**Proposal for Research: Root Nutrient Uptake Capacity**

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**Introduction**

Nutrient uptake kinetics at the root surface is a common means for quantifying direct nutrient inputs into an ecosystem. Modelling nutrient uptake utilizes direct morphological measurements of the associated root systems, and a steady state model of nutrient uptake to quantify nutrient inputs (Yanai 1994). However, tree species are known to exhibit differential nutrient uptake rates based on nutritional status of the individual and nutrient availability on site (Chapin et al. 1986). Mycorrhizal symbionts are the chief drivers of nutrient acquisition for terrestrial plants and receive over 70% of vegetative carbon allocation, but are excluded from most nutrient budgets and many root  uptake models (Smith and Read 1997). Current study lacks a comprehensive insight into the relative roles of nutrient availability and mycorrhizal community composition on uptake rates at a fine scale.

Study has shown the relative impacts of nutrient limitation (mainly nitrogen or phosphorus) on uptake rates, but few studies to date have directly addressed the idea of co-limitation by two or more nutrients. Recent metadata analysis by *Elsar et al. 2007*, suggests that terrestrial and aquatic ecosystems are most often co-limited by nitrogen and phosphorus. Of these studies, only four were conducted in the northeastern United States, none of which included northern hardwood forests. *Fisk et al 2014*, found a synergistic response in resin available nitrogen in a nitrogen and phosphorus treatment plot compared to just a nitrogen treatment, suggesting nutrient co-limitation. Historically, temperate forests have been thought to be nitrogen limited, while tropical forests are thought to be phosphorus limited. However, certain factors, such as repeated forest harvest and base cation depletion due to acid rain deposition, may lead to the gradual shift of temperate forests to a phosphorus limited state. Study by *Fahey et al. 1998*, has shown that early successional stands tend to be phosphorus limited, while mature stands tend towards nitrogen limitation. Synchronization of these cycles is gradually attained over time and critically important in maintaining long term sustainability of nutrients in forests. The role of nutrient limitation and co-limitation in fine scale root nutrient uptake kinetics is of critical importance as our forests change in response to broad scale global factors. Importantly, the resiliency of certain species to acquire and maintain nutrients under variable concentrations and how mycorrhizal associations impact those uptake rates will be examined by this research.

Utilizing study sites in the Bartlett Experimental Forest in Bartlett, NH, which have been maintained annually under the MELNHE project since 2011 with fertilizer application treatments of nitrogen, phosphorus, and NP, will provide a unique insight into the relative roles of nutrient co-limitation in root nutrient uptake capacity. Roots less than 2mm in diameter from two early successional species, Yellow Birch (*Betula alleghaniensis*), predominantly colonized by ectomycorrhizae, and Pin cherry (*Prunus penssylvanica*), predominantly colonized by arbuscular myccorrhizae, will be excavated and analyzed utilizing the depletion method (Lucash et al. 2005,2007). Varying concentrations of nutrient solution will be applied to roots of each tree to ensure adequate concentration for marked changes in uptake capacity. Roots utilized in nutrient uptake experiments will be excised and analyzed for root length and diameter, as well as mycorrhizal communities. Colonization of mycorrhizae, AM vs EM, will be quantified for a subsample of the roots utilized in the uptake experiment for a given root length and AM vs EM root tips will be quantified. EM species composition will be analyzed utilizing DNA extraction from EM root tips to provide relative species distribution and abundance between different individuals and sites.

*Objectives*

* Investigate the response of nutrient uptake capacities of fine roots between tree species (*Betula alleghaniensis* and *Prunus penssylvanica*)  to various nutrient treatments (N, P, NP, and control).
* Asses the role of nitrogen and phosphorus limitation on root nutrient uptake capacity.
* Asses the role of co-limitation of nitrogen and phosphorus on root nutrient uptake capacity.
* Assess the impact of AM vs EM colonization on nutrient uptake rates. Asses the impact of EM species diversity and abundance on nutrient uptake rates.
* Address how the mycorrhizal composition impacts nutrient uptake capacity across a range of treatments.

*Hypotheses*

* Root nutrient uptake capacity will be greater for nitrogen in areas which are nitrogen limited and phosphorus in areas which are phosphorus limited.
* Root nutrient uptake capacity with treatments of both nitrogen and phosphorus will exhibit greater cumulative effects on uptake compared to treatments of nitrogen or phosphorus alone. Nitrogen and phosphorus treatments will stimulate a greater nitrogen uptake response in roots (building off of previous MELNHE work by Fisk et al. 2014).
* Mycorrhizal abundance (AM vs EM) will remain the same or decrease in response to nutrient treatments compared to control plots. AM species will proliferate under phosphorus limiting conditions, EM species will proliferate under nitrogen limiting conditions.
* Ectomycorrhizal species composition and abundance will be less in nutrient treated plots compared to control plots.
* Mycorrhizal colonization (AM vs EM) and EM species composition/abundance will provide unique insight into the roles of these fungi in affecting nutrient uptake capacity under nutrient treated conditions.

**Methods**

Root nutrient uptake capacity measurements will take place at the Bartlett Experimental Forest in Bartlett,NH. Samples will be taken from two early successional species,  Pin Cherry (*Prunus penssylvanica*) and Yellow Birch (*Betuala alleghaniensis*), at three early successional (aged 25 - 35 yrs) stands of northern hardwood forest with similar site characteristics. Young stands have been chosen for this study due to their high relative nutrient uptake rates, and to determine the relative effectiveness of sampling methods. If consistent results can be attained utilizing proposed methodology, study may be expanded in future years to include middle aged, and mature forest stands. Each stand consists of four treatment plots, which are 50m x 50m plots, and have been maintained with annual fertilizer treatments of nitrogen (30 kg N/ha/yr as NH4NO3) and phosphorus (10 kg P/ha/yr as NaH2PO4), since 2011; treatment plots consist of: N, P, NP, and a control. The treatment plots are subdivided into a 30m x 30m inner plots with a surrounding 10 m buffer zone, all of which are manually fertilized each year. Measurements will take place inside the buffer zones of all four treatment plots (N, P, NP, control). Three subplots in each buffer zone will be chosen based upon presence of individuals of the two species, as well as a desired distance between trees, and dbh class (*to be determined*).

Nutrient uptake capacity will be measured via the depletion method as described by Lucash et al. 2005, 2007. This method consists of excavating fine roots from the desired tree species, and identifying them by following the main root back to the host tree, or to a coarse root with distinguishable bark characteristics. Those roots will be left attached to the host tree, while being extracted from the organic and upper mineral soil horizons. Roots less than 2mm in diameter will be washed in distilled water, and placed in 27 mL of nutrient solution, in a 50 mL centrifuge tube. Tubes will be sealed over with parafilm, covered with tarps to prevent evaporation, and aerated with oxygen, via tubing and fish pumps, to prevent formation of depletion zones. In order to ensure nutrient concentrations are available enough for measurable uptake, solutions consisting of 1x, 5x, and 10x nutrient solution concentration will be utilized for each subplot.  An aerated, control nutrient solution consisting of no roots will be utilized for comparative purposes. Solutions will be subsampled after a two hour period, this should provide sufficient time for roots to recover from the shock of excavation and provide more reliable estimates of nutrient uptake capacity (McFarlane and Yanai 2005). Samples will be immediately filtered utilizing a 0.4 micron polycarbonate filter, kept on ice in the field, and frozen until processing. Solutions will be analyzed for ammonium utilizing a continuous flow analyzer; nitrate and phosphate utilizing ion chromatography, and calcium, magnesium, potassium, and sodium will be analyzed utilizing ICP spectroscopy. Data collected will be analyzed using a  two-way ANOVA with fertilizer treatments (four levels) and tree species (two levels) as two factors.

Following the nutrient uptake experiment in  the field, roots utilized for uptake will be excised and placed in CTAB (cetyltrimetylammonium bromide) solution for preservation for DNA extraction. These roots will first be analyzed for root length and diameter by scanning the roots into identification software (*to be determined*). Following this, mycorrhizal analysis will be performed by two different methods to identify arbuscular and ectomycorrhizal colonization rates, as well as the species composition of the ectomycorrhizal communities. Under 50x magnification, colonization of AM vs EM along a given root length, as well as AM vs EM root tips can be readily quantified. A subsample of the EM root tips will be utilized in DNA extraction for community analysis. DNA will be extratced from EM root tips, amplifying the ITS region of the nuclear rDNA genes, constructing clone libraries, and matching clone sequences to available databases. Data collected will be analyzed using a  two-way ANOVA with fertilizer treatments (four levels) and tree species (two levels) as two factors.

**Expected Results**

Following up on previous results from nutrient uptake capacity experiments, expected results will vary based upon a few determinant factors, including current nutritional status of the individual trees, as well as the nutrient concentrations in solution. We expect to see greater net uptake of nutrients in fine roots where those nutrients are limiting. In a nitrogen limited system (such as a P treatment plot), nitrogen uptake capacity will be greater than an area where nitrogen is abundant ( such as a N treatment plot). Likewise, the same will be true for phosphorus limited sites, greater uptake capacity of phosphorus in P-limited sites (N treatment plots) and vice versa. Nutrient uptake capacity in treatment plots containing both nitrogen and phosphorus can be expected to behave synergistically, exhibiting greater impacts on nutrient uptake capacity than in sites which are limited by either nitrogen or phosphorus.

Mycorrhizal analyses can be expected to reveal similar fluxes in species composition and species abundance as a function of nutrient limitation. Community composition under nitrogen and phosphorus limited conditions will vary, and it is commonly known that species diversity diminishes with increasing nutrient concentrations. EM species diversity is expected to be higher in the control plot than the N and P plots. Few studies have assessed the role of both N and P together on species composition. Results from colonization analysis can be expected to depict less arbuscular mycorrhizae in phosphorus limited plots and more ectomycorrhizae in nitrogen limited plots. EM species composition and diversity as well as Am vs EM colonization are expected to provide unique insights into differential nutrient uptake capacities between tree species and treatments. A window into the relative functioning of mycorrhizal communities in response to variable treatments and provided nutrient concentrations will prove valuable in nutrient budgeting and modelling, as well as the potential for carbon sequestration.

**Future Study**

Commonly, there are a few issues with the depletion method for measuring root nutrient uptake capacity. Importantly, previous study by *Lucash et al. 2005* has documented net efflux of certain important nutrients during uptake measurements utilizing the depletion method (ammonium and potassium), which does not accurately depict nutrient uptake by roots in undisturbed systems. Disturbance associated with excavating the root systems sever extramatrical hyphae of associated mycorrhizae which are utilized in nutrient uptake. This leads to efflux of certain nutrients from roots associated with those mycorrhizae. One way to work around this issue is to transplant, or train, roots to grow into 4 micron mesh bags, which are filled with a mixture of sand and soil (for ease of extraction), allowing extramatrical hyphae to grow into these bags while excluding other roots. This will allow the root time to recover following excavation, as well as enable hyphae to regrow into the bag for more accurate readings of nutrient uptake capacity in the future. Similar studies can be imagined utilizing seedlings planted in ingrowth cores or greenhouse studies and are of a strong interest to expanding upon this work. Future comparison of nutrient uptake capacities obtained via the depletion method and mesh bag/ingrowth core method with existing nutrient budgets will provide unique insight into the practicality of budgeted versus measuring rates of nutrient uptake on the scale of fine roots.

One way to measure the seasonal variation of nutrient uptake capacity is to repeat uptake experiments in the fall when aboveground productivity has diminished and greater carbon has been allocated underground for nutrient uptake. This would also provide a stark contrast in mycorrhizal abundance as well as community composition, as mycorrhizal activity is highest during the fall.

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