INFLUENCE OF NITROGEN IN AMOUNT AND FORM ON DECOMPOSITION OF RED SPRUCE NEEDLES AT HOWLAND FOREST, HOWLAND, ME

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ABSTRACT

In the life cycle of a plant, nitrogen can be a limiting factor, affecting growth and decay. Therefore, nitrogen can affect how quickly fallen plant litter decays, a process by which much of the carbon stored in the litter is released to the atmosphere in the form of carbon dioxide. Carbon dioxide is a greenhouse gas, which leads to global warming, the problem many experiments, including this one, attempt to address. This study uses O horizon soil and red spruce needles to examine biologically assisted litter decomposition in the laboratory. O horizon soil was exposed to four different treatments of ^{15}N to simulate natural rainfall, fast and slow moving nitrogen pools, and varying concentrations of nitrogen. The soil was separated from the litterbag by wet sand to break capillary action, and limit abiotic movement of ^{15}N into the decaying litter. Litter decay will be examined, and the effects of added nitrogen in different forms will also be determined on litter decomposition. This experiment focuses upon excess nitrogen effects on biological decomposition, and the expectant increase in the amount of CO_2 released, which leads to global warming.

INTRODUCTION

In northern and northeastern U.S. forests, the majority of nitrogen (N) in precipitation is in the form of nitric acid (HNO_3) with a minor contribution in the form of ammonium (NH_4^+) and dissolved organic N (DON). Forest productivity can be limited by the availability of N, and thus increased N in deposition as a result of human activity may cause increased tree growth –a result that has important implications regarding atmospheric carbon dioxide levels. Northern forests are also suspected of providing a substantial sink for the global overproduction of carbon dioxide and additional N may fuel photosynthesis and storage of this additional C. When gauging the importance of forest C storage in response to increases in N availability, it is also necessary to measure the soil processes that may affect overall ecosystem C storage. These include the mineralization of soil organic matter (SOM) and the decay of plant litter (Swift et al., 1979; Melillo et al., 1989).

A DOE-funded study into the significance of atmospheric-N and forest response in central Maine is currently assessing the fate of added nitrate and ammonium-N to a forest canopy (Figs. 1a,1b). This study will focus on the fate of added ^{15}N in tree tissues and the forest floor, soils and export through soil water. The central hypotheses suggest that the 18 Kg N ha^{-1} y^{-1} additions will fuel measurable productivity and that a substantial fraction of added N (>30%) would be retained in the canopy tissues rather than in the forest floor (as suggested by many previous studies which added N directly to the forest floor (Nadelhoffer et al. 1999)). However, if true, there will still be a modest amount of N (ca. 70%) making it to the forest floor, which may fuel important microbial processes that return CO_2 to the atmosphere. In order to assess the importance of additional N on forest *sink* dynamics, it is also important to look at the influence of N upon forest soil microorganisms as a potential source of carbon dioxide.

Figure 1a. Helicopter spraying of canopy takes place 6 times during the growing season of this red spruce-hemlock dominated forest in central Maine. The annual N input above ambient is 18 kg N per ha per year (roughly 6 times ambient for the growing season). Nitrogen is added as ammonium nitrate and potassium nitrate, reflecting ambient inputs of 2/3 nitrate and 1/3 ammonium.

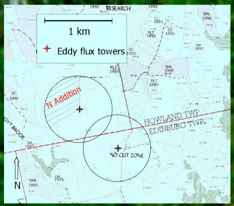

Figure 1b. Nitrogen addition to a 23 ha area centered on an Eddy Flux tower

PURPOSE

I sought to test the influence of additional N on litter decay by using N additions that were relevant in form to those in natural precipitation at this site. I prepared a multi-factorial microcosm experiment to test the importance of additional N upon litter decay using needles from a dominant tree species in Maine, red spruce (*Picea rubens* Sarg.). Needles were added to soil microcosms in which soils were pre-exposed to varying N forms (^{15}N-nitrate and ammonium) and at differing concentrations (10 and 100 µg N g-1 soil dry mass). Additionally, I pre-exposed soil to ^{15}N-nitrite to create a soil with additional N, however in a form that is insoluble and thus may be unavailable to microorganisms. Soil microcosms were built in such a way as to test the microbially mediated translocation of N into decaying litter. My hypotheses are:

•Additional N in microbially available forms (ammonium and nitrate) will increase the decay rate of litter. Little or no difference in decay rate in nitrate vs. ammonium amended soils.

•Nitrite interaction with insoluble organic matter (see Azhar et al, 1986; Dail et al., 2001) will nominally increase N content of soil but will not increase the decay rate of litter. •To fuel decay of needles, microorganisms will translocate soil N into the litters during the decay process.

•Organic matter N (labeled with 15N, will be utilized to decompose litter C (e.g., 15N immobilized into decaying litter).

MATERIALS AND METHODS

O-horizon soil (O_{e+a}) and red spruce needles were obtained from the Howland Forest Site, Howland, Maine. Soils were sieved (2mm) and roots removed by hand. Soils were stored field moist at 4ºC until ^{15}N additions. Needles were dried (50ºC, 96h) and then hand separated from twigs, to be placed in mesh bags with a nominal hole size of 50 micrometers. The soils were exposed to 5 differing N regimes. Two treatments added 99% enriched ^{15}N in forms of nitrogen common to rainfall; NO_3^- and NH_4^+. A third treatment added 99% enriched ^{15}N in the form of nitrite, which has been shown to react with organic matter and form insoluble organic-N. This latter addition was used to simulate slow moving nitrogen pools. The fourth treatment was to add 10% $^{15}NH_4Cl$ and 90% $^{14}NH_4Cl$ to determine effects of concentration on decomposition. All ^{15}N additions as well as a deionized water control were made over the course of 14 days in solution amounts that did not exceed 60% of the water holding capacity of this soil.

Exposed soils were then added to microcosm jars (a 375 mL glass jar was used; **Fig. 2a**); 3 replicates were made for each N regime and each time point for the decay study. For each rep of each sample, 20 grams (air dry) of enriched soil was added to the bottom, distilled water was used to wet the soil, and on top of that soil a layer 4 mm think of sand was added. Sand was added to break capillary action of the exposed soil into a mesh bag in an effort to differentiate microbial (biotic) translocation of ^{15}N to litter from capillary (abiotic) N-flow. On top of the sand layer a mesh bag containing 2 g of dried litter (**Fig 2b**). This was then spray wetted with distilled water (ca. 3ml). On top of the mesh bag I placed 20 g *unexposed* soil, and then wetted this cover soil with distilled water. A cap with a hole drilled to avoid CO_2 buildup was placed on top of the glass jar, and the entire microcosm was weighed. Initially, weight loss in the microcosm over time is to be attributed mostly to water loss and this will be added back on a weekly basis via both spray and syringe needle, when litter mass loss is measured, we will adjust our water additions concomitant to these data.

At 20, 40, 80 120, 200, 280 and 365 d, microcosms will destructively sampled. Mesh bags will be removed, cleaned and dried to a constant weight and then weighed to determine mass loss. A sub sample of exposed and unexposed soil will be collected to measure soluble (NO_3^-, NH_4^+ and DON) and insoluble (organic) nitrogen and mass spectroscopy will be used to determine the ^{15}N enrichment of these N fractions as well as that of the needle material remaining (**Fig. 3**).

Figure 2a: A decomposition microcosm, with a lower layer of 15N-exposed soil, a layer of sand, a litterbag on top of the sand (see Fig 1b) and A layer of unexposed soil on top. A hole in the lid allows for free exchange of air.

Figure 2b: Spruce needle litterbag placement in an uncompleted Microcosm.

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METHODS Continued…

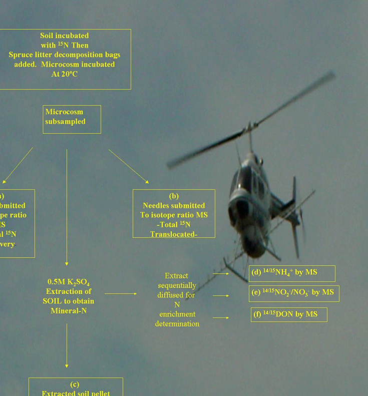

FIGURE 3. Litter decomposition processing flowchart for 15N analysis

EXPECTED RESULTS

Litter decomposition is affected by many factors including temperature, litter quality (e.g., C:N or Lignin:N) and the availability of water and nutrients. This experimental design seeks to hold many of these variables constant while manipulating nitrogen in both form and quantity. Previous studies have found that increasing nitrogen does not necessarily cause an increase in litter decomposition rate (Prescott et al., 1991), while others indicate that increasing nitrogen does have an effect on litter decomposition (Fenn, 1991; Thirukkumaran et al, 2000). In the latter study as well as many others, ammonium nitrate was added to litter or soil and litter and decomposition observed, but these methods add two different forms of nitrogen and the individual effects of N source could not be discerned.

My study set up multiple treatments, including one for ammonium and one for nitrate. Ammonium is a highly available and reduced form of N and thus closer in form to its likely fate (amino-N), so it should be easier for microbes to assimilate. Thus, ammonium should enhance decomposition more than nitrate, but both *could* enhance decomposition, as both inorganic forms are present in precipitation at this site. In addition, I used a greater concentration of ammonium as a treatment, and this should enhance decomposition further by raising the availability of this limiting nutrient. The final soil treatment I used was to add nitrite as a surrogate for slow moving (organic) N pools in soil. Nitrite is known to react strongly with organic matter producing insoluble nitroso- compounds (Azhar, 1986) and while these compounds may not represent all slow moving N pools in soil, this experiment will provide a comparison to the fast moving N pools in the other treatments. Increasing insoluble soil N should have little to no effect on decomposition; however, no previous work has been done with this N form. Therefore, I hypothesize that the nitrite treated soil should show the same decomposition rate as the control.

The sand layer, placed between the litterbag and the underlying treated soils, could initially slow decomposition because it is a barrier for microbes to pass, but the sand layer was added to reduce the possibility that soluble 15N would move by capillary action into the litterbags. The overlying soil is not enriched in 15N and thus leaching N from this soil may increase the N content of litter, however not the enrichment. Indeed, the enrichment of litter may appear reduced if N from untreated soils is incorporated into the decaying litter.

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