**Nutrient Co-limitation of Decomposition in Northern Hardwood Forests**

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**Introduction**

Decomposition is the key process returning nutrients to ecosystems. In forest ecosystems much primary production is returned to the forest floor as litterfall. Primary factors suggested for regulating rates of decomposition include: carbon quality (Melillo et al., 1982; Hobbie, 2000; Hättenschwiler and Jørgensen, 2010); initial litter nutrients (Melillo et al., 1982; Mooshammer et al., 2011; Barantal et al., 2012); site nutrient status (Hobbie and Vitousek, 2000; Hobbie, 2005, 2008); decomposer fauna (McBrayer et al., 1977; Seastedt, 1984; Schädler and Brandl, 2005; Carrillo et al., 2011; Barantal et al., 2012); litter species identity (Cornelissen, 1996; Hättenschwiler et al., 2005); and litter species traits (Cornwell et al., 2008).

In ecosystem studies estimates of decomposition are often based on litterfall. This assumes that decomposition mediates an equilibrium between litter input and nutrient availability although this assumption is generally not tested. Work by Hobbie and Vitousek, 2000 suggest that nutrient limitation of decomposition may function differently than for ANPP. Furthermore, despite positive correlations between nitrogen and decomposition (Melillo et al., 1982) and numerous investigations, the effects of N on decomposition are not clear (Fog, 1988). Studies have shown positive effects, no effect and negative effects (reviewed in Knorr et al., 2005). In contrast to N, little work has investigated the effects of phosphorus on litter decomposition rates and only two tropical studies have looked for interacting effects of N and P (Hobbie and Vitousek, 2000; Barantal et al., 2012). Both of these studies provided some limited evidence of nutrient co-limitation of decomposition under specific circumstances. This study will ask how nurtients limit decomposition of leaf litter and specifically whether litter mass loss shows evidence of nutrient co-limitation.

Experiments testing for effects of litter dwelling invertebrates on rates of litter decomposition have had mixed results although studies suggest that invertebrates can increase the effects of other f factors on decomposition (González and Seastedt, 2001; Hättenschwiler and Gasser, 2005; Schädler and Brandl, 2005; Barantal et al., 2012). Often the fauna present in these studies include earthworms and isopods, groups seen as ecosystem engineers. As the effects of ecosystem engineers are large by definition and that much of the northern hardwood forests lack both earthworms and isopods, it becomes important to understand the role the smaller but more numerous litter arthropods in decomposition.

**Motivation for Involving Middle School Students**

There has been increased emphasis on the importance of student involvement in authentic research however, such activity is rarely achieved. This experiment is very accessible to students. Simple measurements and experimental design will allow up to about 100 middle school students to work as part of creating this experiment as well as analyzing and presenting data.

**Objectives**

1. Assess the overall effects of nutrient additions on leaf litter decomposition.
2. Experimentally test for nutrient co-limitation of decomposition.
3. Assess interacting effects of stand age, decomposer fauna and nutrient additions on leaf litter decomposition.

**Hypotheses**

Evidence for nutrient co-limitation of decomposition may be found in three different areas: site effects; litter quality effects; and stand age effects. This experiment integrates possible litter and site effects and looks for interactions with stand age and invertebrate mesofauna. Overall I expect mass loss from litter should be greatest in fertilized plots but more so in plots fertilized with both N + P. Previous work on foliar resorption at these sites has shown evidence that younger stands are more limited by P availability while mature stands are more N limited. If the same pattern holds I expect decomposition in young stands to show stronger responses to P while decomposers in mature stands will show stronger responses to N fertilization. The presence of mesofauna is expected to increase the effects of fertilization and stand age on decomposition. Mesofauna communities in young and mature stands should show qualitative differences.

**Methods**

*Study Site*

This project uses a long-term co-limitation fertilization study at the Bartlett Experimental Forest in the White Mountains of New Hampshire. Two mature (>100 years) maple-birch-beech stands (C7 and C9) and two young (C1 and C2) stands (<30 years) will be used. Each stand contains four 50 m x 50 m treatment plots. Nutrient additions to these plots began in spring 2011. In each stand one plot receives 30kg N /ha/yr , a second plot receives 10 kg/Ha/yr P, and a third plot receives both 30kg/ha/yr N and 10 kg/Ha/yr P. The fourth plot in each stand is a control, receiving no fertilization. I think you have Ca plots in one young and one old stand. Think about whether you want to include them in your study.

*Experimental Design*

In order to assess the overall effects of N and P fertilization on leaf litter decomposition I will decompose litter from each fertilization treatment in their respective plots. The experiment will be repeated at young stands using stands C1 and C2 and mature stands (C7 and C9). In order to control for differences in species abundance, representative litter from young and mature stands will be reciprocally transplanted between different aged stands. Faunal exclusion and access will be included as well to assess any potential interactions between decomposer fauna and site fertilization.

*Litter Collection*

Leaf litter will be collected this fall during peak litter-fall at each plot using litter nets. At each collection plot litter nets will be placed in each of the middle edge subplots within one meter of the center of the inside boundary to the center subplot for four total litter nets per plot. Litter nets will be approximately 0.25 m2 and positioned about 0.5 m from the forest floor. Litter will be collected as often as possible. Only litter collected prior to rainfall will be included in litterbags. Collected litter will be bagged, labeled, returned to the lab sorted by target species and air dried. To correct for moisture content in air dried samples, subsamples will be oven dried at 60 degrees C and weighed.

All drying, sorting, litter bag construction and weighing will be conducted by 7th and 8th grade students at Kennett Middle School. The building has ample space for this project and the support of the building Principal. Each part of the process will pass through a two-step quality control procedure to eliminate errors.

*Litterbag Construction*

Litterbags will be 100 cm2 and made from fiberglass or polyester mesh. Two types of litter bags will be made. Faunal exclusion bags will be made of 20 µm x 20 µm size mesh both top and bottom. This size allows microbes, and fungal hyphae but excludes mesofauna. A second set of bags will be made to allow mesofauna access by using 5 mm size mesh on the top, with the smaller 20 µm mesh size on the bottom. Bags will be sewn or stapled shut after being filled with litter.

Litter bags will contain either young-stand litter or mature-stand litter. Young-stand litter will contain 1g American Beech, 0.5g each Pin Cherry and Red Maple, and 0.75g White Birch. Mature stand litter will contain 1.25g American Beech, 1g Sugar Maple, and 0.5g Yellow Birch. Litter collected from each treatment plot will be combined within same aged stands. In both cases the mix represents typical litter ratios of the most dominant species collected in these plots. Each litterbag will be assigned a number and exact masses will be recorded when constructed.

All litter bags will be placed in the field as soon as possible after all bags are made and before snow accumulation. Ten subplots will be chosen from each treatment plot. Each subplot will receive one of each litterbag for each combination, Young-stand litter, mature-stand litter, large mesh and small mesh for four bags per subplot. Litterbag locations will be marked using colored flagging and noted on maps. All litterbags will be collected during July 2013.

**Analysis**

Decomposition will be expressed as percent mass loss compared to the initial oven dry weight. Initial analysis can include two-factor ANOVA to test for differences between nutrient treatments and stand age. Mesh size can be analyzed with a paired t-test.

*Fauna Survey*

A fauna survey will be conducted across the same study sites to look for differences in litter meso and macrofauna. Samples will be collected late June and July 2013. Twenty-five 10 cm x 10 cm litter samples will be randomly collected from the buffer zone of each plot, combined, stored and transported in a closed paper bag back to the laboratory. Each sample will be weighed then placed in an individual Tullgren funnel. The tops of each funnel will be covered with cheese cloth to prevent escape. Funnels will be lit and heated with 60 W bulbs positioned directly above the funnel. Specimens will be collected in 70% ethanol below each funnel. Litter samples will remain in the funnels for a minimum of 72 hours. After extraction of invertebrates, all litter will be removed from the funnels, dried at 60°C and weighed. Collected arthropods will be identified under a dissecting microscope. Specimens collected over the summer will also be used to develop middle school student friendly keys to allow students to later work on samples.

**Analysis**

Decomposition will be expressed as percent mass loss compared to the initial oven dry weight. Initial analysis can include two-factor ANOVA to test for differences between nutrient treatments and stand age. Mesh size can be analyzed with a paired t-test.

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