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DEB - Ecosystem Studies

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TITLE OF PROPOSED PROJECT **Preliminary Proposal: RUI: Causes of Nutrient-Enhanced Transpiration in Northern Hardwoods: Leaves, Stems, Roots, or Mycorrhizal Fungi?**

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
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☐ BEGINNING INVESTIGATOR (GPG I.G.2)
☐ DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.C.1.e)
☐ PROPRIETARY & PRIVILEGED INFORMATION (GPG I.D, II.C.1.d)
☐ HISTORIC PLACES (GPG II.C.2.j)
☐ EAGER* (GPG II.D.2) ☐ RAPID** (GPG II.D.1)
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Preliminary Proposal: RUI: Causes of Nutrient-Enhanced Transpiration in Northern Hardwoods: Leaves, Stems, Roots, or Mycorrhizal Fungi?

Research Team: Dr. Mark Green (PI), Dr. Tom Horton (co-PI), Dr. Michele Pruyn (co-PI), Dr. Ruth Yanai (co-PI), Dr. Heidi Asbjornsen (Senior Personnel), Dr. Melany Fisk (Senior Personnel)

Summary

The overall goal of this project is to describe the effect of nutrient addition on transpiration in northern hardwoods and to gain insight into mechanisms responsible for transpiration changes, using a set of N, P, and Ca-treated plots in six stands in New Hampshire that differ in nutrient availability. We will determine whether plot-level water use – as indicated by sap flow, soil moisture, stomatal conductance, and water use efficiency – is enhanced by nutrient addition. We will explore a suite of possible explanatory variables, including root hydraulic conductivity, leaf area, stomatal density, mycorrhizal colonization of roots, and the composition of the ectomycorrhizal (EM) fungus community. We expect to find differences in the response of EM and arbuscular mycorrhizal (AM) tree species (beech, birch, maple, and cherry), based on differences in the mycorrhizal anatomy and hyphal networks. We will also test whether water use and plant and fungal responses are consistent with nutrient optimization theory. We will make use of seedling studies, in the field, in addition to working with trees, in order to observer whole-plant responses in a more controlled setting. It is not possible to conduct this research in the greenhouse because the majority of mycorrhizal taxa cannot be cultured.

Intellectual Merit

Current models of forest hydrology consider evapotranspiration to be a function of vegetation type, atmospheric evaporative demand, and water availability. The role of nutrient availability in determining plant water use has not been adequately explored, and our questions concerting the role of mycorrhizal fungi in this relationship are novel. Preliminary results are consistent with our hypothesis that water use by EM-associated tree species will be more sensitive to nutrient perturbations; the fungal mantle may interfere with water uptake, while AM roots can continue to take up water directly even with extensive colonization. Molecular genetic characterization of EM fungal communities will begin to identify guilds of fungi with different functions in water uptake; because of our comprehensive experimental design, we will also test for patterns in fungal communities as a function of N, P, and Ca availability.

Broader Impacts

The findings from this study will contribute to graduate and undergraduate student theses and a series of articles in open-access peer-reviewed journals. The PIs are committed to increasing participation in science by under-represented groups, and will thus recruit minorities for student positions. The work will also help build research capacity in an EPSCoR state and at a primarily undergraduate institution. Further, first-generation college students comprise 40% of the Plymouth State University undergraduate student population, thus this project is likely to engage individuals from this population. The experimental design allows us to make inferences beyond a single site, thus the work may have broad implications. We expect to demonstrate greater water use by trees following fertilization, which is novel and important to understanding the societal tradeoffs between forest biomass production and water use.

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I. Personnel

PI and co-PIs

Dr. Mark Green (PI); Assistant Professor of Hydrology; Plymouth State University
Green will lead the analysis of soil water dynamics and organization of the research team.

Dr. Tom Horton (co-PI); Associate Professor, Mycology; State University of New York - Environmental Science and Forestry
Horton will lead the data collection and analyses focused on mycorrhizal fungi.

Dr. Michele Pruyn (co-PI); Associate Professor, Plant Biology; Plymouth State University
Pruyn will lead the sap flow and root conductivity data collection and analysis.

Dr. Ruth Yanai (co-PI); Professor, Forest Ecology; State University of New York - Environmental Science and Forestry
Yanai will lead the nutrient uptake measurements and modeling and will serve as liaison to the project on multiple element limitation that maintains the nutrient treatments.

Collaborators

Dr. Heidi Asbjornsen (Senior Personnel); Associate Professor, Ecosystem Ecology; University of New Hampshire
Asbjornsen is collecting sapflow data at existing sites at Bartlett and Hubbard Brook, and will analyze isotopes in plant tissues to estimate water use efficiency measurements.

Dr. Melany Fisk (Senior Personnel); Associate Professor, Ecosystem Ecology; Miami University
Fisk will measure nutrient limitation with in-growth cores; she will also provide data on nutrient availability in the nutrient-amended plots.

II. Project

Preliminary Proposal: RUI: Causes of Nutrient-Enhanced Transpiration in Northern Hardwoods: Leaves, Stems, Roots, or Mycorrhizal Fungi?

1. Objectives

The goal of this project is to describe the effect of nutrient addition on transpiration in northern hardwoods and to gain insight into mechanisms responsible for transpiration changes, using a set of N, P, and Ca-treated plots in six stands in New Hampshire that differ in nutrient availability.

Objective 1: Characterize the above- and belowground controls on transpiration and water use. This includes particular emphasis on understanding the role of ectomycorrhizal (EM) and arbuscular (AM) mycorrhizal fungi.

Objective 2: Distinguish whether the observed responses are optimal for plant nutrition or whether they result from other effects of nutrients on above- or belowground processes.

Our questions are motivated by preliminary observations from one of our Ca-treated plots that show an overall increase in sap flow, but the responses differ by species (Fig. 1). For both objectives, we will use both AM- and EM-associated tree species, at spatial scales ranging from the ecosystem (e.g., LAI), trees (e.g., sap flow), and seedling (e.g., whole-plant response).

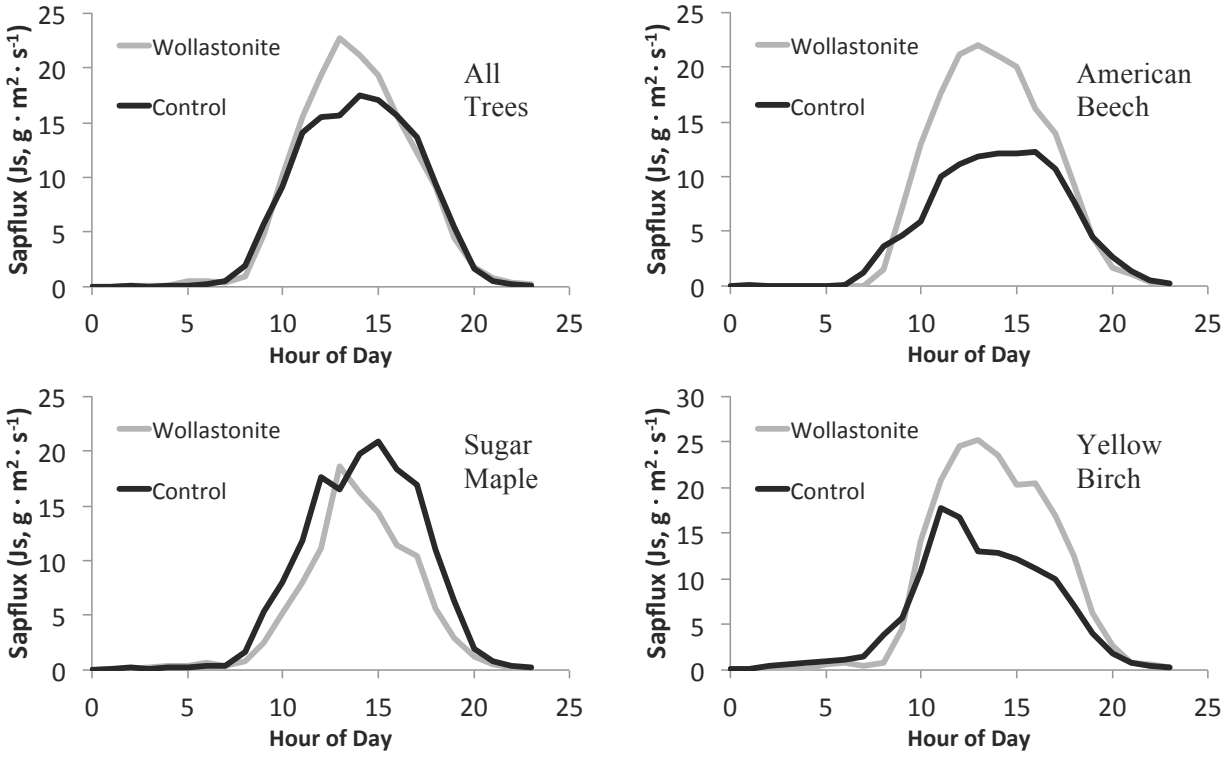

Figure 1. Median sap flow rates for nine trees (top left), and three trees of individual species in a plot amended with wollastonite ($CaSiO_3$) in 2011 compared to trees in a control plot. The data were collected over a seven day period in August 2012. Error bars are not shown due to substantial variation within individual species, however, there is no overlap of the inter-quartile ranges for all trees (top left) at the peak sap flow. Interestingly, sugar maple, an AM species, shows no increase in sap flow and yellow birch and American beech, EM species, show increased transpiration. We hypothesize (articulated further below) that the different water use responses are related to the difference in mycorrhizal groups.

2. Background

Transpiration constitutes a major water loss from forested ecosystems, and thus impacts the amount of surface water flowing from forests. Current models of forest hydrology consider evapotranspiration (ET) to be a function of vegetation type, atmospheric evaporative demand, and water availability. For example, the Budyko model uses energy and water limitation to explain differences in ET between different ecosystems; this model works reasonably well across many sites, but there remains substantial unexplained variance (Jones et al., 2012). Improved understanding of forest-water dynamics is vital as climate change and global population growth increases pressure on forest and water resources.

Nutrients are not considered in these ET models, even though multiple studies have observed changes to transpiration after fertilization. Increased leaf and sapwood area have been observed alongside increased sapflow and decreased soil moisture in response to a suite of nutrients added to coniferous stands (Phillips et al., 2001; Ewers et al., 2001). Similarly, a Eucalyptus forest showed increased sapflow, leaf area, and sapwood area, but no change in stomatal conductance after multi-element fertilization (Hubbard et al., 2004). Nitrogen fertilization of a pine plantation showed increased transpiration, leaf area, sapwood area, stomatal conductance, and specific leaf hydraulic conductance (Samuelson et al., 2008). A Ca and P fertilization experiment in Amazonia resulted in increased photosynthesis, stomatal conductance, and transpiration on Ca plots but not P plots (da Silva et al., 2008). At Hubbard Brook (New Hampshire), a whole-watershed $CaSiO_3$ addition resulted in unexpected reductions in runoff for three years, suggesting a 20 to 25% increase in transpiration (Green et al. 2013). Across all of these studies, there are diverse changes in tree functional characteristics that support increased transpiration. Further, experiments that test fertilization by individual elements in isolation are rare, thus it remains unclear whether this increase in water use is stimulated by only a limiting element, or whether fertilization with non-limiting elements can stimulate transpiration for reasons not associated with optimization theory.

Mycorrhizae are known to impact water and nutrient dynamics in trees. Mycorrhizal hyphae, like roots, have been observed to transport water from depth to surficial soil horizons during the night in other systems (Querejeta et al. 2001). One important paper showed increased number of hyphae and diameter of rhizomorphs was associated with decreased xylem water potential of seedlings (Lamhamedi et al. 1992). Nonetheless, there is very little information about the role of mycorrhizae in transpiration responses to fertilization. In our preliminary data (Fig. 1), we predict that the Ca addition chemically disturbed mycorrhizal fungi as has been shown with N additions for EM fungi (Peter et al. 2001; Lilleskov et al. 2011). This treatment reduces hyphal networks, which may impact water movement into the plants and subsequently transpiration. However, arbuscular mycorrhizal and ectomycorrhizal plants will respond differently based on their different mycorrhiza anatomy or root interaction. EM plants with low levels of colonization potentially move a substantial amount of water directly into the roots given the low number of mantles on the root tips. In the absence of drought or water stress, transpiration may be quite high following Ca treatment as water is flowing freely into the roots. As the EM fungal networks recover, colonizing the majority of root tips, transpiration slows because there is now a physical barrier (the fungal mantle composed of 5-10+ cell layers) to the flow of water into the majority of the EM root tips. At this point also, differences in water movement into the roots would occur as a function of fungal species.

The contrasting water use patterns in EM and AM tree species stands (Fig. 1) raises questions about the role of mycorrhizae. George et al. (1992) showed no differences in water depletion in a hyphal compartment whether plants were well watered, water-stressed or whether the hyphae were severed from the hosts. Kothari et al. (1990) found that mass flow of water through AM hyphae was negligible. In addition to the apparent lack of flow through hyphae, we suggest that unlike EM roots with well-developed fungal mantles, AM roots continue to take up water directly even with well colonized roots because the fungi do not form a mantle that inhibits flow of water into the root. We therefore predict that because water movement into the roots occurs with and without AM fungi, transpiration should not change in AM plants as AM fungal networks recover and colonization rates increase after fertilization.

3. Hypotheses

- Plot-level water use – as indicated by sapflow, soil moisture, stomatal conductance, and water use efficiency – will be higher in fertilized plots compared to controls.
- Root conductivity, LAI, and stomatal density will be higher in fertilized plots relative to controls.
- The type of fungi colonizing roots, AMF or EMF, is a primary determinant of the transpiration response to fertilization. Transpiration will increase in EM trees with the addition of N, P, and Ca.
- Other plant characteristics (e.g., stomatal density, root conductivity, LAI) determine water use.
- The composition of the EM community and the colonization rates of EM or AM fungi will change in response to all nutrient additions.
- Water use, leaf, stem, root, and mycorrhizal fungi measurements will show the greatest stimulation in the plot receiving a nutrient limiting to growth.

4. Research Approach

We will test our hypotheses by making measurements on an existing plot-scale fertilization experiment in the White Mountains of New Hampshire (DEB-0949324). The experiment involves three sites – Jeffers Brook, Hubbard Brook, and Bartlett Experimental Forest (~ 50 km apart) – of differing nutrient availability in soils (data not shown), thus we expect to have different degrees of nutrient limitation. We will study a mature (>100 yr post harvest) stand at each site, a mid-aged (~50 years old) stand at Bartlett and Jeffers Brook, and a young (~25 years old) stand at Bartlett. Each stand has four 50 x 50 m plots: a control and plots treated with N (30 kg N/ha/yr as NH_4NO_3), P (10 kg P/ha/yr as NaH_2PO_4), and Ca (1150 kg Ca/ha in the form of $CaSiO_3$). The form of nutrient addition is important: our P source does not contain Ca, unlike most P fertilizers, and the Ca source does not contain carbonate (which incurs a pH treatment) as is typical of Ca treatments. N and P have been added annually since spring 2011; Ca was applied as a one-time addition in October 2011. We will use trees and plant seedlings in the central 30 x 30 m area at the center of the treatment plots.

We will measure water use, canopy and rhizosphere characteristics, and nutrient limitation of each of two EMF tree species and two AMF tree species - sugar maple, American beech, pin cherry, and yellow birch - in the nutrient treatment and control plots. Three trees of each species will be selected for study in each plot. In addition, measuring similar dynamics in newly established seedlings will provide a more controlled test of the fertilization impacts. For the seedling experiment, seedling bags 15 x 15 x 30 cm containing autoclaved quartz sand will be prepared from mesh screens with a pore size of 45 μm, which allows hyphal but not root ingrowth (Wallander et al. 2001). Seeds of sugar maple and American beech will be surface sterilized and germination will be assessed to identify the number of seeds required per bag. The bags will be installed vertically in slits in the ground in the spring in all the treatment plots. After germination, the seedlings will be thinned to one per bag. Seedlings will be harvested in the fall of year 3 for measurements of root, mycorrhizal colonization, and EM community when present.

The response variables for water use in trees will be sap flow, soil moisture, stomatal conductance, and water use efficiency. Sap flow in the trees will be measured using a thermocouple and a constant heat source (Granier et al. 1996). Soil moisture and tension will be measured in three locations and at three depths in each plot using electrical capacitance moisture probes and automated tensiometers (Decagon, Inc.). Water use efficiency will be estimated using $\delta^{13}C$ as a robust measure of the time-integrated C_i/C_a (the ratio of intercellular (C_i) to atmospheric (C_a) CO_2 mole fractions), referred to as intrinsic WUE (iWUE, Ehleringer and Cerling 1995). Tree cores will be collected and analyzed for stem increment growth (Grissino-Mayer 2001) for the 10 years pre-treatment and all post-treatment years. Cellulose will be extracted from bulk wood for each ring-year (Brendel et al. 2000) and analyzed for $\delta^{13}C$ and $\delta^{18}O$ on an isotope ratio mass spectrometer interfaced with an elemental analyzer at the Stable Isotope Laboratory at UNH. The $\delta^{18}O$ data will enable us to ascertain whether differences in iWUE are due primarily to variation in photosynthetic capacity or stomatal conductance (Scheidegger et al. 2000, Grams et al. 2007). $\delta^{15}N$ will be analyzed on bulk wood samples on the same instrument to provide an additional measure of plant nutrient status in response to the treatments (Guerrieri et al. 2011). Stomatal conductance of leaves sampled with a shotgun will be measured with a LiCOR chamber system.

Leaf area index (LAI) of the canopy will be monitored with a LiCOR-2000. Sapwood depth will be estimated from the cores collected for WUE. Density of leaf stomata will be measured from their impressions in dried nail polish, mounted on microscope slides. Canopy branches from trees will be collected via shotgun, and stem sections from those branches along with roots from the same trees will be measured for hydraulic conductivity (Ks) in the lab. To obtain Ks, water is applied to one end of a 1-2 cm stem at constant hydraulic pressure head and the rate of flow through the stem sample measured.

For seedlings, a similar suite of measurements will be made. Water use will be assessed directly via sapflow collars (Baker and Van Bavel, 1987), stomatal conductance measurements, and water use efficiency will be estimated by analyzing foliar tissue for $\delta^{13}C$ and $\delta^{18}O$ isotopes. LAI for seedlings will be estimated by collecting fallen leaves and scanning them with a flatbed scanner. Stomata density will be analyzed via the methods described above on the scanned leaves. Stem conductivity will be measured (via the same methods as for roots and branches) at the end of year 3 when seedlings are harvested.

We will measure root conductivity, root abundance, AM and EM colonization of roots and EM community composition from roots in the in-growth cores with roots and seedlings. Mycorrhizal colonization will be measured for EM (beech and yellow birch) and AM (sugar maple and pin cherry) species from trees and seedlings following Brundrett et al. (1996). We will examine cross-sections of representative EM root tips to record the presence of a mantle and Hartig net, the key structural features of the EM symbiosis (Allen 1991; Tedersoo et al. 2010). We will identify fungal symbionts using BLAST search of the internal transcribed spacer (ITS) region (the official fungal barcode; Horton and Bruns 2001; Schoch et al. 2012). Plant hosts identity will be confirmed using a combination of the official plant barcode loci, maturase K (matK) and ribulose-1,5-bisphosphate carboxylase (rbcL). We will use PCR-based methods to identify the EM fungi on beech and birch trees and seedlings. The fungal rDNA ITS region will be amplified and sequenced and BLAST searched in Genbank (Horton and Bruns 2001, Horton 2002). We will ingrowth core bags with 45 μm mesh screens to harvest mycorrhizal hyphae in the soil in the different nutrient addition plots adjacent control plots. These should be mycorrhizal hyphae because saprotrophic fungi are carbon limited (Wallander et al. 2001). Fungal hyphae will be extracted from the sand, dried, and weighed.

Fertilized ingrowth cores without mesh will be used to test for nutrient limitations in control, Ca, N, and P amendments in all plots (Naples and Fisk, 2010). Nutrient uptake capacity can also be used to indicate nutrient limitation (Harrison 1995). We will measure nutrient uptake capacity of fine roots in the final year of the project, using intact root branches in the field (Lucash et al. 2005, 2007). We have used this method in mixed species forests by tracing each root back to a tree or to a root coarse enough to have distinguishing bark characteristics. We will assess nitrate, ammonium, phosphate and calcium uptake capacity of the three dominant species in each stand to indicate the relative limitation of nutrients in each of the treatment plots. These observations will provide the parameters necessary to model nutrient uptake at the root surface (Yanai 1994), allowing us to describe the degree to which increased transpiration increases nutrient uptake (Williams and Yanai, 1996).

5. Broader Impacts

The findings from this study will contribute to graduate and undergraduate student theses and a series of articles in open-access peer-reviewed journals. The PIs are committed to increasing participation in science by under-represented groups, and will thus recruit minorities for student positions. The work will also help build research capacity in an EPSCoR state and at a primarily undergraduate institution. Further, first-generation college students comprise 40% of the Plymouth State University undergraduate student population, thus this project is likely to engage individuals from this population. The experimental design allows us to make inferences beyond a single site, thus the work may have broad implications. We expect to demonstrate greater water use by trees following fertilization, which is novel and important to understanding the societal tradeoffs between forest biomass production and water use.

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