a Fisher's least significant difference test was run *a posteriori*.

The statistical analysis for total respiration, mineralization, and nitrification for the three temperatures within the O<sub>a</sub> horizon was a two-way analysis of variance with species and treatment as main effects. A test was also run for species x treatment interaction, but this was not significant for any variable. To determine specific differences between the three treatments, a Fisher's least significant difference test was run *a posteriori*. The statistical analysis for these three processes for the 13° and control treatments across the three horizons was a three-way analysis of variance with species, treatment, and horizon as main effects. There were also tests for species x treatment, species x horizon, and treatment x horizon interactions, some of which were significant.

## RESULTS

The rate of CO<sub>2</sub> evolution increased ( $p < 0.01$ ) from 57.2 to 331  $\mu\text{g C g}^{-1}$  soil day<sup>-1</sup> immediately following freezing to 13° in the O<sub>a</sub> horizon (Fig. 1). Freezing to 3° resulted in an increase from 56.6 to 131  $\mu\text{g C g}^{-1}$  soil day<sup>-1</sup>, but this increase was not statistically significant. These increases were similar for both tree species. The emission of CO<sub>2</sub> demonstrated a very small species effect ( $p < 0.05$ )

at the start of the experiment, with rates of  $47.9\mu\text{g C g}^{-1}\text{ soil day}^{-1}$  in the maple samples and  $64.7\mu\text{g C g}^{-1}\text{ soil day}^{-1}$  in the birch samples. Respiration remained consistently higher in birch relative to maple in both the control and freeze treatments, but the difference was not significant.

Nitrous oxide emissions also increased immediately following treatment in the  $\text{O}_a$  horizon, with the  $-13^\circ$  treatment resulting in an increase from 3.07 to  $28.9\text{ng N g}^{-1}\text{ soil day}^{-1}$ , and the  $-3^\circ$  treatment causing an increase from 9.05 to  $20.8\text{ng N g}^{-1}\text{ soil day}^{-1}$  (Fig. 2). However, due to high variability between samples, neither of these increases was significant. A significant treatment effect was not observed until one week following the treatment, at which time the  $\text{N}_2\text{O}$  evolution rate of the  $-13^\circ\text{C}$  samples,  $6.70\text{ng N g}^{-1}\text{ soil day}^{-1}$ , was significantly ( $p<0.05$ ) higher than that of the control samples,  $1.81\text{ng N g}^{-1}\text{ soil day}^{-1}$ . The  $\text{N}_2\text{O}$  evolution rate of the  $-3^\circ\text{C}$  samples,  $3.85\text{ng N g}^{-1}\text{ soil day}^{-1}$ , was not significantly different from either of the other two. In addition,  $\text{N}_2\text{O}$  evolution demonstrated a significant ( $p<0.05$ ) species effect, with soil from the maple plots evolving more of the gas than soil from the birch plots throughout the experiment, especially immediately following freezing to  $-13^\circ\text{C}$ , when the maple samples averaged  $55.5\text{ng N g}^{-1}\text{ soil day}^{-1}$ , while the birch samples averaged only  $2.28\text{ng N g}^{-1}\text{ soil day}^{-1}$ .

The  $-13^\circ\text{C}$  treatment caused an increase ( $p<0.01$ ) in  $\text{NH}_4^+$  leaching in the  $\text{O}_a$  horizon, but this effect was not observed until one week after treatment

ended, when the  $-13^{\circ}\text{C}$  samples averaged  $76.9\mu\text{g N g}^{-1}\text{ soil day}^{-1}$ , while the control samples averaged only  $39.1\mu\text{g N g}^{-1}\text{ soil day}^{-1}$  (Fig. 3). The  $-3^{\circ}\text{C}$  samples, with an average  $\text{NH}_4^+$  leaching rate of  $42.5\mu\text{g N g}^{-1}\text{ soil day}^{-1}$  were not significantly different from the controls. Ammonia leaching also demonstrated a significant ( $p<0.01$ ) species effect, with between 8 and  $33\mu\text{g N g}^{-1}\text{ soil day}^{-1}$  more  $\text{NH}_4^+$  leached from the soil from the birch plots than that from the maple plots throughout the experiment.

In contrast to ammonia leaching, nitrate leaching in the  $\text{O}_a$  horizon actually decreased ( $p<0.10$ ) immediately after freezing to  $-13^{\circ}\text{C}$ , with average  $\text{NO}_3^-$  leaching rates of  $2.05\mu\text{g N g}^{-1}\text{ soil day}^{-1}$  in the  $-13^{\circ}\text{C}$  samples, and  $4.56\mu\text{g N g}^{-1}\text{ soil day}^{-1}$  in the controls (Fig. 4). The  $-3^{\circ}\text{C}$  treatment, with rates averaging  $2.21\mu\text{g N g}^{-1}\text{ soil day}^{-1}$ , showed no significant difference from either of the other groups. Nitrate leaching also demonstrated a large species effect ( $p<0.01$ ), with between 2 and  $6\mu\text{g N g}^{-1}\text{ soil day}^{-1}$  more  $\text{NO}_3^-$  leached from the maple soil than the birch soil throughout the experiment.

There was no significant treatment x species interaction for any of the measured variables. Although  $\text{CO}_2$  and  $\text{N}_2\text{O}$  evolution in the control samples remained approximately constant throughout the study,  $\text{NH}_4^+$  leaching increased while  $\text{NO}_3^-$  leaching decreased, suggesting that the soil disturbance inherent in the experiment had some effect on nitrogen cycling processes, independent of any treatment effects.

Freezing effects varied strongly by soil horizon. For both tree species, the O<sub>e</sub> horizon had the highest respiration and A<sub>1</sub> horizons had the lowest ( $p < 0.05$ ) (Table 1). Respiration increased significantly with freezing to  $-13^{\circ}\text{C}$  in the O<sub>a</sub> ( $p < 0.001$ ) and A<sub>1</sub> ( $p < 0.001$ ) horizons of the maple soil, and in the O<sub>a</sub> ( $p < 0.01$ ) and A<sub>1</sub> ( $p < 0.05$ ) horizons of the birch soil, but did not change significantly with freezing in the O<sub>e</sub> horizon of either tree species. Respiration increased with freezing to  $-3^{\circ}\text{C}$  in the O<sub>a</sub> horizon of both species, but not significantly.

Mineralization also differed significantly among all three horizons, again with the highest rate in the O<sub>e</sub> horizon and the lowest in the A<sub>1</sub> ( $p < 0.05$ ) for both tree species (Table 2). Mineralization decreased after freezing to  $-13^{\circ}\text{C}$  in the O<sub>e</sub> horizon, but increased in both the O<sub>a</sub> and A<sub>1</sub> horizons. This pattern was the same for both species, but only the increases in the O<sub>a</sub> and A<sub>1</sub> horizons of the maple soil and the decrease in the O<sub>e</sub> horizon of the birch soil were significant ( $p < 0.01$ ). Mineralization exhibited almost no response to freezing to  $-3^{\circ}\text{C}$  in either tree species.

Nitrification in the O<sub>e</sub> and A<sub>1</sub> horizons differed significantly in both tree species ( $p < 0.05$ ), but the O<sub>a</sub> horizon differed from the A<sub>1</sub> only in the maple soil ( $p < 0.05$ ) (Table 3). Nitrification decreased somewhat after freezing for all horizons, temperatures, and species. However, this effect was only significant in the O<sub>e</sub> horizon from the maple plots ( $p < 0.10$ ).

Respiration, mineralization, and nitrification all exhibited species x horizon interactions, with a significance of  $p < 0.01$  for respiration and nitrification, and  $p < 0.05$  for mineralization. Of the three, only mineralization showed a treatment x horizon interaction ( $p < 0.01$ ).

## DISCUSSION

The expected post-freezing flush of activity was observed in increased rates of respiration, mineralization, and  $N_2O$  flux, but these effects varied by horizon, temperature, and species.

The differences between horizons in the rates of respiration, mineralization, and nitrification corresponded well with the expected organic content of the horizons: highest in the  $O_e$ , where there is newly available organic material; lower in the  $O_a$ , where the most usable organic matter has already been removed; and lowest in the  $A_1$ , where organic matter is more scarce. The response to freezing differed between the  $O_e$  and the other two horizons. Respiration increased immediately after freezing to  $-13^\circ\text{C}$  in the  $O_a$  and  $A_1$  horizons of plots of both dominant tree species, suggesting that the freezing caused microbial mortality in these horizons, resulting in the release of organic

material and an increase in microbial activity, as predicted. However, this result was not found in the O<sub>e</sub> horizon, perhaps because the amount of available carbon was already so high that the release of carbon following freezing did not significantly increase the pool. Mineralization also increased after freezing to -13°C in the O<sub>a</sub> and A<sub>1</sub> horizons of both tree species, but decreased in the O<sub>e</sub> horizon, suggesting that the previously available N pool in the O<sub>e</sub> horizon was so large that the freezing-induced mortality of the nitrogen-mineralizing bacteria had a larger effect on the overall mineralization rate than the release of organic material did.

Since the O<sub>e</sub> horizon soil had a drastically higher initial rate of respiration than the other two (699 μg C g<sup>-1</sup> soil day<sup>-1</sup>, as opposed to 56.6 and 6.13 μg C g<sup>-1</sup> soil day<sup>-1</sup>), and a higher total mineralization in the control samples (936 μg N g<sup>-1</sup> soil, as opposed to 194 and 33.5 μg N g<sup>-1</sup> soil), it might seem that it would be the most important result to consider. However, the total mass of O<sub>e</sub> horizon soil is extremely small compared with that of the other horizons, so the importance of this horizon is diminished. In fact, the O<sub>a</sub> horizon probably contributes the most to respiration and nitrogen cycling, since it combines a relatively high respiration rate with a comparatively large total mass. Thus, it is likely that the aggregate respiration and mineralization of all the horizons do increase after freezing to -13°C, as in the O<sub>a</sub> horizon.

The increase in respiration in the O<sub>a</sub> horizon after freezing to -3°C is much less than after freezing to -13°C, and is not statistically significant, while mineralization in the O<sub>a</sub> horizon does not increase at all with freezing to -3°C. Due to high solute concentrations which lower their freezing point temperatures, it is likely that most microbes survive such mild freezing, and therefore not much organic material is released.

The overall pattern of the response to freezing is similar between tree species. Despite higher respiration rates in the birch soil, the percent changes in respiration due to freezing were extremely similar for both species. However, the birch soil appears to have more conservative N cycling. This is indicated by the higher respiration (which indicates higher carbon availability), lower N<sub>2</sub>O fluxes, higher NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> ratios, and lower nitrification. Due to the more conservative nature of the birch soils, the mineralization responses to freezing differed somewhat between the species: more decrease in the O<sub>e</sub> horizon in the birch soil, and less increase in the O<sub>a</sub> and A<sub>1</sub> horizons.

The nitrification rate was not strongly affected by freezing. There are at least two possible explanations why the measured nitrification rate would respond so differently to freezing than the other microbial processes measured. Perhaps the denitrification rate was increased by freezing, along with the nitrification rate, such that a significant portion of the excess NO<sub>3</sub><sup>-</sup> produced after freezing was denitrified rather than leached out. Although denitrification itself

was not measured in this experiment, the increased production of  $N_2O$ , which is a byproduct of the denitrification process, upon freezing to  $-13^{\circ}C$  suggests that denitrification increased. If this is the case, then soil freezing may not increase nitrate leaching, but will accelerate nitrogen loss from the system. Another possible explanation is that the population of nitrifiers was disproportionately damaged by freezing and required a long recovery period before nitrification could increase enough to adequately make use of the newly available  $NH_4^+$ . This explanation is supported by the fact that nitrifiers, primarily fungi, unlike the bacteria responsible for most of the measured respiration and mineralization, are quite susceptible to damage and recover very slowly. In this case, soil freezing may actually increase nitrification in the longer term, after a lag period.

The question of whether reduced snow cover due to global climate change will affect soil microbial processes depends not simply on whether the soil will freeze, but whether it will freeze cold enough to cause significant mortality of microbes and fine roots. The response will also be affected by the dominant tree species and whether freezing occurs in all soil horizons. The question of whether soil freezing increases nitrate leaching and/or overall nitrogen loss remains unanswered. A longer-term study, measuring denitrification as well as nitrification, is needed.

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