Sustainable nutrient supply following forest harvest: Characterizing the fungal link from soils to roots

Ruth Yanai
Professor
Forest and Natural Resources Management

Tom Horton
Associate Professor
Environmental and Forest Biology

Budget and Duration: $54,105, 2 years
Proposal Status: This is a first submission.

Project Summary

To develop guidelines for sustainable rates of forest harvest requires understanding the degree to which the supply of nutrients for forest growth can be accelerated by plant allocation to mycorrhizal symbionts. To date, scientific research on ectomycorrhizal fungi (EMF) has focused primarily on nitrogen acquisition, while research on arbuscular mycorrhizal fungi (AMF) has focused on phosphorus acquisition. Few studies have compared rates of colonization of EMF and AMF, although they commonly co-occur in mixed-species forests. Fewer still have studied fungi at the soil depths relevant to mineral weathering, which is clearly important for nutrients other than N.

This study will take the first steps to identify which mycorrhizal fungi are involved in acquisition of specific nutrients. We will assess EMF and AMF colonization in twelve northern hardwood stands at three contrasting sites and identify EMF by molecular genetic techniques. We will analyze roots collected at depth in the soil (30-50 cm) as well as from the forest floor (Oea) and surface (0-5 cm) mineral soils. We will relate the occurrence of EMF types (short- and long-range foragers) and EMF:AMF ratios to soil N, P, and Ca availability, among other factors, such as stand age and species composition. These relationships will be further tested by repeating the sampling and analysis of AMF and EMF after two growing seasons of regular additions of N, P, and Ca to the study plots.

The project takes advantage of a large long-term field study, and thus contributes to the infrastructure for future research and education at these sites. We will train students, produce journal articles, and lay the groundwork for future investigations into the role of AMF and EMF in nutrient cycling in forests. The role of mycorrhizal fungi in obtaining nutrients from soil is key to evaluating the sustainability of forest production for biomass energy and the future of forest nutrient limitation given harvest removals of nutrients, continued N deposition, and Ca loss with soil acidification.

Justification

The increasing demand for renewable energy poses a challenge to the sustainable production of forest biomass. More intensive forest utilization, for example by whole-tree harvest or shorter rotations, yields more biomass but also increases the demand on soil nutrient supply, compared to bole-only harvest for timber products, because of the higher nutrient content of branches and small stems compared to merchantable boles (Yanai 1998).

Nitrogen deposition in the northeastern US is adequate to resupply forest growth, which means that some other nutrient will likely limit sustainable forest production. The nutrient predicted to be depleted first by repeated forest harvest is calcium (Federer et al. 1989). However, recent studies of ecosystem Ca cycling show that young stands have greater accumulation of Ca in living biomass and the forest floor, greater indication of Ca availability in snail shells and leaf litter, and greater export of Ca in streamwater, suggesting biotic control over Ca supply (Hamburg et al. 2003, Yanai et al. 2005).

One possible explanation for greater Ca supply from mineral soils in young stands is the weathering of apatite deep in the soil by mycorrhizal fungi (Blum et al. 2002), driven by demand for phosphorus. Apatite is the primary mineral source of P (Nezat et al. 2008), and both ecosystem modeling and measurements of foliar retranslocation and root foraging (Naples and Fisk 2010) suggest that young stands are more P-limited and mature stands are more N-limited. If forests can influence the rate of nutrient supply through allocation of carbon to mycorrhizal symbionts at depth in the soil, we may have been understimating sustainable rates of woody biomass removals.

Mycorrhizal fungi can increase the soil volume explored by roots and provide access to forms of N (Schimel and Bennett 2004), and P and Ca (Blum et al. 2004) not otherwise available to plants. The morphology of mycorrhizal root tips ranges from a virtually naked feeder root with internal hyphae and few external hyphae (arbuscular mycorrhizal fungi, AMF) to some ectomycorrhizal fungi (EMF) with copious hyphae extending meters from the root tip. Within the EMF, there are important

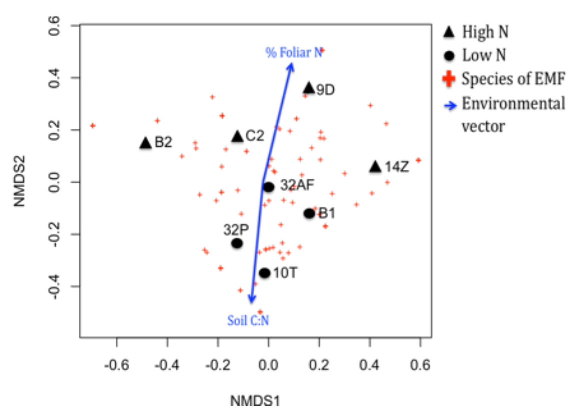

Figure 1. Soil C:N (P=0.04) and % Foliar N (P=0.07) were significant explanatory factors in Nonmetric Multidimensional Scaling (NMDS) analysis of EMF species recovered from root tips and soil at Bartlett (Vineis and Horton, unpublished data).

differences in the roles of taxa in response to N availability (Peter et al. 2001, Lilleskov et al. 2002, Lilleskov et al. in press; Figure 1). The proposed research will extend this analysis to P and Ca, which are clearly important to nutrient supply following forest harvest, and also to deep soils where mineral weathering is most important; these topics have not previously been studied.

Previous Work, Present Outlook, and Future Prospects

An earlier NSF award to Yanai established plots in replicate young and mature stands of northern hardwoods at the Bartlett Experimental Forest, New Hampshire, to explore nutrient cycling in response to prior clearcutting treatments. A recently funded renewal (PI Yanai) will add N, P, and Ca to these plots to determine the response of forest productivity to added nutrients, including root production. However, it will not address the extent of mycorrhizal colonization of roots nor the composition of the fungal community on those roots. Another NSF-funded project (PI Horton) identified a functional group of EMF in plots with low N availability at Bartlett (Figure 1), presumably mobilizing organic N for the trees. We suspect that some of the EMF species that did not respond to the N availability gradient may play roles in the cycling of other nutrients, such as P and Ca.

The goal of the present proposal is to characterize the mycorrhizal fungi on roots in surface soils and at depth at Bartlett (young and mature stands) and in two additional sites that differ in fertility: Jeffers Brook (most fertile) and Hubbard Brook (intermediate). We will interpret differences associated with site, stand age, and soil depth in light of differences in nutrient availability measured in the soils. However, these relationships will be correlational in nature. To better address causal relationships, we will resample roots in the second growing season after the onset of nutrient additions (N, P, and Ca) and test whether the relationships follow those predicted by the pre-treatment patterns.

The proposed project will contribute to future prospects of funding from NSF and other sources (e.g. USDA NIFA, NSRC, DOE). We intend to collaborate with researchers who are developing approaches to AMF; we will preserve AMF root tips from our samples, although identifying AMF species is beyond the scope of the current study. Similarly, samples of the soil collected with our roots have been preserved for analysis of soil fungi by Melany Fisk at Miami University. The results of our study, combined with preliminary results obtained by other researchers on related samples from our sites, will allow us to pose new questions in the next cycle of research funding, as described below under Future Funding Plans.

Objectives and Hypothesis

Objective I: Determine the rate of colonization of roots by AMF and EMF

We will use roots collected from three sites with mixtures of AM and EM trees. The three sites differ in fertility, and there are young and mature stands at each site. We will classify the root length colonized by AMF and EMF in quantitative samples collected from the forest floor, surface mineral soil, and 30-50 cm deep in the mineral soil, and compare these to the ratio of AM to EM trees in the plots.

Site Fertility Hypothesis: We predict that more fertile sites will have greater AMF:EMF, because the trees have less need to invest in fungi that access nutrients at large distances. Within the EMF, fertile sites will have lower abundance of morphotypes with extensive hyphal systems.

Stand Age Hypothesis: We predict that roots in young stands will have greater AMF:EMF due to greater allocation of effort to P acquisition and the putative role of AMF in P uptake. In mature stands, which are thought to be more N-limited, we predict a lower AMF:EMF due to the role of EMF in N acquisition.

Soil Depth Hypothesis: We predict that roots in surface soils have lower AMF:EMF than roots at depth, because of the greater importance of N acquisition in surface soils and P and Ca acquisition at depth.

Objective II: Identify the ectomycorrhizal fungal communities

It is now routine to identify EMF from root and even soil samples (Horton and Bruns 2001, Vineis and Horton, in preparation). However, this is not possible for AMF. We will therefore focus our efforts for Objective II on the EMF communities during the current funding cycle.

Site Fertility Hypothesis: We expect to observe more *Russula, Lactarius* and *Tomentella* in fertile sites, which produce few hyphae for nutrient acquisition at a distance. In less fertile sites, we expect to find more genera in *Cortinairus, Piloderma,* and hydnacious fungi such as *Hydnellum* and *Bankera* because of their putative role in mobilizing organic N.

Stand Age Hypothesis: We predict that roots in young stands will have a different assemblage of EMF species than those in older stands, driven by successional patterns in the fungi, with pioneer fungi dominating early on (*Laccaria, Inocybe, Thelephora, Amanita)* and competitive species dominating later (*Russula, Cortinarius, Tricholoma, Boletus).* We will also test for relationships with N, P and Ca availability across sites and stands, as indicated by analyses of soil nutrients.

Soil Depth Hypothesis: We predict that species richness will increase with soil depth. We will also test for patterns relating fungal community composition to patterns in soil chemistry.

Objective III: Verify Causal Relationships using Nutrient Manipulations

The hypotheses described above depend on naturally occurring differences in nutrient availability and those induced by forest harvest. Nutrient manipulations in our study sites will begin in 2011. By sampling again after the second growing season post-treatment, we will have the opportunity to test whether the correlations reported under Objective I describe causal relationships.

Null Hypotheses: We expect to demonstrate that the patterns observed in root colonization rates by AMF and EMF and the community composition of EMF are explained by differences in nutrient availability, consistent with patterns in nutrient availability associated with our contrasting sites, stand ages, and soil depths.

Alternate Hypotheses: Alternatively, patterns of mycorrhizal colonization and community composition may be due to factors other than the availability of soil nutrients we are testing. We will test for patterns associated with site, stand age, and soil depth. For example, fungal succession has been associated with tree age (Last et al. 1987), possibly due to differences in carbon supply.

Procedures

Field Study Design and Collection of Roots and Soil

This study will take advantage of an extensive system of study plots established in the White Mountains of New Hampshire from 2003 to 2009 for study of Ca cycling (at Bartlett) and N and P co-limitation (including Jeffers Brook and Hubbard Brook as well as Bartlett). The three sites differ in fertility, with Jeffers Brook being highest in N, P, and Ca availability, Hubbard Brook intermediate, and Bartlett the lowest. At each site, we have plots in young (30-35 yr) and mature (>100 yr post harvest) stands. At Bartlett, there are three replicates of each age, including stands of younger age (20-25 years). There are four 50 x 50 m plots at each site, which will be treated four times during the growing season with N as NH_4NO_3 (30 kg N/ha/yr), P as NaH_2PO_4 (10 kg/ha/yr), N&P together, or control, beginning in spring 2011. At five of the stands, including the two at Jeffers Brook, there is a fifth plot that will receive a Ca addition (3500 kg/ha of wollastonite, $CaSi\ O_3$, as a one-time addition, to match a treatment applied at Hubbard Brook to an entire watershed (Peters et al. 2004).

The sampling design for studying mycorrhizal fungi involves a high density of measurements in young and mature stands at the three sites, a lower sampling intensity at six additional sites at Bartlett (two in each of the three age classes), and a comparison of deep roots collected at the Bartlett sites. At all the sites, roots were collected from surface soils using a 5-cm PVC core to a depth of 10 cm. Samples were divided into organic and mineral horizons. At the Bartlett stands, samples were also collected at a depth of 30-50 cm in the mineral soil: five samples per plot were collected in the intensively sampled stands, and two per plot in the six additional stands. These samples were collected for the purpose of soil characterization, but they are perfect for our use as well. The surface soil samples were collected from locations paired with the deep samples in all cases (2 surface cores for each deep sample). All the samples have been frozen and are available to us for analysis.

The same procedure will be repeated in fall 2012, two growing seasons after the nutrient treatments begin. This is enough time for the fine roots to have been replaced, such that the new roots and their mycorrhizal symbionts reflect the altered nutrient regimes. The use of pre-treatment observations improves our power to detect treatment effects; we expect to find high spatial variation within and across plots, and spatially paired samples will allow us to use a repeated measures analysis for both depth effects and treatment effects over time.

Methods to determine the rate of colonization of roots by AMF and EMF

In the laboratory, roots will be thawed, washed, sorted by size (we will use roots <0.5 mm in diameter, including "beaded" mycorrhizae) and preserved in ethanol. AM and two classes of EM root length will be quantified under a dissecting microscope using a line-intercept approach (Newman 1966, Tennant 1975). Identification will be confirmed, if necessary, by clearing, staining, and viewing the arbuscules (AMF) or hartig net (EMF) under a compound microscope (Brundrett et al. 1996). EMF morphological types (morphotypes) will be classified in two groups: those with extensive or limited exploratory hyphae (*sensu* Agerer 2001). For each sample, colonization rates of AM roots will be reported as the frequency of hyphae, coils, and vesicles observed on a subsample of roots cleared and stained to view fungal tissue. For EMF, we will report the number of tips per unit length on subsamples of each morphotype. Because the roots were quantitatively sampled, by taking cores of known diameter and soil depth, results can be reported in extensive units of length per unit ground area, as well as intensive units such as AMF and EMF colonization rates, AMF:EMF root length, and the ratio of EMF exploration types.

Methods to identify the ectomycorrhizal fungal communities

As root systems are cleaned and analyzed, EM root tips for each morphology in each soil sample will be harvested to identify the fungi using molecular bar coding with the fungal nuclear rRNA gene repeat (Horton and Bruns 2001).

EM root tips will be sorted into morphotypes following Agerer (1987 - 2002). Small soil volumes such as those used here are usually dominated by a single fungal species (Horton and Bruns 2001). To sample the dominant fungal types, we will analyze one root tip per soil sample (Gehring et al. 1998). Five EM root tips from each soil sample will be randomly subsampled, stored, and sequentially selected and genetically typed until a sample with high quality DNA is obtained. Typically, 70% of the root tips in field study will yield high quality DNA (Horton, unpublished data). DNA extractions, polymerase chain reaction (PCR) amplifications, and restriction length polymorphism (RFLP) generation will follow White et al. (1990) and Gardes and Bruns (1993, 1996). A unique RFLP type (composite of *Hinf*I and *Dpn*II enzymes) will be considered equivalent to a species (Kårén et al. 1997, Horton 2002). The ITS region of the 100 most abundant genetic types recovered in the study will be identified by querying the Genbank database for similar sequences (Altschul et al., 1997). All sequences will be deposited into the Genbank database for immediate public access.

Data Analysis

Objective I: We will test our hypotheses concerning the colonization of roots by AMF and EMF using analysis of variance (ANOVA) and related statistical models. Dependent variables will be the length of AMF, EMF, and each type of EMF; ratio of AMF:EMF root length; the ratio of EMF morphotypes, and the rates of colonization of AMF and EMF. Independent variables include site, age, soil depth, and soil factors (Ca and other exchangeable cations, available P, and various N fractions). Tree AM:EM ratio (based on basal area at the plot level) can be used as an independent variable or as a correction factor on the dependent variable AMF:EMF.

Objective II: Species richness of EMF will be analyzed as a function of site, age, and soil depth using ANOVA; we can also test whether soil factors explain these relationships. We will use phylogenetic clustering with Picante (R statistical package) to explore the role of ecological filtering in structuring the observed fungal assemblages of species. We will use Nonmetric Multidimensional Scaling (NMDS) and regression analyses to explore relationships between soil chemistry and the EMF communities.

Objective III: Repeated measures analysis will be used to test whether changes following nutrient treatments are explained by soil nutrient factors, as for Objective I. We will also test for patterns associated with site, stand age, and soil depth, which may not be due to soil nutrients.

Relationship to the McIntire-Stennis Program and Other Broader Impacts

Forest management has been a primary focus of the McIntire-Stennis Program since its inception. Clearly, we need to quantify the degree to which the supply of nutrients for forest production can be accelerated by plant allocation to mycorrhizal symbionts, if we are to develop meaningful guidelines for sustainable harvest removal to support the growing demand for biomass energy.

Few studies have documented the presence of EMF in deep soils (Horton 1997); none have been systematic or quantitative. The results of this study will be publishable in high-impact, peer-reviewed journals, and they will likely have influence on other researchers in the field even before they are published, through our informal networks and presentations at meetings. The project will thus advance discovery and understanding, in addition to training graduate and undergraduate students. The students who are interested in working on this project and who have participated in sample collection and methods development are both women from Asia, which promotes diversity and has already improved awareness of cultural issues within our working group. Finally, investments in the long-term nutrient manipulation experiment enhances the infrastructure for future research and education, and the addition of Horton to the Hubbard Brook Committee of Scientists is an addition to intellectual infrastructure that we hope will persist beyond this project.

Probable Duration and Timetable

2010 (pre-award): Samples were collected at depth by power coring at Bartlett in summer 2010 and frozen. Surface soils were collected in fall 2010 and the roots were frozen, after removing sieved soil samples for analysis of soil fungi (not covered in this grant proposal). Additional samples are being collected now at Jeffers Brook and Hubbard Brook. The timing of these pre-award activities is crucial to the success of the project, because N, P, and Ca additions will begin in spring 2011, and thus fall 2010 was the best time to collect samples unaffected by the experimental nutrient manipulations.

2011-2012 (Year 1): Pre-treatment samples will be processed and analyzed for root length colonized by EMF and AMF. EMF samples will be identified genetically. Results will be presented at meetings and prepared for publication, and hypotheses regarding the relationships of EMF types to soil conditions will be refined for further testing under nutrient manipulations. Soil fungi and AMF will be identified by collaborators at other institutions, not covered by this funding.

Fall 2012: At the end of the second growing season following the onset of nutrient additions in May 2011, we will again collect samples by the same methods used in 2010.

2012-2013 (Year 2): Post-treatment samples will be processed and analyzed by the same methods as in Year 1. In each stand, samples from plots assigned to control, N, P, N&P, or Ca treatments will be compared to pre-treatment samples from the same plots. Results will be presented at meetings and prepared for publication, and grant proposals will be prepared in collaboration with other investigators.

Budget Justification

Salary is budgeted for one graduate student, Kikang Bae, who has already been involved in sample collection and sample preparation for this study. Lin Liu, an undergraduate student, is involved in the project for Independent Study, not requiring compensation (she is not eligible to work in the US).

Travel expenses are for field work and professional meetings. We budgeted for two trips per year to Bartlett for 3 people, including 3 nights of housing at $30/night/person and $200 for car rental (cheaper than mileage reimbursement for 950 miles per trip: 375 miles from Syracuse and 100 miles round trip to JB and HB), or $940/year. We budgeted $3000/year to attend professional meetings, including quarterly meetings of the Hubbard Brook Committee of Scientists and the annual meeting of Bartlett cooperators. We budgeted $1000 for publication costs in Year 2.

We budgeted for analysis of 620 root samples in each year, as follows. For the intensively sampled sites, there will be 40 cores in each of 2 stands at Bartlett and Hubbard Brook and 50 at the two stands at Jeffers Brook (where there will be a Ca treatment in addition to N, P, N&P and control), times two soil horizons, for a total of 520 samples. For deep samples, there are 20 from each of two sites at Bartlett and 2 samples from the additional 6 sites at Bartlett (52 samples). Surface soils collected at the additional 6 sites at Barlett, with 4 cores and 2 horizons, contribute another 48 samples. At $5/sample, this amounts to $3100 each year. We will sequence the ITS region of the 100 most common types for identification, at $6/sequence, for $600.

Analytical and Technical Services is for the analysis of soil samples. We will composite these by plot, to reduce the sample load. The number of plot-horizon combinations is 10 at each of the JB sites, 8 at each of the HB sites, 12 at the two intensive Bartlett sites, and 3 at the other 6 Bartlett sites, for a total of 78 samples. We budgeted $30 per sample to allow for analysis of pH, exchangeable cations, available P, and various N fractions ($2340/year).

Personnel

Ruth Yanai will lead the project and coordinate relations with the field project in New Hampshire. **Tom Horton** will direct the laboratory activities. Note that mycorrhizae and fungal genetics are new research interests for Yanai, while ecosystem dynamics and the study of P and Ca present new research opportunities for Horton.

Cooperators

Melany Fisk will be studying soil fungi in samples taken from the same cores used in our study, funded by an addendum from NSF. We will approach other possible collaborators for development of methods for identifying AMF in the samples we prepare. The Hubbard Brook LTER supports the extension of the Nutrient Limitation study at Bartlett to the sites at Hubbard Brook and Jeffers Brook.

Future Funding Plans

Identifying functional groups of fungi important for the acquisition of N, P, and Ca will make it possible to pose more targeted research questions in future proposals. While this proposal uses inexpensive methods for fungal identification, future proposals will use molecular approaches that produce massive sequence datasets for powerful views into fungal communities (e.g. pyrosequencing).

The experimental design in this project expands upon the already funded work at Bartlett to include two additional sites in a fertility gradient (Jeffers Brook and Hubbard Brook). Our preliminary results will allow us to justify inclusion of this expanded network in future proposals, if warranted.

A previously rejected NSF proposal, led by Melany Fisk, Miami University, and including Ruth Yanai, will be more competitive with the involvement of Tom Horton and the results of this McIntire-Stennis supported effort. That proposal includes nutrient-amended ingrowth cores to be installed within the large nutrient addition plots, to test the role of roots and fungi in nutrient acquisition, including weathering of soil minerals and mineralization of organic matter, under conditions of varying nutrient limitation. These future research steps will allow us to close in on the mechanisms by which ecosystems control nutrient supply, which is key to evaluating the sustainability of forest utilization and the fate of forests under future scenarios of nutrient pollution and global change.

References Cited

Agerer, R., 1987-2002. Colour atlas of ectomycorrhizae. Einhorn-verlag, Schwäbisch Gmünd.

Agerer, R., 2001. Exploration types of ectomycorrhizae: A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107-114.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein databse search programs. Nucleic Acids Res. 25: 3389-3402.

Blum, J.D., Klaue, A., Nezat, C.A., Driscoll, C.T., Johnson, C.E., Siccama, T.G. Eagar, C., Fahey, T.J., Likens, G.E. 2002. Mycorrhizal weathering of apatite as an important Ca source in base-poor forest ecosystems. *Nature* 417: 729-731.

Brundrett, M., Bougher, N., Dell, B., Grove, T., Malajczuk, N., 1996. Working with Mycorrhizas in Forestry and Agriculture. ACIAR Monograph, Canberra.

Federer, C.A., Hornbeck, J.W., Tritton, L.M., Martin, C.W., Pierce, P.S., Smith, C.T. 1989. Long-term depletion of calcium and other nutrients in eastern US forests. Environ Manage 13(5): 593-601.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113-118.

Gardes, M., Bruns, T.D., 1996. ITS-RFLP matching for the identification of fungi. *In* Methods in molecular biology, Vol. 50: Species diagnostics Protocols: PCR and Other Nuclieic Acid Methods. *Edited by* J. Walker, Humana Press, Totowa, NJ, pp. 177-186.

Gehring, C.A., Theimer ,T.C., Whitham, T.G., Keim, P. 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in environmental extremes. Ecology 79: 1562-1572.

Hamburg, S.P., Yanai, R.D., Arthur, M.A., Blum, J.D., Siccama, T.G. 2003. Biotic control of calcium cycling in northern hardwood forests: acid rain and aging forests. Ecosystems 6:399-406.

Horton, T., 1997. The below ground view of ectomycorrhizal fungi in three conifer communities. *In* Department of Plant Pathology. University of California at Berkeley, Berkeley, p. 96.

Horton, T.R. 2002. Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant Soil 244, 29-39.

Horton, T.R., Bruns, T.D., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol. Ecol. 10: 1855-1871.

Kårén, O., Hogberg, N., Dahlberg, A. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. New Phytol.136: 313-325.

Last, F.T., Dighton, J., Mason, P.A. 1987. Successions of sheathing mycorrhizal fungi. Trends Ecol. Evol. 2:157-161.

Lilleskov, E.A, Hobbie, E.A., Horton, T.R. (In press) Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecology.

Lilleskov, E.A., Hobbie, E.A., Fahey, T.J., 2002. Evidence for a functional shift in N use by ectomycorrhizal fungal communities with increasing N availability. New Phytol. 154: 219-232.

Naples, B.K., Fisk, M.C. 2010. Belowground insights into nutrient limitation in northern hardwood forests. Biogeochemistry 97:109–121.

Newman, E.I. 1966. A method of estimating the total length of root in a sample. J. Appl.Ecol.: 3(1) 139-145.

Nezat, C.A., Blum, J.D., Yanai, R.D., Park, B.B. 2008. Mineral sources of calcium and phosphorus in soils of the northeastern USA. Soil Science Society of America Journal 72(6): 1786–1794.

Peter, M., Ayer, F., Egli, S. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytol. 149: 311-325.

Peters, S.C., Blum, J.D., Driscoll, C.T., and Likens, G.E. (2004) Dissolution of wollastonite during the experimental manipulation of Hubbard Brook Watershed 1. Biogeochemistry 67:309–329.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591-602.

Tennant, D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63(3): 995-1001.

White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols - a guide to methods and applications.

Edited by M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J., White. Academic Press, New York, pp. 315-322.

Yanai, R.D. 1998. The effect of whole-tree harvest on phosphorus cycling in a northern hardwood forest. Forest Ecol. Manag. 104: 281-295.

Yanai, R.D., Blum, J.D., Hamburg, S.P., Arthur, M.A., Nezat, C.A., Siccama, T.G. 2005. New insights into calcium depletion in northeastern forests. J. For. 103:14-20.

Budget

McIntire-Stennis Program
Title: **Sustainable Nutrient Supply After Forest harvest: Characterizing the Fungal Link from Soils to Roots**
Project Period: 08/15/2011 to 9/30/2011
Budget Period: 08/15/2010 to 9/30/2013

MS - Yanai-Horton:MS-11-12 rev: 11/12/2010

Budget Category (Term Date)	FY 2011 9/30/2011	FY 2012	FY 2013	Total
Salaries & Wages				
Principal Investigator				
Ruth Yanai 10% AY	0	0	0	0
Co-Principal Investigator(s)				
Tom Horton, 5% AY	0	0	0	0
% AY/CY	0	0	0	0
Hourly Personnel (Non-Student)	0	0	0	0
Subtotal	0	0	0	0
Graduate Assistants	1,481	13,750	14,163	29,394
Student Assistants (Hourly)	0	0	0	0
Total Salaries & Wages	1,481	13,750	14,163	29,394
Employee Benefits:				
41.75%; 42.75% and 43.5%	0	0	0	0
13.19%; 14.0625% and 15% Grad Stdts	206	1,814	2,071	4,091
5.0% Undergrad	0	0	0	0
Total Salaries, Wages & Benefits	1,687	15,564	16,234	33,485
Equipment	0	0	0	0
Supplies & Materials	0	3,700	3,700	7,400
Travel	0	3,940	3,940	7,880
Other Direct Costs				
Computer Time	0	0	0	0
Equipment Rental	0	0	0	0
Analytical & Technical Services	0	2,340	2,000	4,340
Publication Costs	0	0	1,000	1,000
				0
				0
Total Project Budget	**1,687**	**25,544**	**26,874**	**54,105**

Responses to Previous Reviews

This is a first submission.

Record of Success with Previous McIntire-Stennis Funding

Horton has not previously received McIntire-Stennis funding as a lead PI.

Yanai has had one previous McIntire-Stennis award, with Ralph Nyland, titled "New measures of spatial variability in uneven-aged stands: distinguishing sustainable from exploitative cutting practices." This project was the brainchild of Dave Ray, then a PhD student in FNRM. Dave left ESF, and the project supported Kimberly Bohn, who obtained her PhD in 2006 and is currently an Assistant Professor at the University of Florida. The project was active from 1999 to 2003, with a total expenditure of $72,000.

Kimberly gave presentations on the results of this work at professional meetings, and two journal articles will result from her dissertation. This work has also been presented in recent lectures and workshops by Ralph Nyland.

Journal Articles

Bohn, K.K., R.D. Nyland, and R.D. Yanai. Comparing selection-system and diameter-limit cutting in uneven-aged northern hardwoods using computer simulation. Canadian Journal of Forest Research (in review)

Bohn, K.K., R.D. Yanai, and R.D. Nyland. Spatial structure of residual trees after simulated treatments of selection system silviculture and diameter-limit cutting in uneven-aged northern hardwood stands (in preparation)

Professional Presentations

Nyland, R.D. 2009. Diameter-limit Cutting and Exploitation. A webinar in the ForestConnect Internet Seminar Series, sponsored by Cornell Cooperative Extension. 18 February 2009

Nyland, R.D. 2008. Sustainable Forest Management in Today's Acadian Forests sponsored by Unama'ki Institute of Natural Resources at Membertou, Nova Scotia. 8-9 July 2008

Nyland, R.D. 2008. Exploitive or Responsible Forestry? Diameter-limit Cutting, High-Grading, Selective Harvesting – How Your Forests Can Recover from These Practices. A workshop in East Rupert, Vermont, sponsored by the Bennington County Sustainable Forest Consortium. 20 August 2008

Bohn, K.K. and R.D. Yanai. 2006. Effects of different partial cutting treatments on structural dynamics in uneven-aged northern hardwood stands. Ecol. Soc. Am. Annual Meeting. Memphis, Tennessee, August 2006.

Bohn, K.K. and R.D. Yanai. 2005. Spatial distribution of trees in northern hardwoods after simulated selection system silviculture and diameter-limit cutting. Ecol. Soc. Am. Annual Meeting. Montreal, Quebec, August 2005.

Bohn, K.K., R.D. Nyland, and R.D. Yanai. 2005. Residual Spatial Structure of Uneven-aged Northern Hardwoods After Simulated Selection System Silviculture or Diameter-limit Cutting. Diameter-limit Cutting in Northeastern Forests Conference. University of Massachusetts. Amherst, MA. May 23-24, 2005.

Bohn, K.K., R.D. Nyland, and R.D Yanai. 2005. Residual Overstory Variability in Uneven-aged Northern Hardwoods Managed with Selection System Silviculture versus Diameter-Limit Cutting. New York Society of American Foresters Winter Meeting. Liverpool, NY. February 3, 2005.

Related Funding

Yanai has not continued in this line of research, but Co-PI Ralph Nyland has, along with John Wagner and David Newman.

Potential Reviewers

Erik Lilleskov
Northern Research Station
USDA Forest Service
410 MacInnes Dr.
Houghton, Michigan 49931
Phone: (906) 482-6303, ext. 18
elilleskov@fs.fed.us
http://www.nrs.fs.fed.us/people/Lilleskov

Erik Hobbie
Institute for the Study of Earth, Oceans, and Space
Morse Hall
University of New Hampshire
8 College Road
Durham, NH 03824-3525
603-862-0322
Fax: 603-862-1915
erik.hobbie@unh.edu

David Rothstein
113 Natural Resources Building
Michigan State University
East Lansing, MI 48824
Phone: (517) 432-3353
Fax: (517) 432-1143
Email: rothste2@msu.edu

Richard Phillips
Assistant Professor
Indiana University
Bloomington, IN 47405
812-856-0593
rpp6@indiana.edu

Ruth D. Yanai

Professional Preparation

Yale College	Geology and Geophysics	B.A., 1981.
Yale Graduate School	Forest Ecology	M.S. 1985, M. Phil. 1987, Ph.D. 1990.
Boyce Thompson Institute for Plant Research	Plant and Soil Modeling	1991.

Appointments

Sabbatical Fellow, Ecosystems Center, Marine Biological Laboratory. Woods Hole, MA. 2008.

Professor, SUNY-ESF. 2007 – present.

Visiting Professor, University of Tokyo, Graduate School of Agricultural and Life Sciences. 2006.

Associate Professor, SUNY-ESF. 1999 – 2007.

Assistant Professor, SUNY-ESF. 1994 - 1999.

Senior Research Associate, Boyce Thompson Institute for Plant Research. 1994.

Research Associate, Boyce Thompson Institute for Plant Research. 1992 -1993.

Postdoctoral Associate, Boyce Thompson Institute for Plant Research. 1991.

Research Support Specialist, Cornell University. 1989-1990.

Fulbright Fellow, Edinburgh University and Institute of Terrestrial Ecology, 1987-1988.

Secondary School Teacher, The Putney School, Putney Vermont. 1982-1983.

Research Assistant, Geology and Geophysics, Yale University. 1981-1982.

Five Publications Most Closely Related to the Proposed Project

Yanai, R.D., M.C. Fisk, T.J. Fahey, N.L. Cleavitt, and B.B. Park. 2008. Identifying roots of northern hardwood species: patterns with diameter and depth. Can. J. For. Res. 38(11): 2862-2869

Nezat, C.A., J.D. Blum, R.D. Yanai, and B.B. Park. 2008. Mineral sources of calcium and phosphorus in soils of the northeastern USA. Soil Sci. Soc. Am. J. 72(6): 1786–1794

Yanai, R.D., B.B. Park, and S.P. Hamburg. 2006. The vertical and horizontal distribution of roots in northern hardwood stands of varying age. Can. J. For. Res. 36: 450-459.

Yanai, R.D., J.D. Blum, S.P. Hamburg, M.A. Arthur, C.A. Nezat, and T.G. Siccama. 2005. New insights into calcium depletion in northeastern forests. J. For. 103:14-20.

Yanai, R.D. 1998. The effect of whole-tree harvest on phosphorus cycling in a northern hardwood forest. Forest Ecol. Manag. 104:281-295.

Five Other Significant Publications

Yanai, R.D., J.J. Battles, A.D. Richardson, E.B. Rastetter, D.M. Wood, and C. Blodgett. 2010. Estimating uncertainty in ecosystem budget calculations. Ecosystems 13(2): 239-248.

Fisk, M.C. , R.D. Yanai, and N. Fierer. 2010. A molecular approach to quantify root community composition in a northern hardwood forest: testing effects of root diameter and species. Can. J. For. Res. 40(4): 836-841

Yanai, R.D, K.J. McFarlane, M.S. Lucash, J.D. Joslin, and S.E. Kulpa. 2009. Nutrient uptake by Engelmann spruce and subalpine fir at two Colorado subalpine forests. Forest Ecol. Manag. 258(10): 2233-2241.

Hamburg, S.P., R.D. Yanai, M.A. Arthur, J.D. Blum and T.G. Siccama. 2003. Biotic control of calcium cycling in northern hardwood forests: acid rain and aging forests. Ecosystems 6:399-406.

Yanai, R.D., W.S. Currie, and C.L. Goodale. 2003. Soil carbon dynamics after forest harvest: an ecosystem paradigm reconsidered. Ecosystems 6:197-212.

Synergistic Activities

- Workshop, Preparing Manuscripts for Publication: Ecological Society of America Annual Meeting, Montreal, Quebec, Aug. 9, 2005; San Jose, CA, Aug. 7, 2007; Milwaukee, WI, Aug. 5, 2008; American Society of Agronomy Annual Meeting, Seattle, Washington, Nov. 2, 2004; Salt Lake City, UT, Nov. 8, 2005; Indianapolis, IN, Nov 14, 2006; New Orleans, LA, Nov. 6, 2007; Houston, TX, Oct. 7 2008; Pittsburgh, PA, Nov. 3, 2009; Long Beach, CA, Nov. 2, 2010. Forestry and Forest Products Research Institute, Tsukuba, Japan, July 28, 2006; April-May, 2007; Chiba Experimental Forest, Tokyo University, Japan, July 25, 2006; Kyoto University, Graduate School of Agriculture, Kyoto, Japan, July 14, 2006; Marine Biological Laboratory, Woods Hole, MA, Dec. 8, 2008
- Workshop, *Estimating uncertainty in ecosystem budgets*. Michigan State University - Department of Forestry, East Lansing, MI, Feb. 20, 2009; Ecological Society of America meeting, Aug. 2, 2009, Albuquerque, NM; Agronomy Society of America Meeting, Nov. 1, 2009, Pittsburgh, PA; Université Laval, Centre de foresterie de Laurentiedes & Le Centre d'Étude de la Forêt, Québec, QC, Oct 13, 2010
- Organizer, ESF Women's Caucus, providing informal and formal programs for students, staff and faculty and for girls interested in careers in science, 1994-present. Instructor of a graduate seminar at ESF: FOR 797 Women in Scientific and Environmental Professions, 1999-2002.
- Panelist, Climate Change Mitigation and Adaption in Agriculture, USDA NIFA Competitive Programs, Sept 21-24, 2010.
- Moderator, *Forest Stress* session, Workshop on Comparison of Forest-Soil-Atmosphere Models, Leusden, The Netherlands, May 10-14, 1993; session on *Management impacts on forest hydrology, biogeochemistry and water quality (C-14)* at the 2010 IUFRO conference in Seoul, Aug. 25, 2010; *COS 38, Soil Ecology* session at the 2010 Ecological Society of America annual meeting in Pittsburgh, PA, Aug. 3, 2010.

Current and Pending Research Funding

Yanai, R.D., and M.J Mitchell. Long-term ecological research at the Hubbard Brook Experimental Forest," 2011-2016. National Science Foundation, overall grant to Cornell University is $5 million, the ESF subcontract is $330,000, and my share is $240,000. 1/1/2011-12/31/2016.

Yanai, R.D. Collaborative Research: Nutrient co-limitation in young and mature northern hardwood forest. National Science Foundation. $905,961 with $529,987 in collaborative awards to Marine Biological Laboratory, Miami University, University of Michigan, and Cornell University. 7/01/10-6/30/15.

Yanai, R., L. Wielopolski, C. Goodale, I. Fernandez, S. McNulty, and S. Hamburg. Non-Destructive Soil Inventory using Inelastic Neutron Scattering: An Application to Nitrogen Controls on Soil Carbon Storage. Northeastern States Research Cooperative. $90,745, 7/1/2009-6/30/2011.

Yanai, R.D. Forest Health GIS Planner. New York Department of Environmental Conservation; $104,274. 7/1/2007-9/30/2011.

Yanai, R.D., and M. Mitchell. Long-term ecological research at the Hubbard Brook Experimental Forest," 2004-2010. National Science Foundation, overall grant is $4.8 million, the ESF subcontract is $331,511, and my share is $163,728. 1/1/06-12/31/10.

Thomas R. Horton

Professional Preparation

Humboldt State University, Biology, B.A. ,1986.

Humboldt State University, Single Subject Credential, Biology with a Supplementary Authorization in English, 1987.

San Francisco State University, Biology: Ecology and Systematic Biology, M.A., with honors, 1992.

University of California, Berkeley, Plant Pathology, Ph.D, 1997.

Oregon State University (Postdoc), 1997 – 2001.

Appointments

Associate Professor, Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, New York, 2007 – present.

Assistant Professor, Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, New York, 2001 – 2007.

Biology teacher, Logan High School (public, grades 9 - 12), Union City, California, August 1987 - June 1989.

Publications (5 most relevant)

Lilleskov EA, Horton TR, Hobbie E (in press) Patterns of fungal species response to elevated soil N. Fungal Ecology. (Should be out early next year)

Hobbie EA, Horton TR (2007) Evidence that saprotrophic fungi mobilise carbon and mycorrhizal fungi mobilise nitrogen during litter decomposition. New Phytologist 173: 447–449.

Horton TR (2002) Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale . Plant and Soil 244: 29-39.

Lilleskov EA, Fahey TJ, Horton TR, Lovett GM (2002) Nitrogen deposition and ectomycorrhizal fungal communities: a belowground view from Alaska. Ecology 83: 104 - 115.

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10 (8): 1855-1871.

Publications (5 additional)

O'Brien, MJ, Gomola CE, Horton TR (accepted) The effect of forest community on ectomycorrhizal soil inoculum potential and seedling establishment success. Plant and Soil. (Should be out early next year)

Molina R, Horton TR, Trappe JM, Marcot BG (available on line) Addressing uncertainty: how to conserve and manage rare and little-known fungi. Fungal Ecology. (Should be out early next year)

Nuñez MTA, Horton TR, Simberloff D (2009) Lack of belowground mutualisms hinders Pinaceae invasions. Ecology 90:2352-2359.

van der Heijden MGA, Horton TR (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. Journal of Ecology 97:1139-1150.

Ashkannejhad S, Horton TR (2006) Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. New Phytologist 169:345-354.

Synergistic Activities

- Elected Counselor: Ecology and Pathology, Mycological Society of America, 2009 – 2011.
- Program Committee, Mycological Society of America, 2006 - 2011. Chair 2010. Organized the annual meeting of the Mycological Society of America, held jointly with the International Symposium on Fungal Endophytes of Grasses, Lexington, Kentucky, June 28 - July 1.
- Peer review: Editorial board, Mycorrhiza September 2003 – present, review panels for NSF and USDA, adhoc reviewer for many journals and funding agencies.
- I teach 200+ General Ecology students each year plus 15 -20 students in advanced mycology (Mycorrhizal Ecology, Basidiomycete taxonomy).
- The following two articles were written to communicate advances in the development and use of molecular tools for mycorrhizal studies and have been cited by many authors.
    - Horton TR (2002) Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale . Plant and Soil 244: 29-39. Cited 73 times
    - Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10 (8): 1855-1871.

Pending Proposals

This proposal.

Current Funding

Simberloff D, Nuñez MA, HortonTR. 2010 – 2013. Collaborative Research: Determinants of ectomycorrhizal fungal spread and its relation to Pinaceae invasion. NSF Population and Community Ecology panel. The total award is $571,637, with $242,040 to Horton.

Powell WA, Maynard CA, Leopold DP, Horton TR, Perry D. 2008 – 2011. Evaluating environmental impacts of transgenic American chestnut trees to chestnut trees produced by conventional breeding. USDA – NRI, BRAG program. $380,00.