Mycorrhizal Colonization Across an Urban to Rural Gradient:

Interactions with Nitrogen and Calcium Cycles

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Abstract

Nutrient cycling in urban ecosystems is changing because of human activities such as burning fossil fuels and fertilizer runoff. N deposition, in particular, is causing a range of changes to forest processes and ecological relationships. This study focuses on the symbiotic relationship between oak trees and ectomycorrhizal fungi. Mycorrhizal fungi, which colonize tree roots, help trees obtain important nutrients from the soil, such as N and Ca, and in return the trees transfer glucose through the roots to the mycorrhizae. I examine how urbanization and N addition impacts mycorrhizal colonization, pH, and soil and leaf litter Ca concentrations. Soil samples were collected along an urbanization gradient from the Boston area to Harvard Forest in Petersham, MA as well as from the experimental manipulation Chronic N Amendment plots at Harvard Forest that receive increased N loads. Mycorrhizal colonization of roots, expressed as a percentage, was determined by identifying the presence or absence of mycorrhizal mantels; soil pH measurements were taken; and exchangeable soil Ca and total leaf litter Ca were quantified using an atomic absorption spectrophotometer. Along the rural to urban gradient, there is a slight decrease in mycorrhizal colonization in addition to lower pH and Ca levels. The high N addition site, compared to the control site, has significantly lower levels of mycorrhizal colonization as well as lower pH and Ca concentrations. These results suggest that urbanization is changing how nutrients flow through forest ecosystems and that this could be altering the relationship between trees and mycorrhizal fungi.

Key Phrases and Keywords

Mycorrhizal colonization, Ectomycorrhizae, Urbanization, Nitrogen deposition, Calcium loss
Introduction

In the age of anthropogenic climate change and rapid global development, it is crucial to study the structure and functioning of urban ecosystems. Urban environments are expanding rapidly and becoming the home of a growing percentage of the globe’s human population. In 1900 only 9% of all people lived in cities, but by 2000 50% of the world population lived in urban environments (McIntyre et al. 2008), and this percentage is predicted to grow to over two thirds of the population by 2050 (Gómez-Baggethun and Barton 2013). The urban environment has been described as one that is polluted, has many non-native species, and is greatly influenced by humans (McIntyre et al. 2008). Despite the high percentage of humans living in urban environments, urban ecosystems are not well studied (McIntyre et al. 2008). As global change continues, investigations of urban ecosystems will help us to better understand this important habitat for humans and discover how to manage cities to improve the health of the environment and the species living there.

Trees are important to urban and rural ecosystems: they provide homes for many species, stabilize the soil, and prevent erosion. The role of trees as a carbon sink is crucial for storing and sequestering CO$_2$ which, if it accumulates in the atmosphere, is a primary driver of climate change. In urban environments, trees also protect infrastructure from the elements, alter the microclimate, help manage water flow and runoff, and create greener places for people to live (Gómez-Baggethun and Barton 2013).

Trees do not live solitary lives. Most trees form symbiotic relationships with mycorrhizal fungi, which colonize the roots of trees. The trees send sugars to the mycorrhizae, which in turn...
send nutrients, such as nitrogen, back in return (Phillips et al. 2013). There are two main types of mycorrhizae in forest ecosystems—arbuscular mycorrhizae and ectomycorrhizae—and they differ in their methods of taking in nutrients and associate with different tree species. Arbuscular mycorrhizae, which associate with trees such as maples and ash, release sugars into the soil which stimulate microbial breakdown of organic matter (Phillips et al. 2013). Oak species, which dominate forests of southern New England, are associated with ectomycorrhizae (Rao et al. 2013), and are the focus of this research. Ectomycorrhizae form a thick hyphal mantle around the root tips and branching hyphal filaments can extend from this mantle. Enzymes are secreted into the soil from the tips of these hyphae and they break down soil organic matter, releasing nitrogen, phosphorous and other nutrients that the mycorrhizal hyphae then take up and send to the tree roots. (Phillips et al. 2013). In this research, I studied how the density of ectomycorrhizal colonization can change, from heavy colonization to light colonization, as nutrient cycling changes across a rural to urban gradient.

In urban areas, land use change, transportation, and fertilizers are increasing nitrogen deposition in the soils (Rao et al. 2014). When humans burn fossil fuels, the gaseous emissions to the atmosphere contain sulfur and nitrogen compounds that can cause acid rain. Efforts have been put into regulating sulfur emissions, but nitrogen emissions continue to impact water quality and forest nutrient cycling. Temperate forests of New England are typically nitrogen limited, so nitrogen deposition is altering ecosystem processes such as N mineralization, nitrification, and nitrate leaching that then have other effects on these ecosystems in addition to fertilization (Aber et al. 1989). Industrialization and wind patterns have created a nitrogen deposition gradient in the northeastern United States where the highest N levels are in western New York and Pennsylvania and the lowest are in eastern Maine.
Northeastern forests are also experiencing calcium loss due to acid rain. Although the deposition of acid in forest ecosystems is decreasing, the reduction in calcium content associated with decades of acid inputs to forest soils can limit their recovery following a range of disturbances (Blum et al. 2002). Calcium is an important element for plant growth and development (Hepler 2005), and the Ca addition experiment at the Hubbard Brook Experimental Forest in NH resulted in improved tree health and growth (Huggett et al. 2007). Since urban environments are experiencing acid rain (Chen 2007), it would be expected that soil acidification would also be occurring in urban areas in New England. Mycorrhizae have been found to be involved in soil mineral weathering that helps make calcium available to trees in calcium-poor systems (Blum et al. 2002), so a decrease in soil Ca could be countered by increased mycorrhizal colonization. I investigated how calcium availability can impact the relationship between trees and mycorrhizal colonization.

For this research, I worked alongside students Maggie Anderson of Lawrence University and Talia Michaud of Mount Holyoke College, and we were mentored by Jerry Melillo and John Hobbie, both Distinguished Scientists at the Ecosystems Center at the Marine Biological Laboratory. We collaborated with Professors Pamela Templer and Lucy Huttyra, both professors at Boston University, who have established a set of plots along an urban-to-rural gradient from Boston to Harvard Forest. In addition to the urbanization gradient, we also sampled in the Chronic Nitrogen Amendment plots at Harvard Forest, which are part of an experiment led by Professors Serita Frey and Scott Ollinger of the University of New Hampshire. That enabled us to compare our mycorrhizal colonization values in an urban setting to a rural setting that also experienced nitrogen deposition.
I hypothesized that the urban sites would have a lower percent of mycorrhizal colonization than the rural sites. Since the urban environment has higher nitrogen levels than rural sites, it would be expected that the urban trees would have less nitrogen limited. This may cause a decrease in mycorrhizal colonization, since the urban trees may have less need for their mycorrhizal symbionts and may not send as much carbon to the mycorrhizae to fuel their colonization.

The Chronic Nitrogen Amendment plots served as an analog for the urban to rural gradient. Those plots with added N and the urban plots both have higher levels of nitrogen deposition than do the rural plots, but the Nitrogen Amendment plots isolate just one variable – the addition of nitrogen. However, there are many factors that could be impacting the mycorrhizal colonization in the urban plots. If the results of mycorrhizal abundance in the urban sites are similar to that in the high nitrogen addition plots, then this will indicate that N is one of the main factors driving percentage of mycorrhizal colonization of tree roots.

I hypothesized that the urban sites would have less mycorrhizal colonization than the rural sites, because of increased N deposition. Since urban sites are predicted to have a low amount of Ca, I would expect mycorrhizae would help make Ca more available for the trees (Blum et al. 2002). If this is the case, I hypothesized that there will be a lower ratio of leaf litter Ca to soil Ca in the urban plots than in the rural plots, since with less mycorrhizal colonization, the trees will get less access to Ca from the soil. I also predicted that the urban leaf litter would have a low Ca to N ratio because of the high N deposition and low Ca levels in comparison to the rural sites. An alternative hypothesis is that since mycorrhizae help trees take in Ca, this may cause an increase in mycorrhizal colonization in the calcium-poor urban sites.
Methods

Field Sites

Urban to Rural Gradient

The urbanization gradient was set up by Drs. Lucy Hutyra and Pamela Templer, to investigate how forest fragmentation (including edge effects and urbanization) at these sites are impacting nutrient cycling. The gradient consists of eight sites that each contain a 90 m transect that goes from an edge to the center of a plot of forest. We sampled at four of the eight sites: Hammond Woods (Newton, MA), Sutherland Woods (Lexington, MA), and two or three sites at Harvard Forest (Petersham, MA) (Fig. 1). The Hammond Woods site is near the Hammond Pond Parkway and part of the Webster Conservation Area and gets a large amount of human and dog foot traffic. Sutherland Woods is also frequently visited by humans and dogs and is located near a suburban residential area. The first Harvard Forest site (HF04) is located in near a field bordered by a rock wall. The edge effect in the second Harvard Forest site (HF06) is caused by Barre Road that cuts through the forest. The 2014 Rao et al. paper reports that Harvard Forest had an annual N input of 3.7 kg N/ha/yr and the city of Newton had an N input of 8.8 kg N/ha/yr. This paper did not include a site in Lexington, but Waltham, a city near Lexington, had an N input of 17.3 kg N/ha/yr (Rao et al. 2014).

Harvard Forest Chronic N Amendment Plots

The experimental site at Harvard Forest consist of three 30x30 m plots: a control which gets no N treatment, a 50 kg N ha\(^{-1}\) yr\(^{-1}\), and a 150 kg N ha\(^{-1}\) yr\(^{-1}\). The N treatments are applied in six equal doses to add up to the annual N addition amounts (Aber et al. 1993).
**Field Protocol**

At each of the Hutyra-Templar urbanization gradient sites, each plot is set up as a 90 m transect from the forest edge to the center of the forest. To create a more homogenous set of samples, we sampled from about 40-90 m from the edge, and I collected soil samples beneath five trees at each site. Every 10 meters along my 50 m transect, I identified the nearest oak tree to the transect that has a DBH larger than 15 cm (Fig. 2). I then located three equally spaced spots that were 0.5m away from the tree and collected a rectangular block of soil from the forest floor (Fig. 3). After cutting and extracting a 10x10 cm soil sample, I measured the depth of each side of the soil sample to calculate the volume of soil. I took three soil samples per tree to try to sample an even representation of the mycorrhizal community around the whole tree.

In the Chronic Nitrogen Amendment Experiment plots, we followed a similar protocol to our methods along the urbanization gradient. We sampled in the control, 50 kg N ha\(^{-1}\) yr\(^{-1}\) addition, and 150 kg N ha\(^{-1}\) yr\(^{-1}\) addition plots. Each of these plots is 30x30 m, so we set up a diagonal transect between two corners of each plot. Since these plots are smaller than the urbanization gradient sites, we sampled every 5 m. I located five trees along the 25 m transect that we created, and I followed the same sampling procedure that I described for the urbanization gradient plots (Fig 4).

**Lab Protocol**

*Wet and dry soil weights*

To increase time efficiency, only soil samples from trees 1, 3, and 5 in each of the seven plots were analyzed (Table 1). Each of these 63 soil samples was weighed, and a quarter of each sample was sieved at 2 mm. For each tree, all three sieved samples were combined into one
composite sample per tree, which resulted in a total of 21 soil samples. Each sieved, composite sample was weighed, put in a drying tin and dried at 68°C for 24 hours. Each sample was then reweighed and 3 g of the 68°C dried soil were saved for Calcium analysis and 3 g were saved for pH analysis. The remaining soil was reweighed and put back into the oven to dry at 110°C for 16 hours. These soils were then reweighed after the 110°C drying (Table 2) to calculate soil moisture content.

**pH Analysis**

For each of the 21 samples, 30ml DI water was added to 3g of 68°C dried soil and mixed into a slurry. The pH of each sample was then measured with a pH meter (SES Lab Manual 2018a).

**Quantifying Mycorrhizal Biomass**

A 2x2 cm block was cut out of each rectangular soil sample block. All three samples from each tree were combined into one composite sample per tree and stored in water to help loosen the soil from the roots. For each sample, all of the roots that were about 2 cm or longer were extracted from the organic soil, rinsed with water, and then laid out on a cutting board. All of the roots were then cut into 2 cm long segments and counted to find the total root length in the sample. These 2 cm segments were then put into a plastic container filled with water and shaken to mix all the roots. Fifty of the 2 cm root segments were randomly chosen and analyzed under a dissecting microscope. Each root segment was categorized as either colonized with mycorrhizae or not colonized with mycorrhizae based on the presence or absence of hyphal mantles (Kärén and Nylund 1997). Roots with one or more mycorrhizal mantles were considered colonized (Fig. 5). This resulted in a value for the percentage of colonized root tips for each of the 21 samples.
CN Analysis

Maggie Anderson and I worked together to measure C and N concentrations in the soil (sampled to a depth of 10 cm) and leaf litter from each of the sites. Three replicates for each of the sites were measured, and Anderson’s soil samples were separated into O and A layers. This gave us a total of 62 samples: 21 soil O layer, 21 soil A layer, and 21 leaf litter samples. We then packed and prepared the samples and standards and ran them on the elemental analyzer using the SES lab methods (SES Lab Manual 2018b).

Measuring Exchangeable Ca in Soil with Ammonium Acetate Assay

The soil extracts were prepared for analysis in the Atomic Absorption Spectrophotometer (Carter 1993). First the ammonium acetate extracting solution was prepared. Ammonium acetate (77.08g) was weighed and added to about 600 mL water in a 1L beaker with stir bar. The extracting solution was mixed with the stir bar until the ammonium acetate completely dissolved. The pH was then measured, and acetic acid was added to the solution until the pH of the buffer decreased to the goal pH of 7. The solution was then added to a 1L volumetric flask, and water was added to reach 1L of buffer solution. Then for each sample, 3g of 68°C dried soil was added to a 125mL Erlenmeyer flask. Using a re-pipet, 30mL of the ammonium acetate buffer was added to each flask and the flask was then covered with parafilm. All of the samples were then shaken with a reciprocating shaker at 120 rpm for 15 minutes. The samples were then filtered through Whatman No. 2 paper in a funnel. The three replicates from each sample site HW07, SW08, HF04, HF06 and NDC were diluted by 10 and three replicates from each sample site
NDHi and NDLo were diluted by 4. All 21 samples were then run through an atomic absorption spectrophotometer to obtain the concentration of exchangeable Ca in the soil.

**Measuring Total Ca in Leaf Litter Samples**

Leaf litter samples were dried in a 100°C oven for about 12 hours and then ground using a Retsch mixer mill. For each sample, 0.5g was weighed into glass scintillation vials and then ashed in a muffle furnace at 450°C for 4 hours. To extract the Ca from the sample, 13mL of 4M HCl was added to each of the scintillation vials. Each sample was then poured through a #1 Whatman filter paper in a funnel into a 100mL volumetric flask. Then 5mL of 4M HCl was added to each scintillation vial and dumped into the funnel to try to filter most of the sample that had remained stuck to the vial. After the filters drained, they were removed, and the volumetric flasks were all filled to 100mL with 4x HCl. These extracts were then poured into falcon tubes. Samples HW07, SW08, HF04, HF06 and NDC were diluted by 50 and samples NDHi and NDLo were diluted by 10. The samples were run through an atomic absorption spectrophotometer to obtain the total concentration of Ca in the leaf litter.

**Statistical Analysis**

The statistical tests ANOVA (analysis of variance) and Tukey-Kramer HSD comparisons for all pairs were done for all of the data in the figures that will be presented. Only the p-values and connecting letters of significant results are included in this paper.

**Results**

My results indicate that the urban sites had a slightly lower percent of mycorrhizal colonization than the rural sites, but the difference was not statistically significant (Fig. 6).
Anderson measured ammonium and nitrate pools in the soil samples of O and A layers combined. These data suggest that Sutherland Woods, in Lexington, had higher amounts of ammonium and nitrate in the soil than Hammond Woods, in Newton, and the two rural sites (Fig. 7). Anderson also measured N-mineralization in the combined O and A layer soil samples and found a trend of higher rates of N-mineralization in the urban plots than in the rural plots, although the rates were not significantly different from each other (Fig. 8).

In the analysis of the Chronic N Amendment plots, the high N addition plot had significantly lower mycorrhizal colonization than the control and low N addition plots. The percent mycorrhizal colonization in the high N addition site was also significantly different than all of the other sites except for Sutherland Woods (Fig. 6, Table 3). Anderson’s soil ammonium and nitrate pool values indicate that ammonium and nitrate values increase with N addition treatments (Fig. 7). The rate of N-Mineralization followed an increasing trend with higher amounts of N addition (Fig. 8).

Linear regressions of the data from all seven sites suggest relationships between mycorrhizal colonization and nitrogen. Plotting a linear regression of percent mycorrhizal colonization against soil nitrate in the O layer resulted in a negative correlation with a p value of 0.0142 (Fig. 9). There was also a negative correlation between percent mycorrhizal colonization and N-mineralization in the O layer (p=0.0011, Fig. 10). Comparison of total C and N pools in the O layer resulted in a linear relationship with a p value of <0.0001 (Fig. 11).

Along the urbanization gradient, soil pH was lower in the urban sites than in the rural sites (Fig. 12). The pH of the soil at Hammond Woods was significantly different than the Harvard Forest-04 plot (Table 3). Exchangeable Ca in the soil also had a decreasing trend with
urbanization (Fig. 13). Total Ca in the leaf litter of Hammond Woods was slightly lower than in the other sites along the urbanization gradient (Fig. 14).

In the Chronic N Amendment plots, soil pH followed a decreasing trend with increase in N addition (Fig. 12). Exchangeable Ca in the soil and Ca concentrations in the leaf litter also reflected the same decreasing trend along the N addition gradient (Fig. 14). The ratio of Ca in the litter to the soil was calculated to estimate how much Ca was transferred from the soil to the trees. These ratios were then examined in contrast to mycorrhizal colonization data for the Chronic N Amendment sites, and the results indicated that there could be less transfer of Ca from the soil to the trees in the plots with less mycorrhizal colonization (Fig. 15). Across all seven sites, the ratio of leaf litter N to Ca was significantly higher in the high N addition plot than in the urban, rural, and control Chronic N Amendment plot (Fig. 16, Table 3). An analysis of the relationship between N and Ca in the leaf litter in all plots resulted in a significant negative relationship for the linear regression of litter N with litter Ca (p= 0.0167, Fig. 17). This significantly higher N:Ca ratio in the leaf litter in the high N addition plot (which had lower mycorrhizal colonization) could suggest a relationship between mycorrhizal colonization and Ca availability for trees.

Discussion

The overall trends of my results matched my hypotheses about mycorrhizal colonization, N, pH and Ca. Results indicated a slight, though insignificant, decreasing trend in percent mycorrhizal colonization with urbanization. In other words, tree roots in the urban plots had slightly less mycorrhizal colonization than those in the rural sites. The high N addition plot had a significantly lower percent of mycorrhizal colonization than did all the other sites (Fig. 6). This
could indicate that trees reach a possible N addition threshold beyond which they greatly decrease carbon supply to the mycorrhizae. The results suggest that this threshold could lie somewhere between N addition of 50 kg N ha\(^{-1}\) yr\(^{-1}\) and 150 kg N ha\(^{-1}\) yr\(^{-1}\).

The lack of significant difference in the percent mycorrhizal colonization across the urban to rural gradient could be due to the fact that the increase of N in the urban sites is not drastic enough to cause a significant change in mycorrhizal colonization. The urban sites have an annual N deposition range from about 8.8 kg N/ha/yr to 17.3 kg N/ha/yr (Rao et al. 2014). If urban deposition continues to increase to very high levels, I would predict that mycorrhizal colonization could decrease following a similar trend as the mycorrhizae in the high N addition plot.

Anderson’s data on soil nitrate and ammonium pools indicate that nitrate and ammonium levels were higher in Sutherland Woods and in the high N addition plots than in the other plots, although the results were not statistically significant (Fig. 7). The N-mineralization data, also from Anderson, followed a similar trend where N-mineralization rates were highest in the urban and high and low N addition plots (Fig. 8). The C:N ratio in the soil O layer remained constant (Fig. 11). Linear regressions with percent mycorrhizal colonization and both O layer soil nitrate pools and O layer N-Mineralization showed significant decreasing trends. A decrease in mycorrhizal colonization corresponded with both an increase in soil nitrate and N-mineralization (Fig 9, Fig 10). This result that mycorrhizal colonization decreases with higher nitrogen availability supports my initial hypothesis that mycorrhizal colonization would decrease with N addition.

Although my data did not prove that the amount of mycorrhizal colonization significantly changes with urbanization, a previous study by Alexandrea Rice found that increased N from
Industrialization can change abundance of different mycorrhizal species and enzymes (Rice 2015). She examined soil samples taken from the high and low range of the east coast N deposition gradient. Harvard Forest in MA receives 8 kg N ha\(^{-1}\) yr\(^{-1}\), while Bousson Forest in western PA receives 13 kg N ha\(^{-1}\) yr\(^{-1}\). Both of these forests have long-term N Amendment experiments, so Rice was able to investigate the impacts of increasing N deposition, and she found that this severe increase in N caused by industrialization changed the ectomycorrhizal species and enzymes that they produced. Based on the changes in enzymes, Rice predicted that with an increase in N, the Bousson Forest would become more phosphorus limited while the Harvard Forest would become more carbon limited (Rice 2015). This suggests that anthropogenic N deposition can have a range of impacts on mycorrhizal fungi as well as nutrient cycling beyond N cycling.

In my study, along with the expected higher N levels in the urban sites compared to rural sites, there was also a decreasing trend in soil pH (Fig. 12). The urban sites and the high N addition site at Harvard Forest had the lowest pH values. N deposition can cause acidification which can lead to a loss of Ca in the soil (Högberg et al. 2006). My results indicate that the sites with higher N and lower pH also have lower Ca in the soil and leaf litter (Fig. 13, Fig. 14). This suggests that Ca content in the soil and leaves decreases with urbanization. Although the urban sites are experiencing less N limitation than in the rural sites, urban Ca limitation could cause other changes in tree growth and productivity. The Hubbard Brook Experimental Forest in New Hampshire executed a Ca addition experiment. Hubbard Brook has a long history of acid rain input that has led to increased Ca leaching (Likens et al. 1998). The experimental addition of Ca at Hubbard Brook resulted in an increase in tree health and growth (Huggett et al. 2007).
Mycorrhizae do not just help trees obtain N; they help with other nutrients as well, such as Ca. If mycorrhizal colonization decreases due to N deposition, trees could become stressed by lack of other nutrients. In the Chronic N Amendment plots, mycorrhizal colonization significantly decreased as N addition increased (Fig. 6), and the ratio of Ca in the leaves compared to that in the soil decreased as well (Fig. 15). This could indicate that a decrease in the transfer of Ca from the soil to the leaves could be partially due to a decrease in mycorrhizae. Another line of evidence is the significant negative relationship between leaf litter N and Ca (Fig. 17). The high N addition plot, which had the lowest mycorrhizal colonization across all plots, also had the significantly highest ratio of N to Ca in the leaf litter compared to the ratio in the urban, rural and control Chronic N Amendment plots (Fig. 16). These results indicate that with higher N addition, there can be less Ca available for trees. A decrease in mycorrhizal colonization with high N addition values could be one of the reasons for the higher N:Ca ratio in the leaf litter.

Mycorrhizae are known to help trees with Ca acquisition (Blum et al. 2002), so the results of my study fit my hypothesis that trees will get less access to Ca in the plots with lower mycorrhizal colonization. More research needs to be done to confirm these results. It would be interesting to investigate whether mycorrhizal colonization in the N addition plots would be lower if there was more Ca available. Even when N deposition is high, in order to maintain the mycorrhizal relationship which provides other elements such as Ca, trees might continue to send some sugars to the mycorrhizal fungi (Phillips et al. 2013). A multiple element experiment with addition of N, Ca, and N plus Ca could explore this possibility. If mycorrhizal colonization is lower in the N plus Ca addition treatments then in just the N addition, this could support the above hypothesis.
In this study, there was a noticeable difference in levels of exchangeable Ca in the soil between the two Harvard Forest urbanization gradient plots (the “rural” plots) and the N addition control plot at Harvard Forest (Fig. 14). It would be expected that these three plots would be similar since they are all untreated sites at Harvard Forest. However, the N addition control had less Ca than the other two untreated Harvard Forest sites, although was not statistically different (Fig. 14). The difference in Ca levels could also be due to different cation exchange capacities (CEC) of the soil. Sandy soils have a low CEC while soils with more clay have a higher CEC, and organic material has a very high CEC. Analysis of the soil texture could indicate how CEC could differ among the plots (Brown and Lemon 2018).

This study indicates that urbanization has the potential to impact many aspects of nutrient cycling and ecosystem dynamics. An immense increase in N as demonstrated with the high N amendment plot was associated with a decrease in mycorrhizal colonization (Fig. 6). Increase in soil nitrate and N-mineralization was also correlated with a decrease in mycorrhizal colonization (Fig. 9, Fig. 10). The high N addition plot had a low pH level that was similar to that of the urban sites (Fig. 12). Soil acidification and N deposition was also correlated with a loss of exchangeable Ca in the soils, which in turn was related to lower Ca concentrations in the leaves (Fig. 14). Ca limitation could lead to tree stress and decreased tree productivity. The Ca limitation experience by trees could become exacerbated as amounts of mycorrhizal colonization decrease as well. Mycorrhizae and trees both sequester carbon from the atmosphere, so a decrease in mycorrhizal colonization or an increase in tree stress could decrease their carbon sink capabilities (Bidartondo et al. 2001).
Conclusion

Overall, this study suggests that nitrogen levels affect the relationships among trees, mycorrhizal fungi and other nutrients, though further research is needed. For future studies, increasing the sample size by adding more sites and replicates could help yield more statistically significant results. It would also be interesting to execute a multiple element manipulation study with a Ca addition, N addition, Ca plus N addition, and control plot to further investigate the interactions among N, Ca mycorrhizae, and trees. Studying dendrochronology or photosynthetic rates of tree leaves could help in understanding how urbanization, Ca limitation, and changes in mycorrhizal colonization will impact tree health, growth, and productivity. Since increase of N in cities due to burning of fossil fuels and other human activities are affecting ecosystems in many ways, it is important to continue to research the relationships between trees and mycorrhizae in environments affected by anthropogenic change.

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Literature Cited


Rice, A. 2015. Effects of Long Term Nitrogen Deposition on Ectomycorrhizal Fungal Communities in Forests of the Northeastern United States. Ecosystems Center, Marine Biological Laboratory SES.


Figures and Tables

**Figure 1:** Sampling locations of Harvard Forest (Petersham, MA), Sutherland Woods (Lexington, MA), and Hammond Woods (Newton, MA) along the urbanization gradient as well as annual N deposition values (Rao et al. 2014).

**Figure 2:** Urbanization gradient sampling transect diagram

**Figure 3:** Sampling diagram for three soil samples per sample tree

**Figure 4:** Chronic Nitrogen Amendment sampling transect diagram

**Table 1.** Sample Name Key

**Table 2.** Ratio of wet to 68°C dry weight and ratio of 68°C dry to 110°C dry weight soil samples

**Figure 5.** A: Uncolonized root segment from Hammond Woods (HW07). B: Root segment colonized with *Cenococcum* from Harvard Forest-06 (HF06). Both photos were taken through the lens of a dissecting microscope.

**Figure 6.** (Mean +/- SD) Percent mycorrhizal colonization across an urban to rural and N addition gradient. The high N addition plot had significantly lower mycorrhizal colonization compared to all of the plots except for Sutherland Woods. See Table 3 for significant p-values.

**Figure 7.** Pools of ammonium and nitrate in combined O and A layer soil samples across an urban to rural and N addition gradient. Data from Maggie Anderson, SES Soil Project. These results were not statistically significant, partly due to the large variability among replicates.

**Figure 8.** (Mean +/- SD) Annual N-mineralization rates in combined O and A layer soil. Data from Maggie Anderson, SES Soil Project. Results were not statistically significant (p=0.8191).

**Figure 9.** Mean percent mycorrhizal colonization compared to mean soil nitrate pools in combined O and A layer soil samples. Nitrate Data from Maggie Anderson, SES Soil Project. This negative relationship has a statistically significant p value of 0.0142.

**Figure 10.** Mean percent mycorrhizal colonization compared to mean N-Mineralization Rate in combined O and A layer soil samples. N-Mineralization Data from Maggie Anderson, SES Soil Project. This negative relationship is statistically significant (p=0.0011).

**Figure 11.** Linear regression of C and N pools in the soil O layer. This positive relationship is statistically significant (p=<0.0001).

**Figure 12.** (Mean +/- SD) Soil O layer pH across an urban to rural and N addition gradient. Soil pH in Harvard Forest-04 is significantly different than in Hammond Woods and in the high N
addition plot. The pH in high N addition plot is significantly different than both of the rural sites. See Table 3 for significant p-values.

**Figure 13. (Mean +/- SD)** Soil pH and soil Exchangeable Calcium Concentrations Across an Urban to Rural and N Addition Gradient. Soil pH in Harvard Forest-04 is significantly different than in Hammond W and in the high N addition plot. The pH in high N addition plot is significantly different than both of the rural sites. See Table 3 for significant p-values. An ANOVA found a slight statistical significance in Soil Ca concentrations (p=0.0488), but the Tukey-Kramer HSD compared each pair and did not find a significant difference.

**Figure 14. (Mean +/- SD)** Soil exchangeable calcium and leaf litter Ca concentrations across an urban to rural and N addition gradient. An ANOVA found a slight statistical significance in soil Ca concentrations (p=0.0488), but the Tukey-Kramer HSD compared each pair and did not find a significant difference. Litter Ca concentration in the high N addition plot was significantly different than in Sutherland Woods and the two rural plots. See Table 3 for significant p-values.

**Figure 15.** Comparison of ratio of Ca in litter to soil with mean percent mycorrhizal colonization in the chronic N amendment sites. Percent mycorrhizal colonization was significantly lower in the high N addition plot compared to the low addition and control plots. See Table 3 for significant p-values. There ratio of litter to soil Ca was not statistically significant (p=0.2232).

**Figure 16. (Mean +/- SD)** Ratio of total leaf litter N to total leaf litter Ca. Site NDLo has no standard error bars since that site only had one successful replicate for leaf litter N. The high N addition plot has a significantly higher litter N:Ca ratio than the urban, rural and control N amendment sites. See Table 3 for significant p-values.

**Figure 17.** Linear regression of total N and Ca in leaf litter. This negative relationship is statistically significant (p=0.0167).

**Table 3.** P-values for comparison for all pairs using Tukey-Kramer HSD. All results were tested but only those with significant P values were reported.
Figure 1. Sampling locations of Harvard Forest (Petersham, MA), Sutherland Woods (Lexington, MA), and Hammond Woods (Newton, MA) along the urbanization gradient as well as annual N deposition values (Rao et al. 2014).
Figure 2. Urbanization gradient sampling transect diagram
Figure 3. Sampling diagram for three soil samples per sample tree
Figure 4. Chronic Nitrogen Amendment sampling transect diagram
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Table 2. Ratio of wet to 68°C dry weight and ratio of 68°C dry to 110°C dry weight soil samples

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**Figure 5.** A: Uncolonized root segment from Hammond Woods (HW07). B: Root segment colonized with *Cenococcum* from Harvard Forest-06 (HF06). Both photos were taken through the lens of a dissecting microscope.
Figure 6. (Mean +/- SD) Percent mycorrhizal colonization across an urban to rural and N addition gradient. The high N addition plot had significantly lower mycorrhizal colonization compared to all of the plots except for Sutherland Woods. See Table 3 for significant p-values.
Figure 7. Pools of ammonium and nitrate in combined O and A layer soil samples across an urban to rural and N addition gradient. Data from Maggie Anderson, SES Soil Project. These results were not statistically significant, partly due to the large variability among replicates.
**Figure 8.** (Mean +/- SD) Annual N-mineralization rates in combined O and A layer soil. Data from Maggie Anderson, SES Soil Project. Results were not statistically significant (p=0.8191).
Figure 9. Mean percent mycorrhizal colonization compared to mean soil nitrate pools in combined O and A layer soil samples. Nitrate Data from Maggie Anderson, SES Soil Project. This negative relationship has a statistically significant p value of 0.0142.
Figure 10. Mean percent mycorrhizal colonization compared to mean N-Mineralization Rate in combined O and A layer soil samples. N-Mineralization Data from Maggie Anderson, SES Soil Project. This negative relationship is statistically significant (p=0.0011).
Figure 11. Linear Regression of C and N pools in the soil O layer. This positive relationship is statistically significant (p=<0.0001).
Figure 12. (Mean +/- SD) Soil O layer pH across an urban to rural and N addition gradient. Soil pH in Harvard Forest-04 is significantly different than in Hammond Woods and in the high N addition plot. The pH in high N addition plot is significantly different than both of the rural sites. See Table 3 for significant p-values.
Figure 13. (Mean +/- SD) Soil pH and soil exchangeable calcium concentrations across an urban to rural and N addition gradient. Soil pH in Harvard Forest-04 is significantly different than in Hammond Woods and in the high N addition plot. The pH in high N addition plot is significantly different than both of the rural sites. See Table 3 for significant p-values. An ANOVA found a slight statistical significance in Soil Ca concentrations (p=0.0488), but the Tukey-Kramer HSD compared each pair and did not find a significant difference.
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Table 3. P-values for comparison for all pairs using Tukey-Kramer HSD. All results were tested but only those with significant P values were reported.

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