

Assessing Impacts of Atmospheric Nitrogen Deposition on New Jersey Forests 2002-2003

Final Report Year 1

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Executive Summary

The project scope was conceptualized at an inter-programmatic meeting within the New Jersey Department of Environmental Protection including representation by the Divisions of Science Research and Technology, Forestry, Air, Watershed Management and Endangered Species. A consensus was reached that a suite of bioindicators would prove critical in satisfying State management goals to enhance air and water quality; to restore ecosystems, sustain land and natural resource communities; as well as to preserve biodiversity in the state. The immediate goal was to establish unique baseline terrestrial communities of known structure in the Pinelands Ecoregion of New Jersey, then to evaluate the influence of potential environment stressors. Of particular interest were air deposited nitrogen and its measurable biological effects on forest ecosystems for long term trends-assessment. Methods are needed to benchmark nitrogen status in the Pinelands, and other air sheds in New Jersey, to track deposition trends and effects over time; that is, to develop a means to measure Nitrogen dry deposition effects (i.e., using bioindicators such as mycorrhizal fungi). Changes in macro-fungal species composition and abundance have been used in other parts of the world as biological indicators for terrestrial wooded ecosystems.

The objective of Year 1 of this study was to determine if ectomycorrhizal fungal species of trees in the New Jersey pine barrens could serve as a biological indicator for nitrogen pollution.

A transect of three sites at three State Parks in the New Jersey pine barrens, along a potential N deposition gradient, were established. Soils and root samples from these sites were used in the field and in greenhouse studies to evaluate the effects of additional N inputs.

Major findings were:

- Incoming rainfall at the two northern sites contained up to twice the amount of nitrogen than the southern site during the six months of collection.
- Maximum N input amounted to 0.1 kg ha⁻¹ inorganic N for the half year of collection.
- Abundance and species richness of ectomycorrhizae collected from the field decreased significantly along the transect from south to north.
- Experimental additions of N up to 140 kg ha⁻¹ showed a trend of decreasing mycorrhizal species richness on pine seedlings planted in the field, but no increase in N accumulation by those seedlings.
- Experimental additions of N between 35 and 140 kg ha⁻¹ to pine seedlings in a greenhouse study decreased overall pine seedling shoot mass, increased N content and reduced ectomycorrhizal species richness.
- There are suggestions from the data that *Russula*-like, *Cortinari*-like and *Lactarius*-like ectomycorrhizae are reduced in abundance as N additions to soil are increased. *Suillus*-like mycorrhizae appear to increase in abundance with added N.

In general, we can conclude that additions of N (Greenhouse Study), at significantly higher levels than found in the incoming rain, decreases seedling tree growth and reduces ectomycorrhizal species richness in the year of addition. In addition, field sites receiving naturally elevated levels of N (Rainfall) show a trend for reduced ectomycorrhizal species richness. The functional consequences of the reduced species richness on these ecosystems have not yet been explored.

In Year 2 of this study (ongoing) another transect of sampling locations has been established in three northern New Jersey State forests, on a west to east gradient. The goal of this second year is to measure N air deposition and to determine if a similar fungal response gradient may be discerned in these significantly different physiographic provinces of New Jersey (i.e., higher elevations, different soil, and vegetation types).

In Year 3 (Summer of 2004) the project will return to the New Jersey pine barrens sites (referenced in this report) and will perform a series of ecological measurements on trees and shrubs to investigate whether there are significant ecosystem function consequences for the reported reductions in ectomycorrhizae fungi species richness.

INTRODUCTION

Nitrogen deposition from atmospheric pollutants is a continuing threat to environmental health in the United States. Over the past few decades, studies have been conducted across Europe to describe N deposition effects on ecosystems and, in particular, ectomycorrhizal communities (Arnolds, 1991; Brandrud, 1995; Brandrud and Timmerman, 1998; Jonsson et al., 2000; Karen and Nylund, 1997; Peter et al., 2001; Taylor et al., 2000). As a result, species of ectomycorrhizae are emerging as likely indicators of nitrogen deposition. Determination of species likely to indicate areas of high N deposition in the United States, therefore, is useful.

Forests in Europe occur in areas receiving N deposition ranging from $> 50 \text{ kg ha}^{-1} \text{ yr}^{-1}$ to $\sim 13 \text{ kg ha}^{-1} \text{ yr}^{-1}$ for the lowest ambient amount (Brandrud, 1995; Wright et al., 1995; Emmett et al., 1998; Taylor et al., 2000). Forests in the pine barrens in New Jersey receive around $0\text{-}3 \text{ kg ha}^{-1} \text{ yr}^{-1}$. So even the lowest, most oligotrophic forests in Europe receive almost an order of magnitude more N. In Europe, this condition has lasted for several decades. Only one study, to our knowledge, has been conducted in North America (Lilleskov et al., 2001, 2002), and this was following decades of emissions downwind from an ammonia plant.

Results from these studies indicate that ectomycorrhizal species are more sensitive to N deposition than saprotrophic species (Arnolds, 1991; Peter et al. 2001) and ectomycorrhizae forming resupinate fruit bodies become more abundant in areas of high N deposition than those forming large mushrooms (Peter et al. 2001). Species of ectomycorrhizal fungi that are inhibited by high nitrogen have been termed “nitrophobic”. Genera such as *Russula* and *Cortinarius* have been identified as possible indicators since there is often a reduction in their numbers at high levels of N deposition (Arnolds, 1991; Brandrud, 1995; Taylor et al. 2000; Peter et al. 2001) along with *Tricholoma*, *Lactarius*, and *Hebeloma* identified by fruit body surveys (Lilleskov et al., 2001), and *Piloderma* spp., *Amphinema byssoides*, *Cortinarius* spp. and *Tomentella* spp. identified from root tips (Lilleskov et al., 2002). Other mycorrhizal taxa that are more nitrogen tolerant are called “nitrophilic”. These taxa include *Cantherellus*, *Lactarius theiogalus*, *Lactarius rufus*, *Laccaria*, *Paxillus involutus*, *Hygrophorus olivaceoalbus*, *Tylospora fibrillosa* (Brandrud, 1995; Taylor et al. 2000; Lilleskov et al., 2001; Erland and Taylor, 2002) and *Tomentella sublilacina* and *Thelephora terrestris* from root tips (Lilleskov et al. 2002). Additionally, fungi associated with hardwood species are thought to be more tolerant than fungi associated with coniferous species (Arnolds, 1991; Wallenda and Kotke, 1998; Taylor et al., 2000;), possibly because hardwood stands typically have higher N availability and therefore may associate with nitrophilic symbionts.

Overall decreases in species diversity and mycorrhizal colonization have been seen in some studies (especially, for ericoid mycorrhizae see Stribley and Read 1976; Yesmin et al., 1996 – cited in Rillig et al., 2002; for ectomycorrhizae - Erland et al. 1999 – cited in Erland and Taylor, 2000; Lilleskov et al., 2001), but not in all studies (increases, decreases or no response reviewed in Jansen and Dighton 1990; Wallenda and Kottke 1998; Cairney and Mehar 1999; Taylor et al. 2000; Treseder and Allen 2000).

Ectomycorrhizal fungal growth may increase to a point (especially while under nutrient deficient conditions) then decrease as nutrient saturation occurs. This has important implications since the initial pre-disposition (i.e. nutrient status) of the ecosystem may determine the response. There have also been reports of decreases in root tip density with increased N fertilization (Meyer 1962; Ahlstrom et al., 1988 – cited in Erland and Taylor 2000; Clemensson-Lindell and Persson, 1995) and reductions in percent colonization with increased pollution (Wollecke et al. 1999 – cited in Erland and Taylor 2002). Generally, there appears to be shifts in community composition, reduction in species diversity and in abundance of ectomycorrhizal fungi in response to nitrogen deposition.

Many European studies have measured mycorrhizal responses, but we are now formulating hypotheses regarding more specific mechanisms (than tolerance alone) responsible for the shifts in community composition. Ecosystem responses to N deposition may result in N-mediated declines in soil pH, base cation availability and toxic metal availability, resulting in changes in ectomycorrhizal community composition based on species optima of these parameters. It is also thought that the shift in C:N ratios in the stands may be a potential mechanism causing shifts in the mycorrhizal communities. “Carbohydrate to nitrogen supply ratios affect formation of EMF fruiting bodies, external hyphae, and root tips” (Rillig et al., 2002). Plants in areas of high N deposition are not N deficient since so much inorganic N is available. Consequently, the carbon cost of the mycorrhiza relative to the benefit of receiving N is reduced. Changes in host-plant nutrition may result in subsequent shifts in host-plant carbon allocation and receptivity to ectomycorrhizal fungi. There is a large carbon cost of assimilation of inorganic N into amino acids under high N conditions (Wallander, 1995 – cited in Lilleskov and Bruns, 2001) causing the mycorrhizal community to shift to species that either are more efficient at using the C received from the host, or to species that are C parasites (Lilleskov and Bruns, 2001). Increased inorganic N availability causes fungi to allocate C to amino acid production and away from carbohydrate production, causing a reduction in overall root growth (Peter et al., 2001). The physical structure is also changed in mycorrhizae experiencing high N environments (Soderstrom 1992, Karen and Nylund, 1996). Implications include:

- changes in mycorrhizal community structure resulting in changed plant community structure since below ground biodiversity affects aboveground diversity and productivity (van der Heijden et al., 1998)
- high levels of N input causing the system to be at or near critical load causing an increase in N tolerant species, a decrease in N sensitive species, a decrease in overall productivity leading to a decrease in C sequestration and N immobilization (acting as a feedback to decreased levels at which N saturation takes place) (Wallander and Kotke, 1998)
- long-term N inputs could lead to loss of EMF sporocarp and root tip diversity (Lilleskov et al., 2001; Lilleskov et al., 2002)

Therefore, ectomycorrhizae should be considered in establishing critical loads (Lilleskov et al., 2001).

We were curious whether these patterns would exist in oligotrophic systems that may not yet be saturated. We wondered whether we would observe the elimination of more, rare

and N sensitive species at this early stage that were not even recorded in the European or Alaskan studies (all of these systems had received N deposition for decades prior to study). Since nutrient availability is very low in the New Jersey pine barrens, taxa may be adapted to this condition and could be more sensitive than taxa in more nutrient rich systems. Effectively, a very small input (1-2 kg N ha⁻¹ yr⁻¹) would be doubling the current input. So the pine barrens may not be able to withstand the higher levels at which N deposition is regulated in other regions. An investigation of mycorrhizal responses to N deposition could be used to test this. We also planned to compare the indicator species we identified with those from European studies.

Objectives of the study

The main objective of this study is:

- To determine mycorrhizal species that can be used as indicators for atmospheric deposition of N within the outer coastal plain upland oak/pine forests of the New Jersey pine barrens.

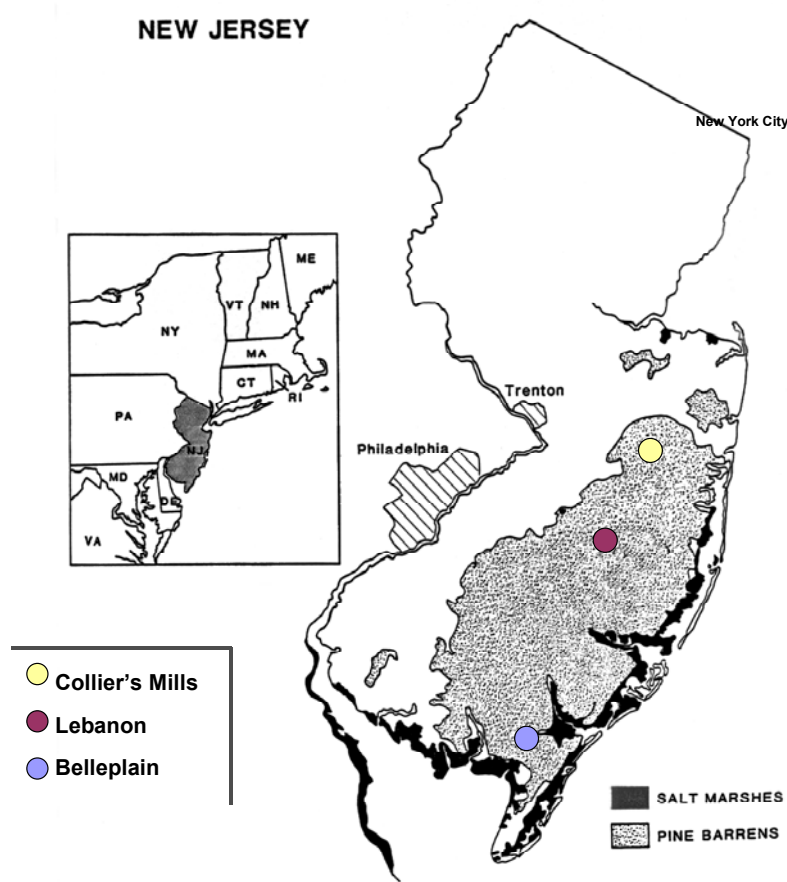
The individual tasks required to address this objective are:

1. Establish three field sites in the outer coastal plain of NJ along a potential N deposition gradient.
2. Quantify natural ectomycorrhizal populations along a deposition/emissions gradient within the outer coastal plain in south NJ.
3. Identify mycorrhizal species that appear to be affected by N levels along this gradient.
4. Quantify N deposition in bulk rainfall.
5. Analyze inorganic N stores in soils.
6. Confirm species sensitive to N by conducting field and greenhouse studies that manipulate N levels to determine whether the suspected species really are affected by N levels.
7. Analyze N in soils used for greenhouse studies.

METHODS

Site selection: Three sites on an approximate southwest to northeast transect of pitch pine (*Pinus rigida*) dominated forests were established in the NJ pine barrens (sites are named Belleplain (N 39° 14.45', W 74° 52.75'), Lebanon (N 39° 51.55, W 74° 29.43), and Collier's Mills (N 40° 04.24', W 74° 25.92') in order from southwest to northeast; Fig. 1). Sites were as similar in vegetation cover and soil type as could be found to ensure comparability except for geographic location. Rainfall collectors were positioned in the open near each site in order to determine the N loading of the site in bulk precipitation. Within each site, eight pitch pine trees were identified and numbered as sampling units. Soil cores were extracted from within 1 m of these trees for chemical analyses, ectomycorrhizal species composition and for experimental units in a greenhouse, N amendment study. Additionally, bait tree seedlings were planted at Lebanon within a 2 m radius on one side of selected trees to sample ectomycorrhizal fungi on their roots under the influence of nitrogen addition.

Fig. 1. Field sites for 2002 study.



Bulk Precipitation and Soil Chemistry

Rainfall collectors were installed at each site. Bulk precipitation, not influenced by the tree canopy, was collected and analyzed for nutrient content, particularly N.

Soil cores were divided into the upper organic horizon, comprising the leaf litter and partially decomposed humic material (O horizon) and underlying mineral soil (E and B horizon) collected to a depth of 5 cm below the O horizon. Total nutrient content (TN and TP) of the soil was determined on acid digested soil. Available nutrients ($\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) were determined on water extracts of the soil and $\text{NH}_4\text{-N}$ by KCl extraction. In addition, the amount of nitrogen contained in the microbial community was analyzed by a fumigation-extraction technique where the N is released from microbial biomass by chloroform fumigation (see Brookes et al., 1985) and referred to as microbial biomass N (MBN).

Ectomycorrhizal identification

Soil cores were taken from standardized distances from the base of individual pitch pine trees in each site. Roots were washed from the soil and ectomycorrhizal fungal species associated with these roots identified by morphological characters identified by observation through a stereoscope as well as microscopic features such as mantle

structure. Community structure analysis and diversity indices were compared between sites.

Bait Seedlings

In addition to the study of roots from mature trees, pitch pine seedlings were planted in the field at one site (Lebanon) to act as mycorrhizal bait plants. These seedlings were subjected to differential N addition at zero, 17.5, 35, 70 and 140 kg ha⁻¹ equivalent. Seedlings recovered from the field were assessed for biomass, nutrient content (TKN and TP) and mycorrhizal community structure.

Greenhouse Study

A greenhouse experiment was established using soil from all three field sites. Intact soil cores were planted with pitch pine providing a factorial experiment of tree/no tree cores, within which was a three level N addition treatment (zero, 35 and 140 kg ha⁻¹ equivalent), simulating potential N deposition. Plant growth parameters and the ectomycorrhizal community structure on the seedlings were measured, along with soil chemistry. Soils were compared between N treatments, sites, and cores that contained trees and those that did not in order to observe the effect of a tree seedling as a sink for nitrogen.

Statistical Analyses

Statistical analyses were conducted in SAS (release 8.02 TS Level 02MO, Windows version 4.90.3000; 1999-2001). All data were tested for normality using the SAS procedure UNIVARIATE and for homogeneity of variance by performing Levene's test for equality of variance with the absolute value of residuals option in SAS. All soil chemistry, water chemistry, and field seedling data were ranked (Conover and Iman 1981) and analyzed by General Linear Model (GLM) Analysis of Variance (ANOVA). All field core ectomycorrhizal data were square root transformed; greenhouse measures were square root transformed except for root:shoot ratios, which were log transformed, then all were analyzed by GLM ANOVA. For the field cores, two-way ANOVAs were conducted with site and date as the independent variables. For field seedlings, one-way ANOVAs were conducted with N addition treatment as the independent variable. For greenhouse seedlings, two-way ANOVAs were conducted with site and N addition treatment as the independent variables. ANOVAs were followed by Tukey means separation where appropriate.

RESULTS

Field Study

Relation of Nitrogen Loading of Sites to Ectomycorrhizal Community Properties

The nitrate nitrogen loading (mg N m⁻² (100 mg m⁻² ≡ kg N ha⁻¹)) of the sites is presented in Fig. 2, along with inorganic nitrogen from ammonium and nitrate (Fig. 4). It can be seen that there is a general trend of increasing N deposition from the southwestern to northeastern sites (see also Appendix 1). These data suggest that we have identified a true N deposition gradient, at least for the year of study. When compared to ectomycorrhizal richness (in spring; Fig. 3) and abundance (in fall; Fig. 5), mycorrhizae are decreased both in numbers of species and in numbers of root tips along this gradient. This relationship is supported by a regression between NO₃⁻ and ectomycorrhizal richness

(Fig. 6). Though the relationship between input in rainfall and ectomycorrhizal response was significant, there were no significant regressions between ectomycorrhizal parameters and soil nutrient chemistry or microbial biomass N.

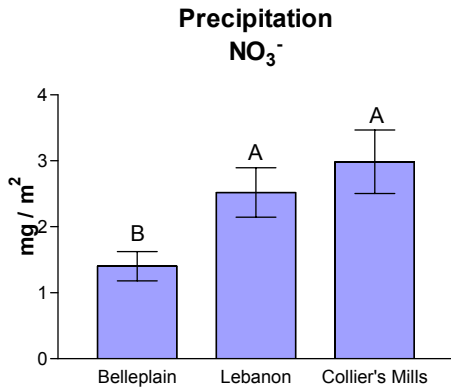


Fig. 2. Total nitrate nitrogen deposited on the three sites during the summer months of 2002. Differences between sites are significant at $P < 0.001$.

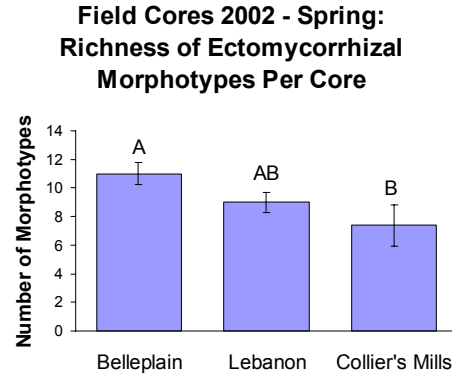


Fig. 3. Ectomycorrhizal richness decreased from Belleplaine to Collier's Mills. Differences between sites are significant at $P < 0.05$.

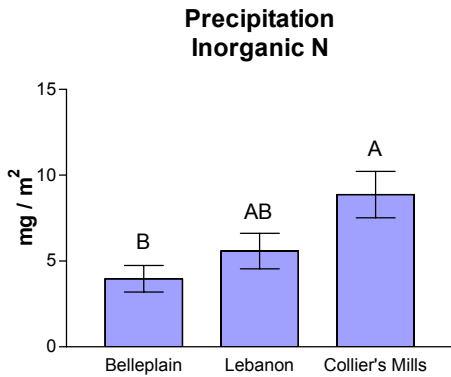


Fig. 4. Total ammonium plus nitrate nitrogen deposited on the three sites during the summer months of 2002. Differences between sites are significant at $P < 0.05$.

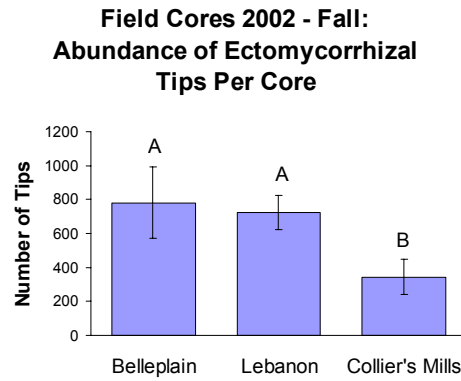


Fig. 5. Abundance of ectomycorrhizal root tips decreased from Belleplaine and Lebanon to Collier's Mills. Differences between sites are significant at $P < 0.05$.

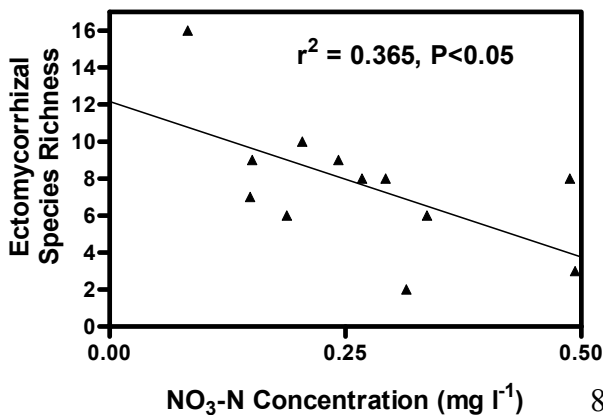


Fig. 6. Regression of fall ectomycorrhizal species richness and nitrate N concentration in rainfall over all data between the three sites, Belleplaine, Lebanon and Collier's Mills. Collier's Mills has the highest N deposition rates.

Soil Chemistry

Results of these analyses are shown for spring in Figs. 7, 8, and 9 for the upper, organic soil horizons, and Figs. 10 and 11 for the mineral soil. Likewise, results from fall are shown in Figs. 12, 13, and 14 for the litter and humus layer and in Figs. 15 and 16 for mineral soil. There were no differences in soil chemistry in spring between the sites. Consequently, there is no detectable increase in nitrogen to match the elevated levels of deposited N in Collier's Mills, compared to the other sites. In fall, however, microbial biomass N was significantly higher at Collier's Mills ($P < 0.01$) than the other sites, suggesting that, at this time of year, atmospherically deposited N may be immobilized in the microbial community. Phosphate phosphorus is significantly lower in availability in Collier's Mills than Lebanon ($P < 0.05$; see Appendix 1). Similar to spring, there appear to be no differences between sites in organic or inorganic N. Overall, levels of nutrients in the mineral soil horizon are much lower than the organic horizon, but in neither horizon was there an increase in concentration of nutrients relative to increased input.

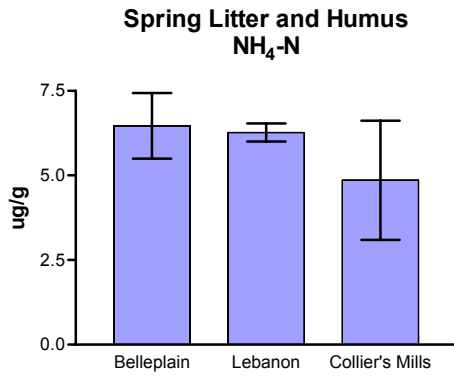


Fig. 7. Ammonium in litter and humus in spring.

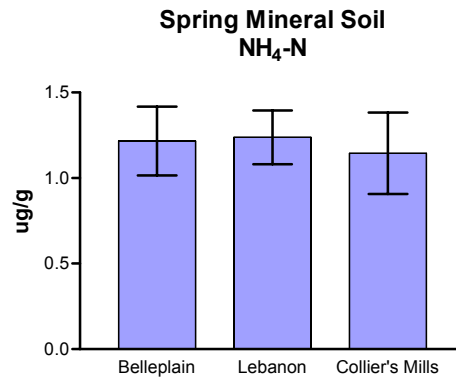


Fig. 10. Ammonium in mineral soil in spring.

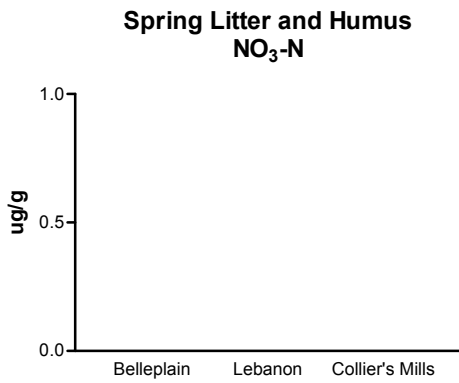


Fig. 8. Nitrate in litter and humus in spring.

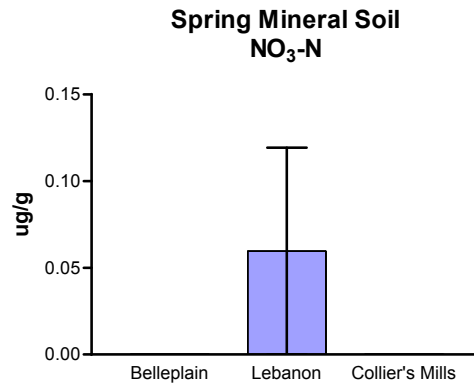


Fig. 11. Nitrate in mineral soil in spring.

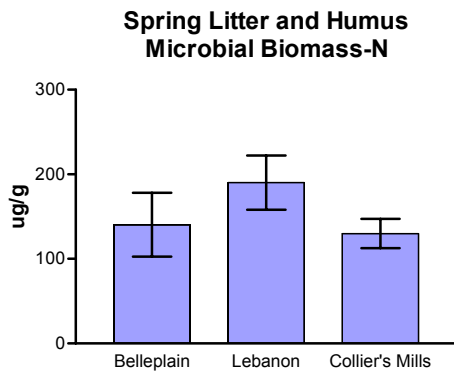


Fig. 9. Microbial biomass nitrogen in litter and humus in spring.

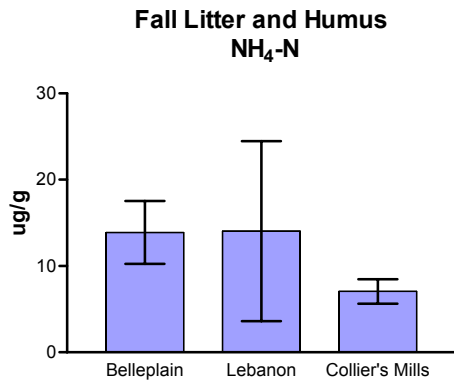


Fig. 12. Ammonium in litter and humus in fall.

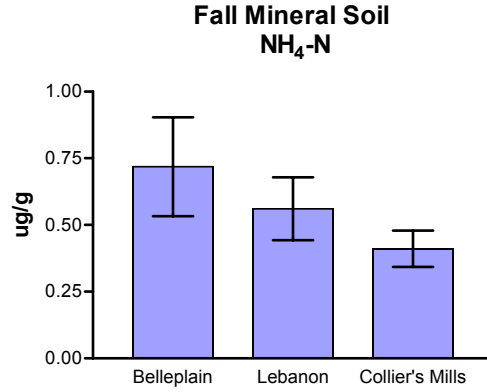


Fig. 15. Ammonium in mineral soil in fall.

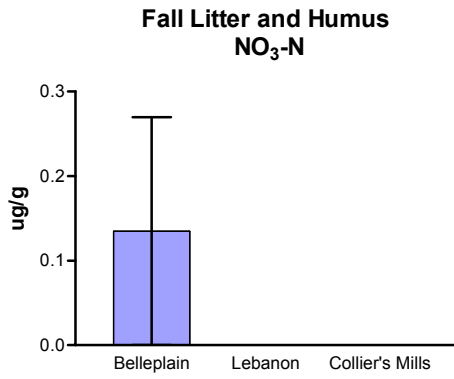


Fig. 13. Nitrate in litter and humus in fall.

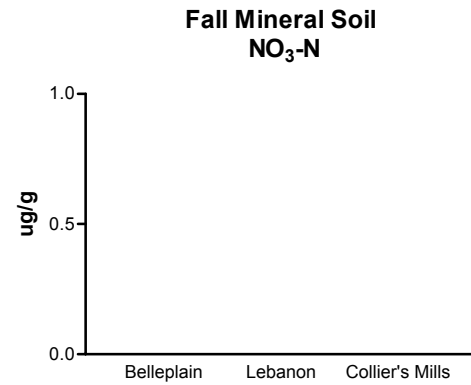


Fig. 16. Nitrate in mineral soil in fall.

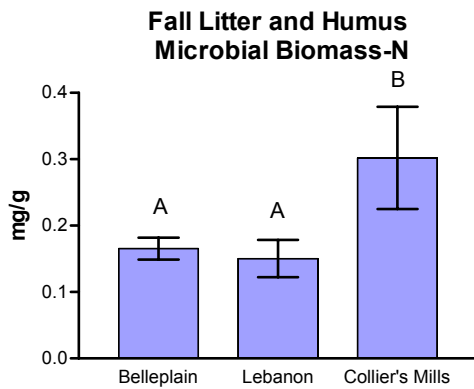


Fig. 14. Microbial biomass N in litter and humus in fall.

Relation of Bulk Precipitation Chemistry to Soil Chemistry

Interestingly, regressions between rainfall chemistry and soil chemistry show significant negative regressions between concentration of ammonium N in rainfall, total amount of ammonium in rainfall, inorganic N concentration in rainfall, and total inorganic N in rainfall with soil ammonium N concentration (Table 1). In opposition, there is a positive relationship between the above noted rainfall parameters and soil PO₄-P concentration.

Table 1. Regression parameters between rainfall chemistry and soil nutrient content.

Rainfall	Soil	r²	Slope	Significance of slope from zero
NH ₄ -N (mg l ⁻¹)	NH ₄ -N (μg g ⁻¹)	0.549	- ve	P<0.01
NH ₄ -N (mg)	NH ₄ -N (μg g ⁻¹)	0.530	- ve	P<0.01
Inorg-N (mg l ⁻¹)	NH ₄ -N (μg g ⁻¹)	0.518	- ve	P<0.01
Inorg-N (mg)	NH ₄ -N (μg g ⁻¹)	0.358	- ve	P<0.05
NH ₄ -N (mg l ⁻¹)	PO ₄ -P (μg g ⁻¹)	0.351	+ ve	P<0.05
NH ₄ -N (mg)	PO ₄ -P (μg g ⁻¹)	0.437	+ ve	P<0.05
Inorg-N (mg l ⁻¹)	PO ₄ -P (μg g ⁻¹)	0.362	+ ve	P<0.05
Inorg-N (mg)	PO ₄ -P (μg g ⁻¹)	0.446	+ ve	P<0.05

Bait Seedling Study

Effectively, there were no differences between fertilizer levels in seedling performance (seedling height, shoot weight, root weight or root:shoot ratio; Appendix 2). The only statistically significant difference was in shoot weight, between 17.5 and 35 kg ha⁻¹ N treatments. Similarly, ectomycorrhizal colonization of roots of seedlings placed in the field yielded few differences between levels of N addition in abundance, richness (Fig. 19) or diversity, when expressed on a per seedling basis. However, when total ectomycorrhizal species richness was calculated over all seedlings, there was a trend of decreasing richness as N deposition increased (Appendix 2). Seedling TKN shows a bit of the opposite trend, somewhat mirroring patterns seen in greenhouse seedlings (see below) and at field sites: where there is higher N availability through increased input, richness decreases and N content in seedlings increases. This pattern is not significant in the field seedling study.

Field Seedling Study 2002: Total Richness

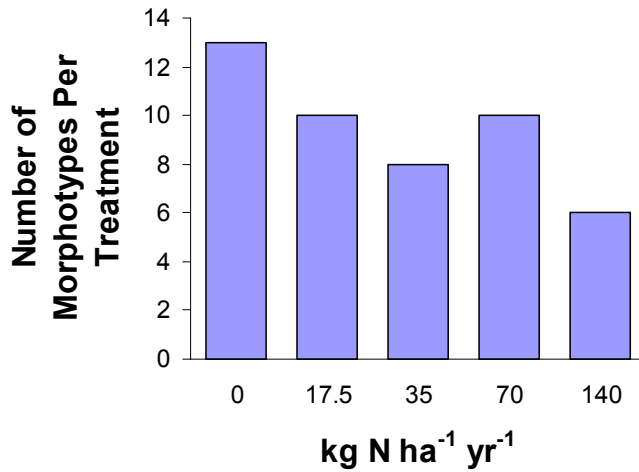


Fig. 19: Total ectomycorrhizal species richness on tree seedlings planted in the field at all of the field sites and exposed to differential levels of N addition. The trend of decreasing richness with increasing N deposition is apparent.

Field Seedling Root TKN

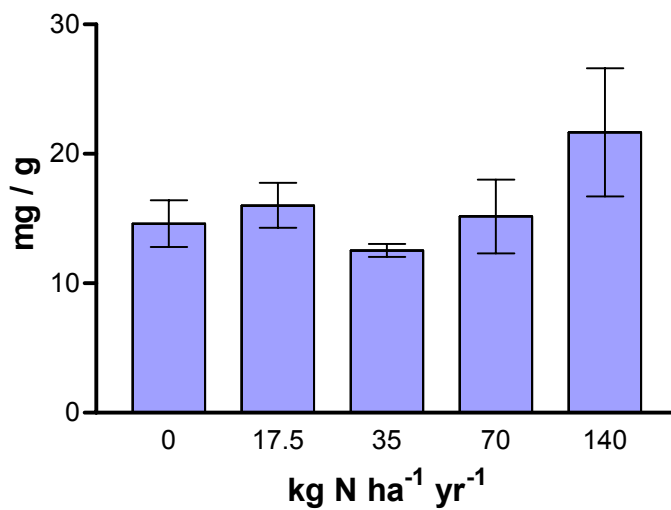


Fig. 20. Total Kjeldahl nitrogen from roots of seedlings planted in the field at all of the field sites and exposed to differential levels of N addition. The trend of increasing N content in roots with increasing N deposition is only somewhat apparent and not significant.

Greenhouse Study

Seedling tree growth showed significant decreases with increasing N addition in the greenhouse for seedling height, shoot and root mass (Fig. 21, Appendix 3), but the shoot:root ratio remained similar over all levels of N addition and across all sites. Likewise, seedling height, shoot and root mass were decreased at the site with the greatest amount of N deposition, Collier's Mills (Appendix 3).

Ectomycorrhizal community structure evaluated on the greenhouse seedlings showed that there were significant reductions in mycorrhizal abundance and species richness (e.g. Figs. 22) with increasing N addition and a trend of reduced diversity. There is also a significant difference in overall richness between the ectomycorrhizal community from seedlings in Lebanon soil (highest) and Collier's Mills (lowest).

Total ectomycorrhizal species richness in the greenhouse seedlings also showed a very slight trend of decreasing richness with increased N addition, but a more pronounced decline from Belleplain to Collier's Mills. In a comparison of the dominant ectomycorrhizal morphotypes between N addition levels and across all soils, the abundance of both "Brown/White-DB" and "Brown/White DB-Y/Br", both likely Russuloid types, declined significantly from no addition of N to both N addition levels ($F = 13.02, P < 0.0001$; $F = 5.87, P < 0.01$ respectively – mean occurrences 269.5, 108.4, 57.7 and 93.9, 19.3, 4.4 for 0, 35 and 140 kg ha⁻¹ N respectively). This suggests that these two mycorrhizal morphotypes may be indicators of N deposition. The number of dead and non mycorrhizal roots also declined with increased N addition ($F = 4.76, P < 0.05$ and $F = 10.88, P < 0.001$ respectively).

Seedlings accumulated more N in the shoots (Fig. 23) with increased N addition, but less P (Fig. 24). Soils showed a significant increased extractable N as ammonium, nitrate and total inorganic N in the litter and humus with increasing addition of exogenous N ($P < 0.001$ for all), (Figs. 27-28). However, this accumulation only occurred in soil cores in which tree seedlings were growing (compare with Figs. 25-26). This suggests that where there are no trees planted in the cores, N is in excess of microbial demand, even in soils with no added N. Where trees are planted, only in higher levels of N addition does N availability exceed plant demand (35 kg ha⁻¹ yr⁻¹ for NH₄⁺ and 140 kg ha⁻¹ yr⁻¹ for NO₃⁻), resulting in detection of extractable inorganic forms of N. Similar trends in the extractable nutrients were seen in the mineral soil horizons (Appendix 3), but here a significant decrease ($P < 0.001$) in extractable nitrate N was seen with increased addition of N in the unplanted soil cores. In planted cores there was an increase in extractable ammonium ($P < 0.001$), nitrate ($P < 0.01$) and total inorganic N ($P < 0.001$) with increased N addition.

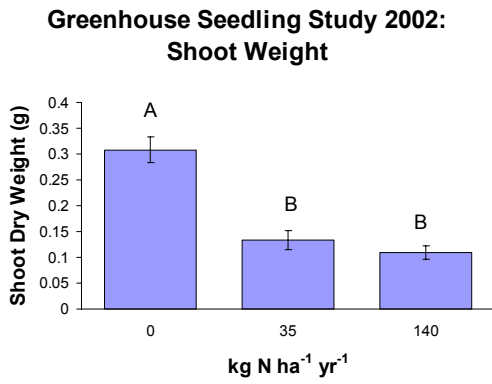


Fig. 21. Decreased seedling shoot weight with N addition.

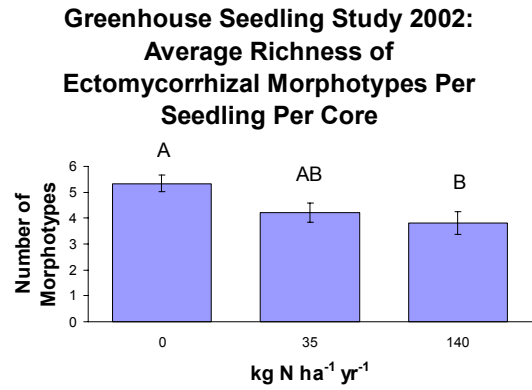


Fig. 22. Decreased ectomycorrhizal richness with high N addition.

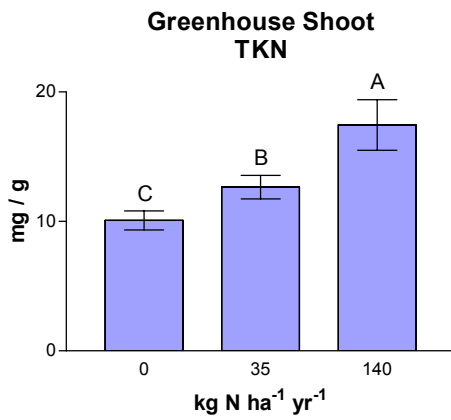


Fig. 23. Greenhouse seedling shoot Total N concentration.

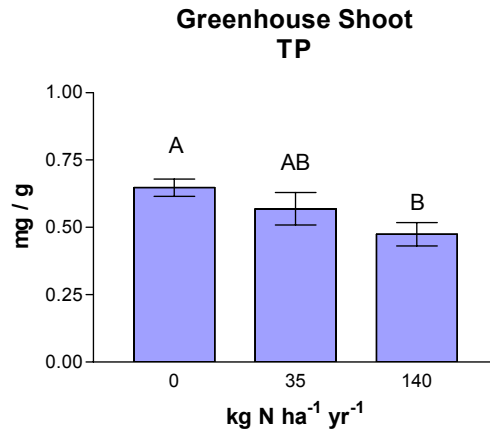


Fig. 24. Greenhouse seedling shoot Total P concentration.

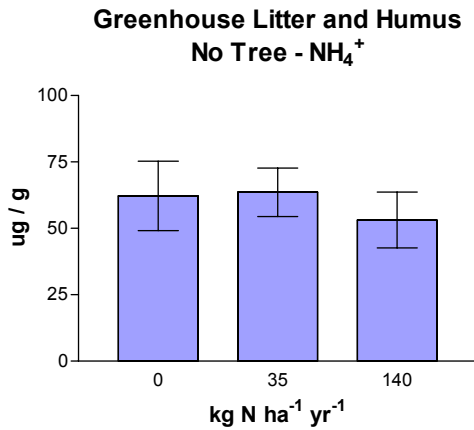


Fig. 25. Ammonium concentration in litter and humus extracts in cores without seedlings.

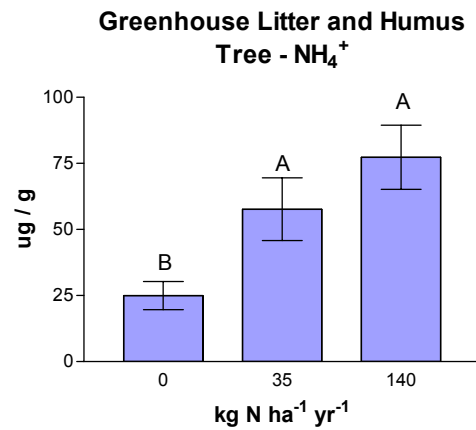


Fig. 27. Ammonium concentration in litter and humus extract in cores with trees.

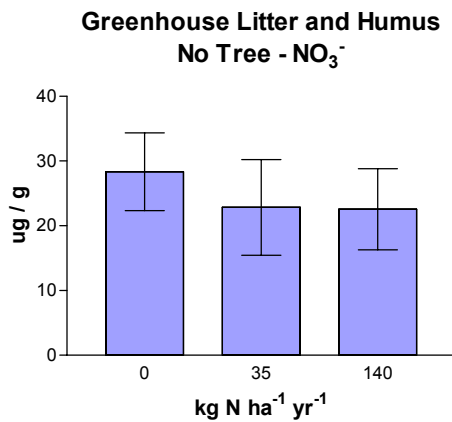


Fig. 26. Nitrate concentration in litter and humus extracts in cores without seedlings.

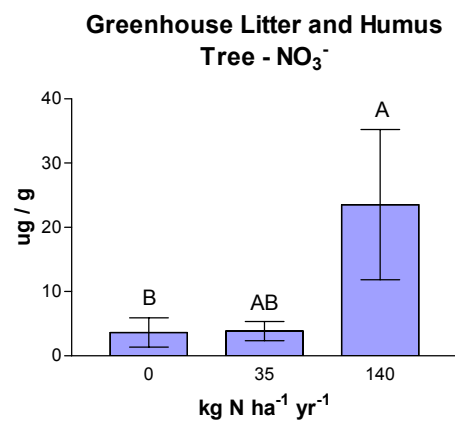


Fig. 28. Nitrate concentration in litter and humus extracts in cores with trees.

Potential Ectomycorrhizal Indicators

Several ectomycorrhizal morphotypes were identified as potential indicator species based on their abundance at high versus low levels of N addition in the greenhouse (Table 2) and at field sites (Table 3) with high versus low levels of inorganic N input (see also Appendix 4.)

Table 2. Abundance of ectomycorrhizal tips counted in the greenhouse seedling experiment (based on average abundance per core).

	0 kg N ha ⁻¹ yr ⁻¹	35 kg N ha ⁻¹ yr ⁻¹	140 kg N ha ⁻¹ yr ⁻¹
<u>Potential Nitrophobic Types:</u>			
Brown-shiny*	71.5	0.0	0.0
Brown/white–DB (Russula-like)	4043.5	1627.0	865.5
Brown/white–DB/YBr	1305.0	111.0	6.0
Copper (Cortinarius-like)	45.0	0.5	1.0
White-bulbous	37.5	1.5	0.5

Table 3. Abundance of ectomycorrhizal tips counted in cores from mature tree roots in the field experiment (based on combined abundance from the spring and fall sample dates).

	Belleplaine	Lebanon	Collier's Mills
<u>Potential Nitrophobic Types:</u>			
Brown/white–DB (Russula-like)	672	232	179
Green/yellow	178	68	0
Yellow-lactarius (Lactarius-like)	509	10	0
Brown-bristly, pinnate	71	0	0
Chartreuse	97	0	0
Yellow-bulbous	123	0	0
<u>Potential Nitrophilous Types:</u>			
Coral	0	0	418
Pumpkin (Suilloid-like)	11	24	49
White/brown-rhizopogon-like	0	0	90
Y/Br (Ascomycete)	693	1066	1391
Yellow/white	0	0	49
Yellow-tuber	4	8	79

Of the mycorrhizal morphotypes described from the field seedling study, only the "Y/Br" Ascomycete morphotype was seen to respond to N addition over all sites with a significant increase in abundance at 70 kg ha⁻¹ of N addition compared to 35 kg ha⁻¹ (F=3.24, P<0.05), similar to results from field cores (Table 3, Appendix 4). Non mycorrhizal root tips were more abundant in 35 kg ha⁻¹ N than in either zero or 17.5 kg ha⁻¹ (F=2.97, P<0.05). However, there were not enough differences to suggest any mycorrhizal morphotype could be used as an indicator species from this small field fertilization study.

After initial problems with the PCR methodology, we have resolved the major difficulties and have established RFLP types for all of the observed morphotypes in field cores and the greenhouse study. We are continuing to analyze the banding patterns using computer software to allow us to make comparisons with patterns obtained from fungal fruitbodies and published databases.

Discussion

From the Alaska study, environmental data explaining nitrophobic and nitrophilic species were soil base cations, organic horizon mineral N, organic horizon net mineralization for fruit bodies (Lilleskov et al., 2001) and organic horizon mineral N and foliar nutrient ratios (N:P and P:Al) for root tip richness (Lilleskov et al., 2002) and organic horizon NO₃⁻ availability for abundance (Lilleskov et al., 2002). We did not find an association between ectomycorrhizal community properties and any soil chemistry variables, but communities did shift in areas of or treatments with high inputs of inorganic N. This is expected from results of other published studies. We might also expect to find generalists (both resource generalists and host generalists) at Collier's Mills or in our greenhouse high N treatment. Ectomycorrhizal species that have the enzymatic ability to utilize proteins are reduced in number or eliminated in areas with high N deposition (Taylor et al., 2000). Ectomycorrhizal species that do utilize protein, but that can switch the enzyme pathway off and take up inorganic N (resource generalists, e.g. *Paxillus involutus*) can survive in areas with high N deposition. Ectomycorrhizal species that are host generalists do better in areas of high N deposition than do host specialists (Wallenda and Kotke, 1998).

Lilleskov and Bruns (2001) suggest that atmospheric deposition may lead to long-term shifts in ectomycorrhizal community structure, possibly resulting from decreased abundances of fruit bodies dispersing spores. We may have already seen this change occur in our northern most site, Collier's Mills, as indicated by both field and greenhouse species richness data.

It is likely that the field fertilization study did not show the same patterns, or at least significant patterns, since the summer was relatively dry and nutrient availability is linked to moisture (Tuininga et al., 2002). However, when total ectomycorrhizal species richness was calculated over all seedlings, there was a trend of decreasing richness as N deposition increased. This concurs with information from the literature that plants become less dependent upon mycorrhizae as soil fertility increases. In this case it is possible that species richness of ectomycorrhizae decreases as the community becomes less dependent on mycorrhizal species capable of obtaining N from organic sources as inorganic N supply increases.

We have demonstrated strong indications that ectomycorrhizal community structure is likely influenced by the N deposition pattern both in field and greenhouse studies. A reduction in ectomycorrhizal abundance and species richness is in line with published data that show a reduced dependency of plants on their mycorrhizal symbionts as soil nutrient availability increases. The reduction in species richness probably represents the reduced functional diversity required under conditions of increased soil fertility. In

oligotrophic conditions, ectomycorrhizal diversity will be maintained, allowing the presence of species, which can both access nutrients from readily available inorganic sources and from organic sources by virtue of enzyme production. With increasing availability of inorganic forms of nutrient, species exhibiting high enzyme expression are likely to be competed against. The trends observed here might be shown to be more significant with increased sample size.

The significant reduction in extractable phosphate in the litter and humus and strong, but not significant trend in the mineral soil, with increasing N addition may be a factor relating to tree seedling performance. In these oligotrophic soils, P may be a more limiting factor than N for plant and microbial growth, though it is clear that increased N levels inhibit plant and mycorrhizal growth. It is suggested here that the addition of N increases P deficit, such that there is no available P for extraction. It is likely a combination of P limitation and N toxicity causing the responses we observed. In the unplanted cores we suggest that microbial immobilization is increased sufficiently with added N that PO_4 is reduced to a greater extent than that seen for N decrease. In the planted cores, tree uptake of N decreases the availability of N at low N supply, but reduced plant growth and a high availability of inorganic forms of N appear at high rates of N addition as plant growth.

The scale of nutrient addition is of importance. It is possible that the New Jersey pine barrens would become saturated with very small additions of N. The amount of N being added at Collier's Mills over the amount at Belleplain is only $0.03 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, yet it is triple. We have seen changes in mycorrhizal communities between these sites and taxa that have been identified as indicators in other systems are among those that shift in abundance or presence. The implication is that in oligotrophic systems such as the New Jersey pine barrens, plants and microbes may be adapted to very low levels of nutrient availability and may respond to extremely small increases in deposition of N.

Conclusions

A gradient in NO_3^- and inorganic N deposition was identified increasing from southwestern to northeastern field sites. Curiously, nitrogen concentration in both field and greenhouse soils did not vary by site, indicating either that soils are already saturated and excess nutrient was leached or, more likely, that additions are moderated by biological activity, either saprotrophic or mycorrhizal. Changes in mycorrhizal abundance and richness followed the predicted pattern, decreasing at the higher N deposition sites. Indicator species were identified that follow those predicted from European studies, though exact confirmation of species is still required. Similar to previously published studies, we saw reductions in abundance of Russula-like mycorrhizal root tips in areas of higher N deposition. This pattern was confirmed by the greenhouse study. We also saw reductions in a Cortinarius-like type and a Lactarius-like type from the southwestern to northeastern sites. Unlike other reports, we found potentially nitrophilic Suillus-like and Ascomycete types. This method for identification of expected indicator species appears useful.

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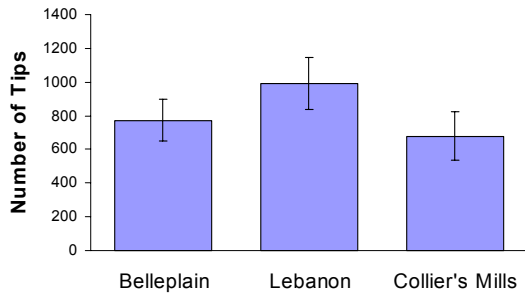
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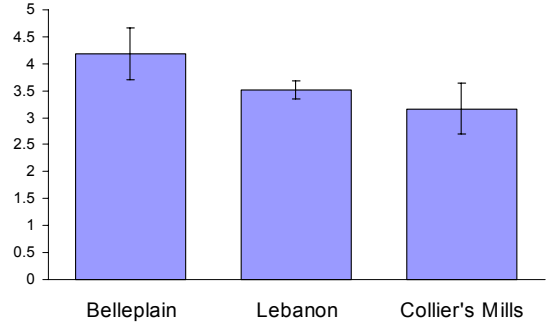
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Appendix 1. Field core ectomycorrhizal, precipitation, and soil chemistry results.

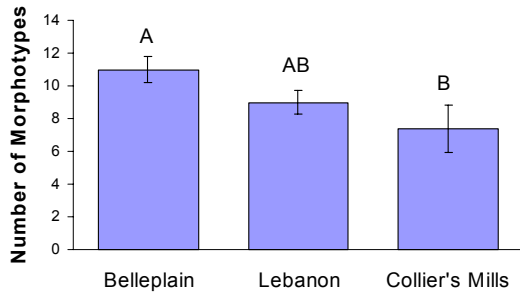
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Root Tips Per Core**



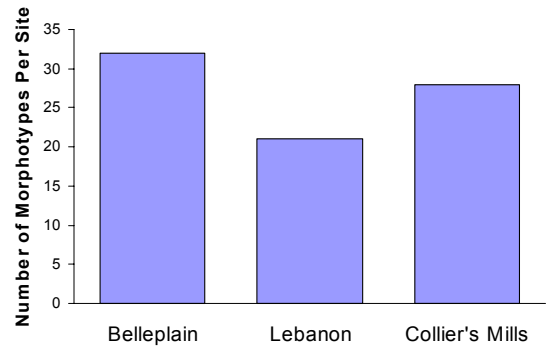
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Simpson's Diversity Per Core**



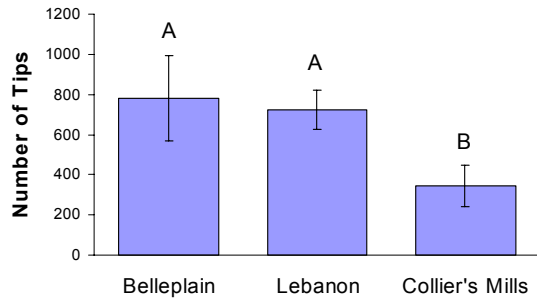
**Field Cores 2002 - Spring:
Richness of Ectomycorrhizal
Morphotypes Per Core**



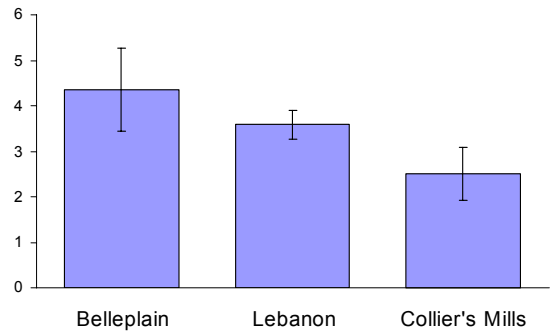
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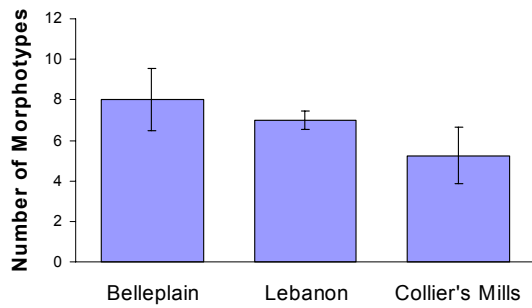
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Abundance of Ectomycorrhizal
Tips Per Core**



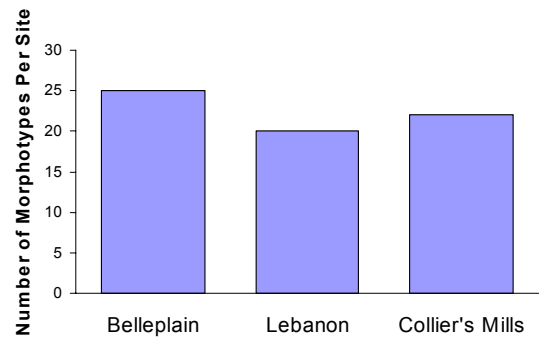
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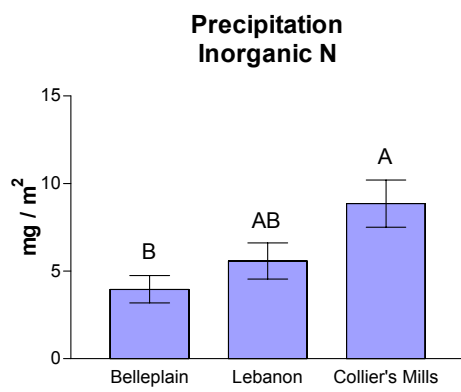
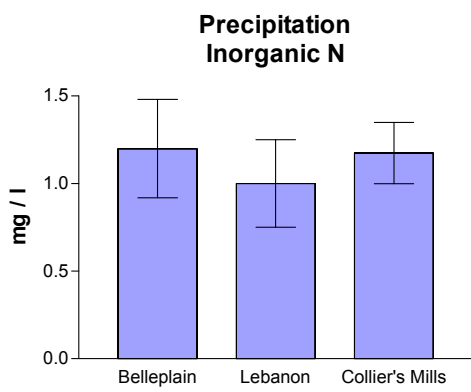
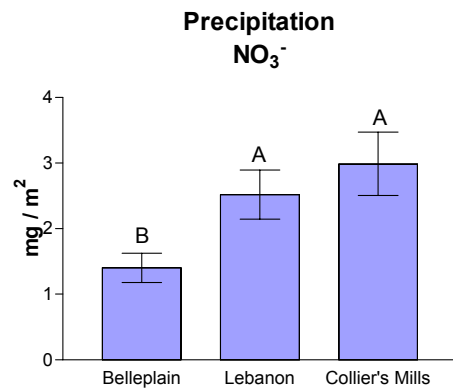
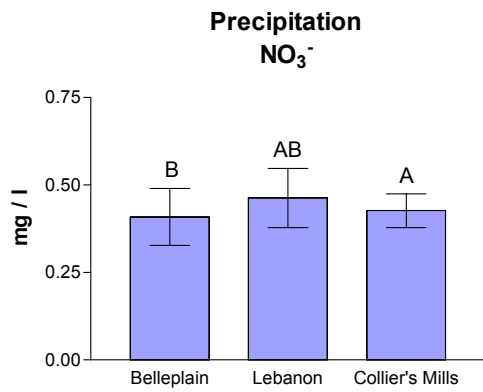
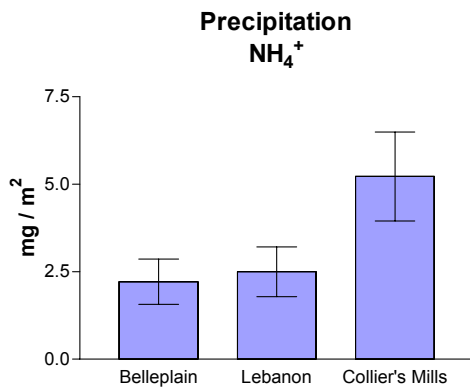
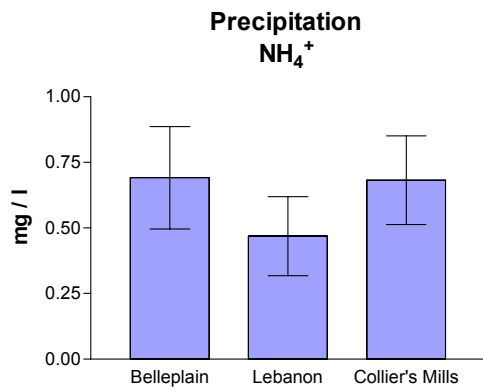


**Field Cores 2002 - Fall:
Richness of Ectomycorrhizal
Morphotypes Per Core**

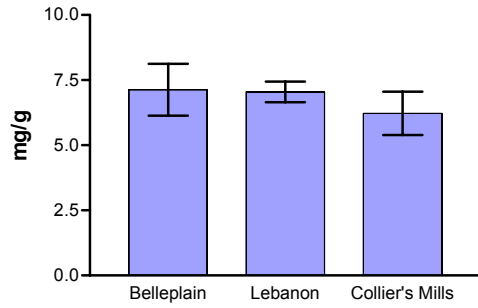


**Field Cores 2002 - Fall:
Total Richness Per Site**

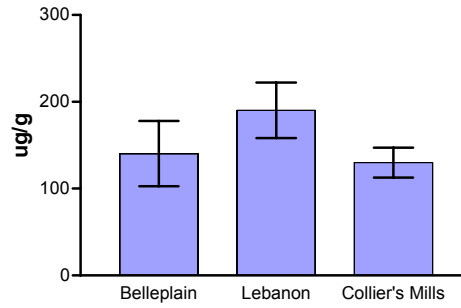




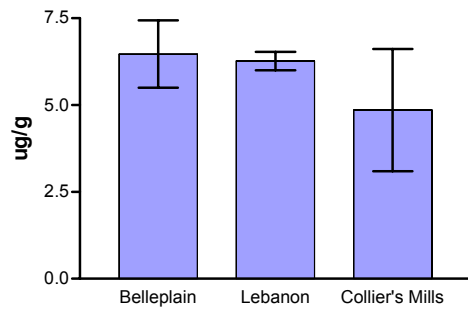
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Total-N**



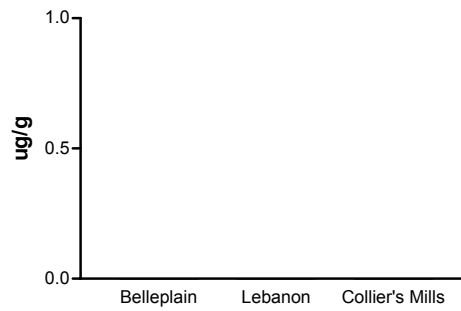
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Microbial Biomass-N**



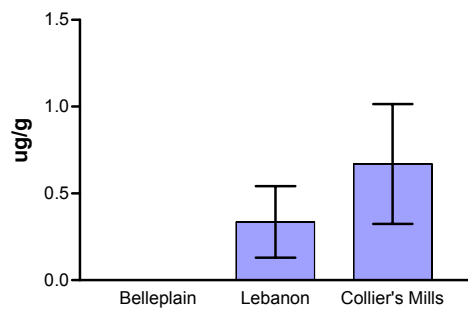
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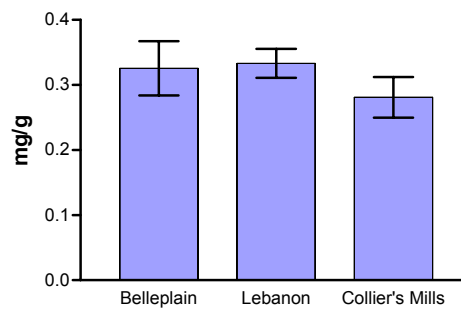
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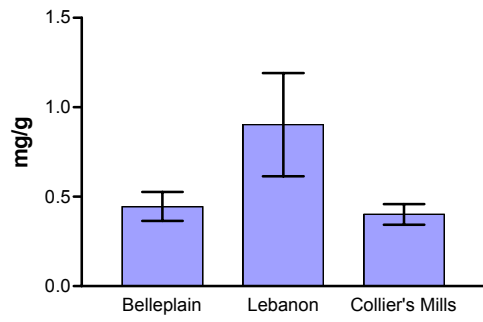
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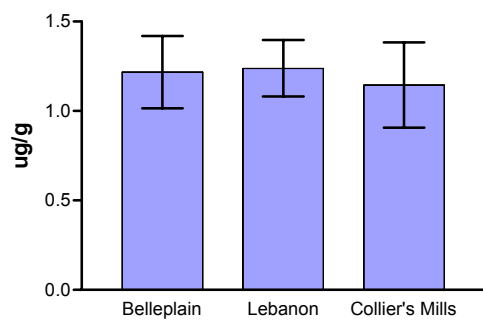
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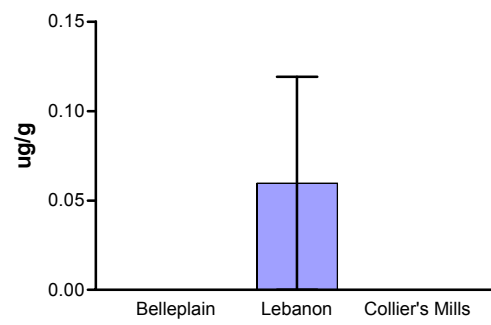
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**Spring Mineral Soil
NH₄-N**



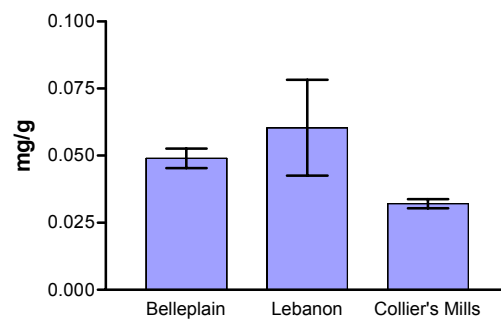
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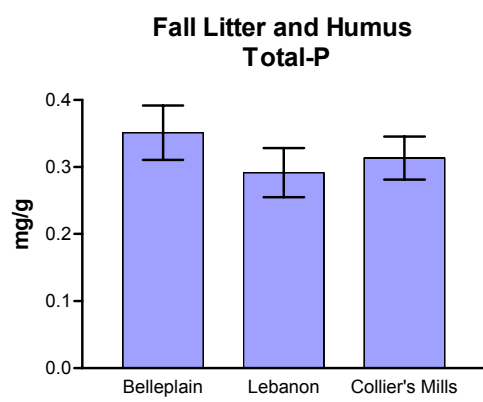
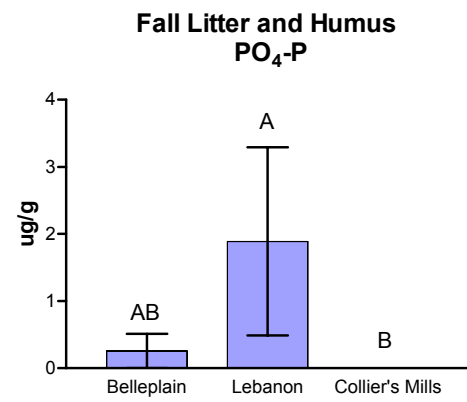
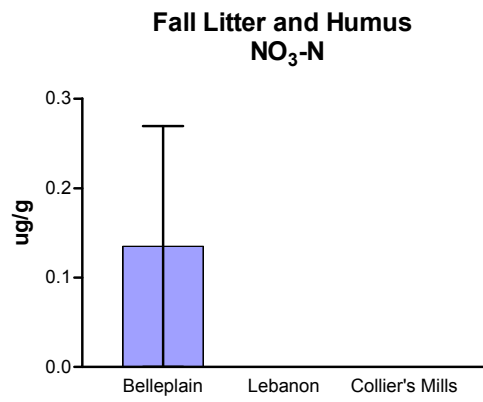
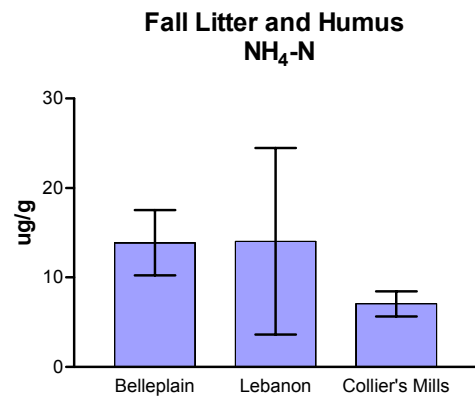
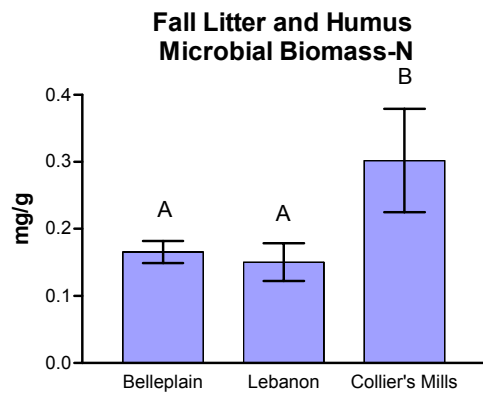
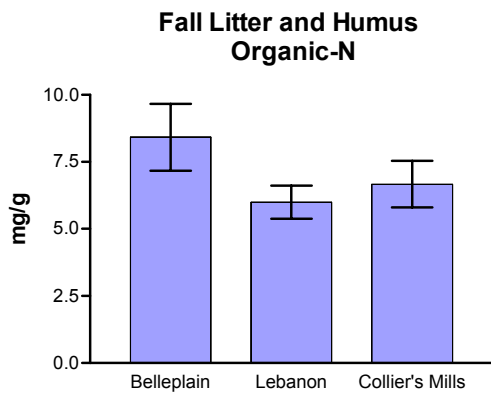


**Spring Mineral Soil
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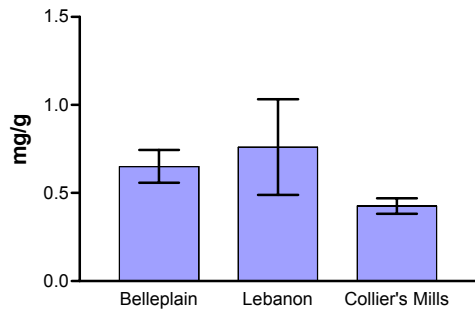


**Spring Mineral Soil
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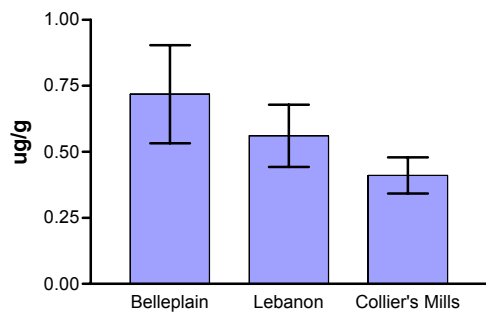




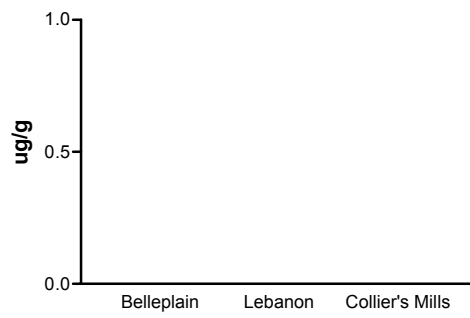
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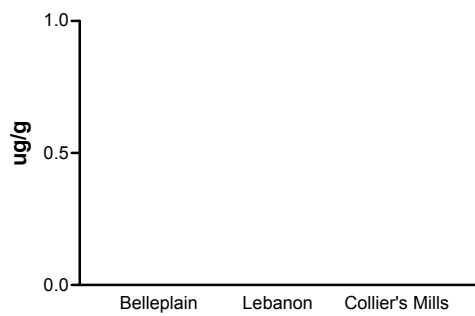
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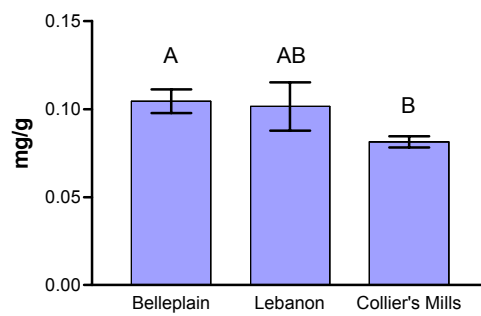
**Fall Mineral Soil
NO₃-N**



**Fall Mineral Soil
PO₄-P**

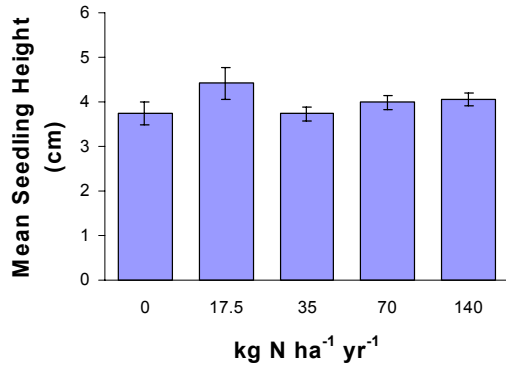


**Fall Mineral Soil
Total-P**

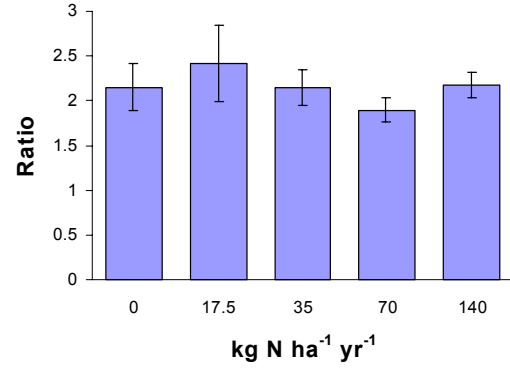


Appendix 2. Field seedling study ectomycorrhizal and chemistry results.

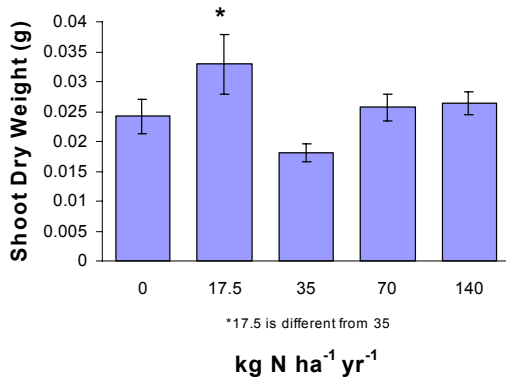
**Field Seedling Study 2002:
Seedling Height**



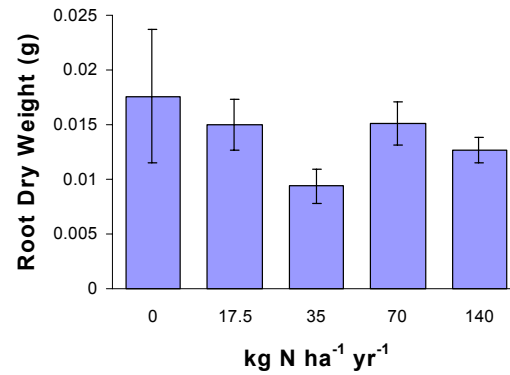
**Field Seedling Study 2002:
Shoot:Root Ratio**



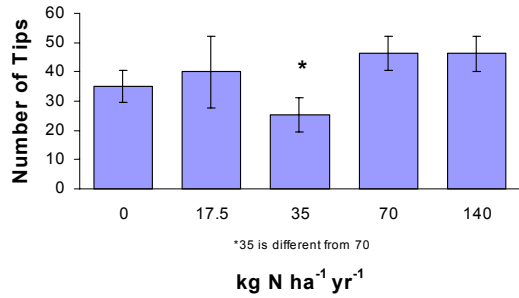
**Field Seedling Study 2002:
Shoot Weight**



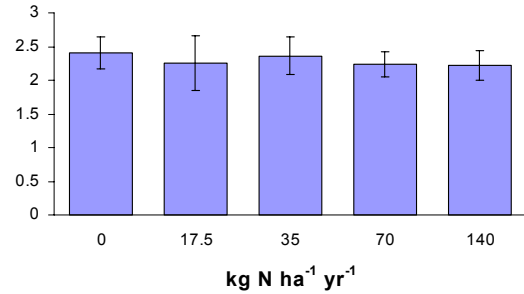
**Field Seedling Study 2002:
Root Weight**



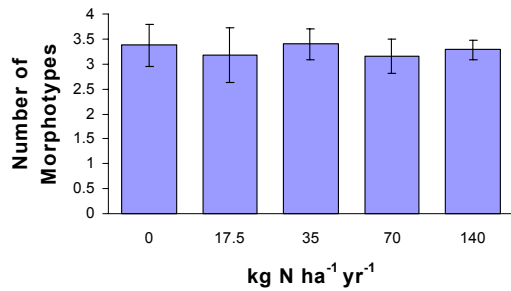
**Field Seedling Study 2002:
Abundance of Ectomycorrhizal
Tips Per Seedling**



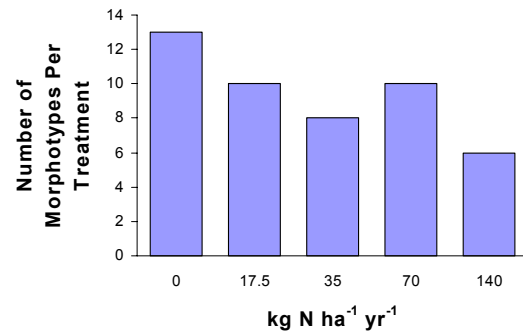
**Field Seedling Study 2002:
Simpson's Diversity Per
Seedling**



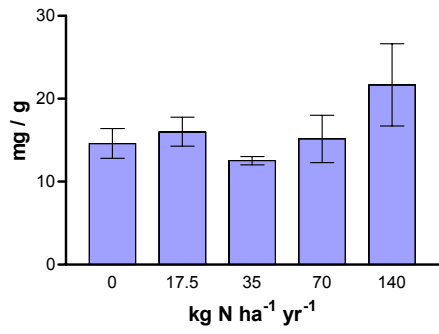
**Field Seedling Study 2002:
Richness of Ectomycorrhizal
Morphotypes Per Seedling**



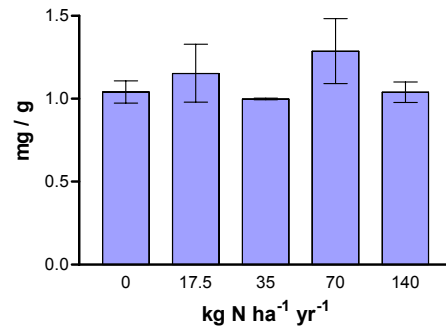
**Field Seedling Study 2002:
Total Richness**



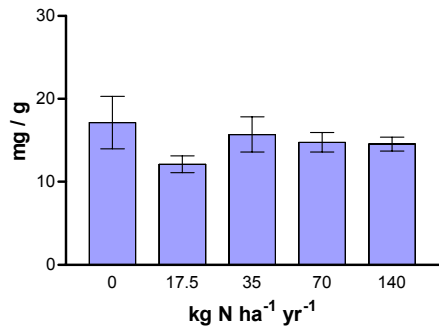
**Field Seedling
Root TKN**



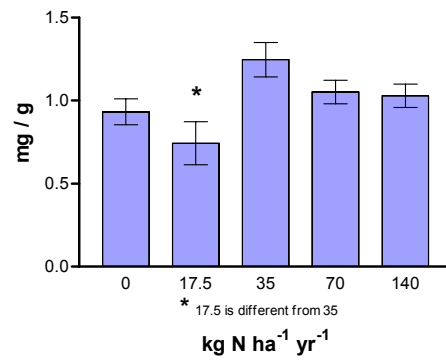
**Field Seedling
Root TP**



**Field Seedling
Shoot TKN**

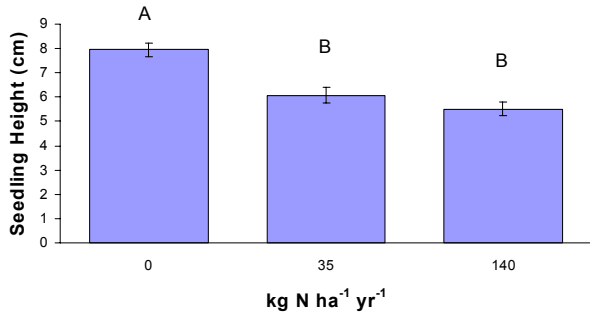


**Field Seedling
Shoot TP**

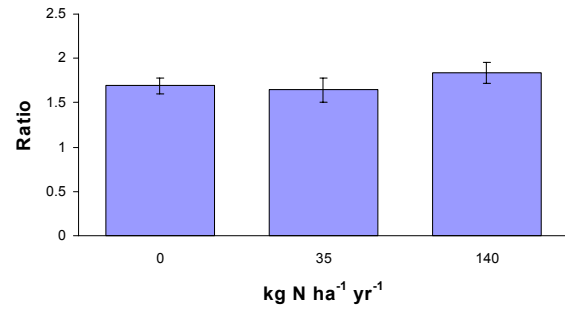


Appendix 3. Greenhouse seedling study ectomycorrhizal and soil chemistry results.

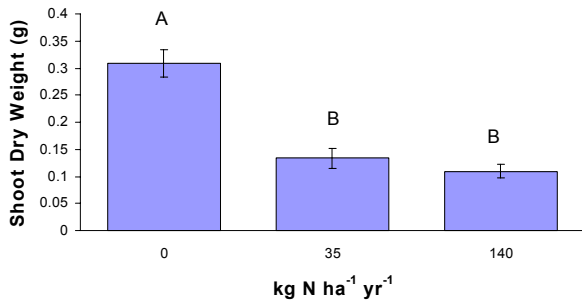
**Greenhouse Seedling Study 2002:
Seedling Height**



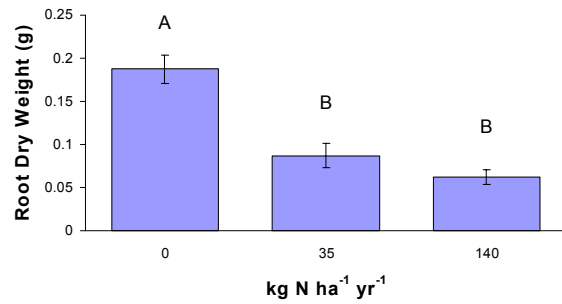
**Greenhouse Seedling Study 2002:
Shoot:Root Ratio**



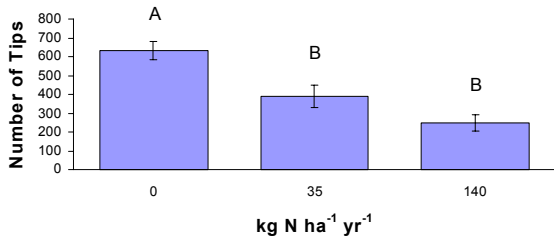
**Greenhouse Seedling Study 2002:
Shoot Weight**



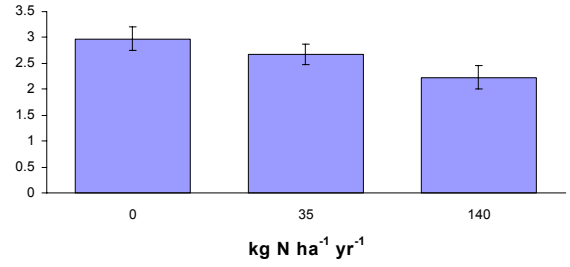
**Greenhouse Seedling Study 2002:
Root Weight**



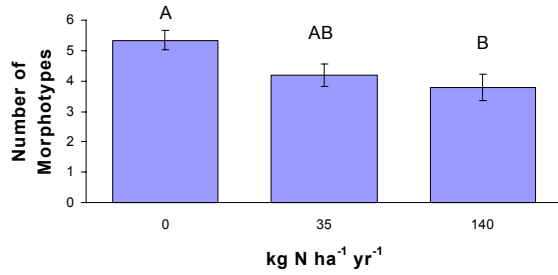
**Greenhouse Seedling Study 2002:
Average Abundance of
Ectomycorrhizal Tips Per Seedling Per
Core**



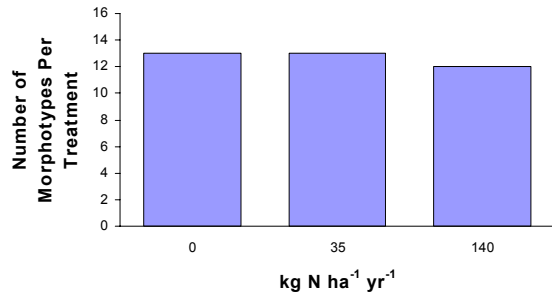
**Greenhouse Seedling Study 2002:
Average Simpson's Diversity Per
Seedling Per Core**



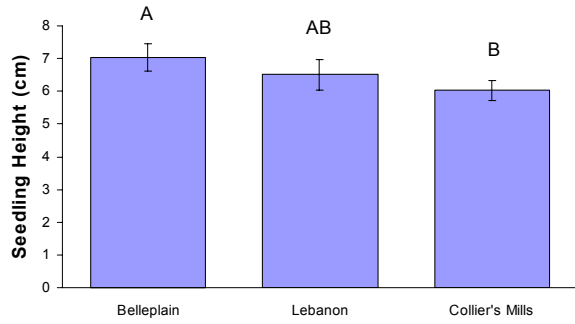
**Greenhouse Seedling Study 2002:
Average Richness of Ectomycorrhizal
Morphotypes Per Seedling Per Core**



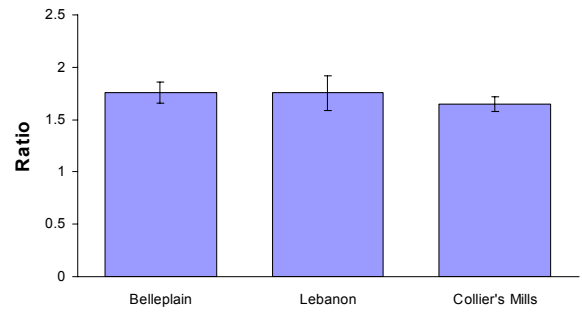
**Greenhouse Seedling Study 2002:
Total Richness**



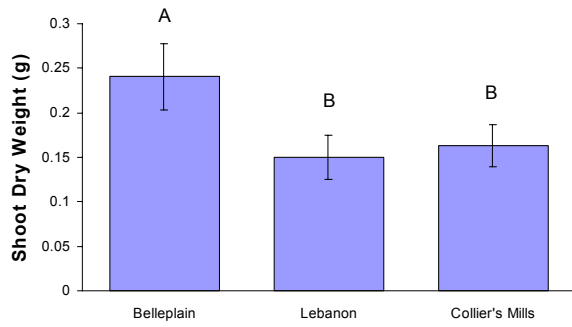
**Greenhouse Seedling Study 2002:
Seedling Height**



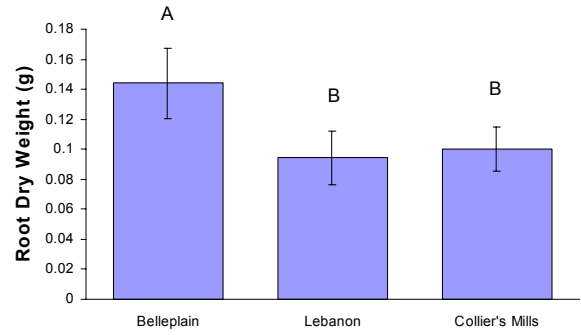
**Greenhouse Seedling Study 2002:
Shoot:Root Ratio**



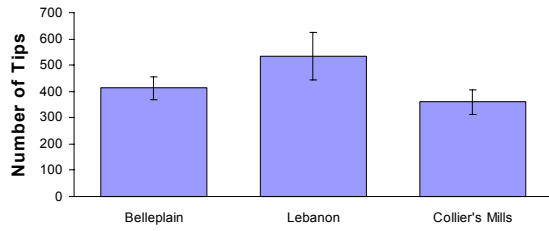
**Greenhouse Seedling Study 2002:
Shoot Weight**



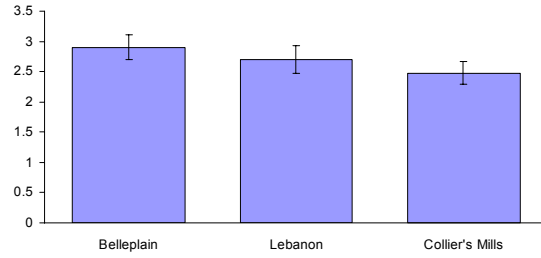
**Greenhouse Seedling Study 2002:
Root Weight**



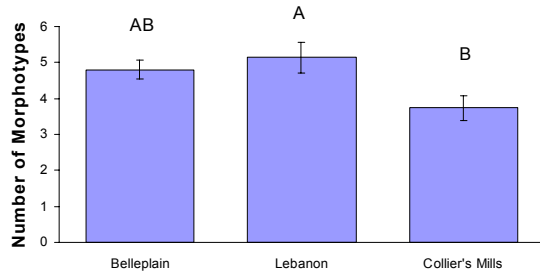
**Greenhouse Seedling Study 2002:
Average Abundance of
Ectomycorrhizal Tips Per Seedling Per
Core**



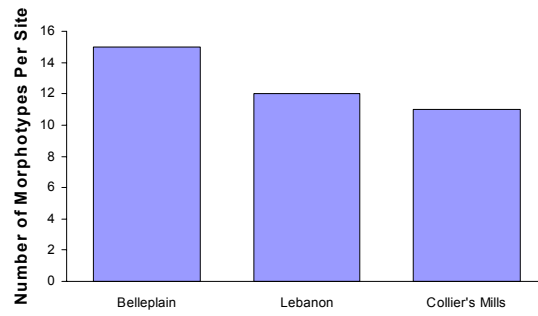
**Greenhouse Seedling Study 2002:
Average Simpson's Diversity Per
Seedling Per Core**

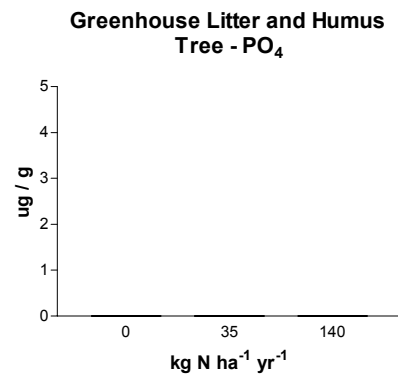
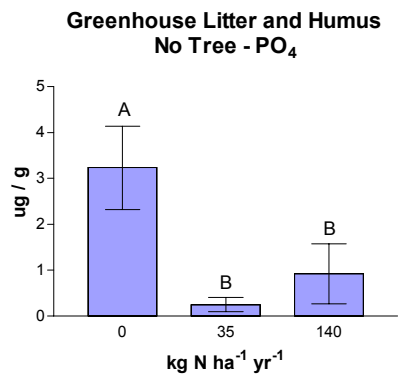
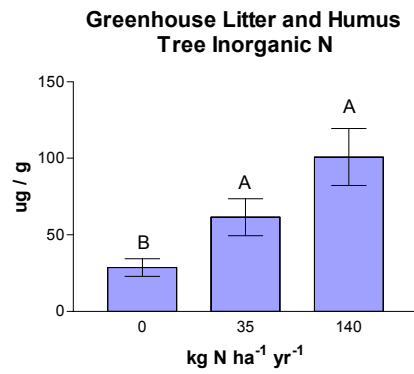
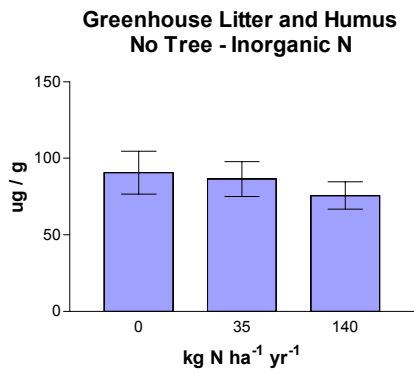
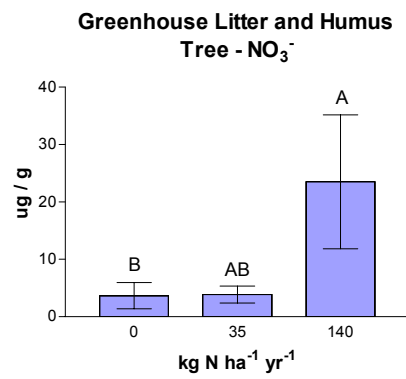
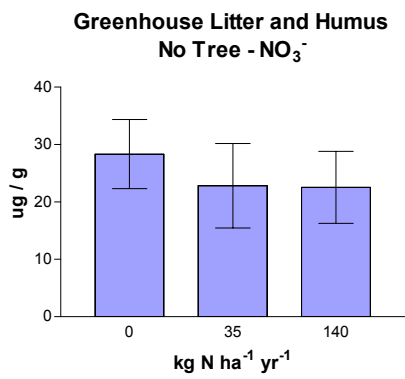
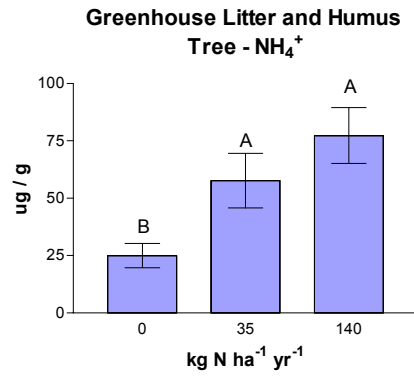
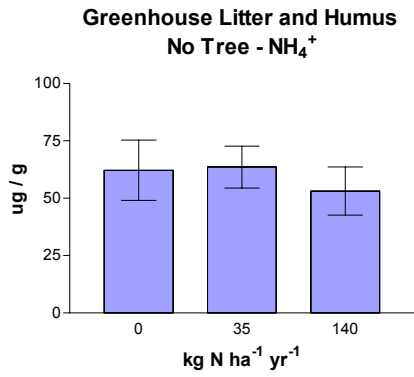


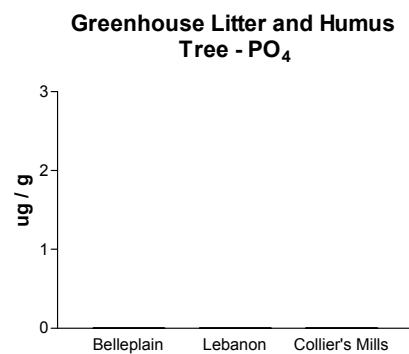
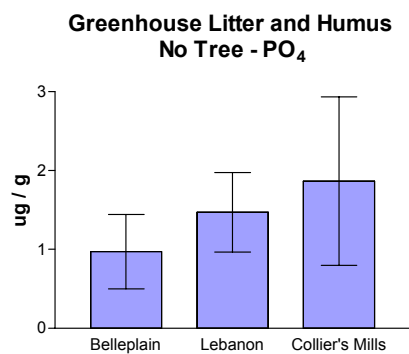
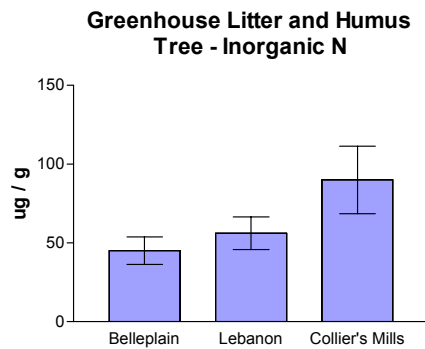
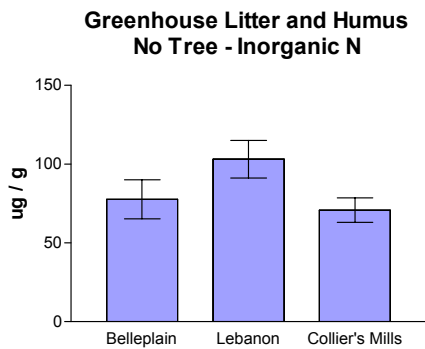
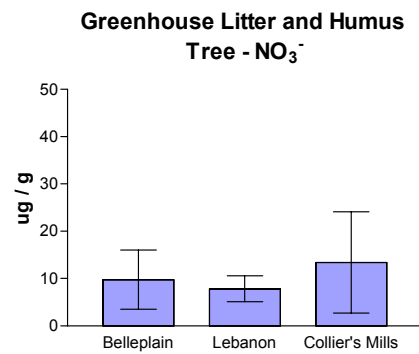
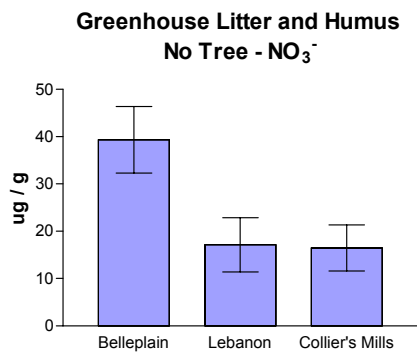
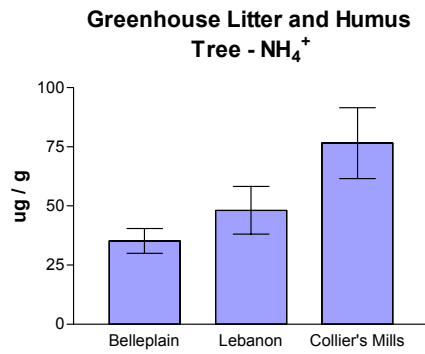
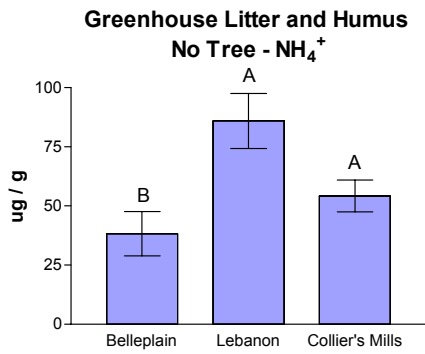
**Greenhouse Seedling Study 2002:
Average Richness of Ectomycorrhizal
Morphotypes Per Seedling Per Core**

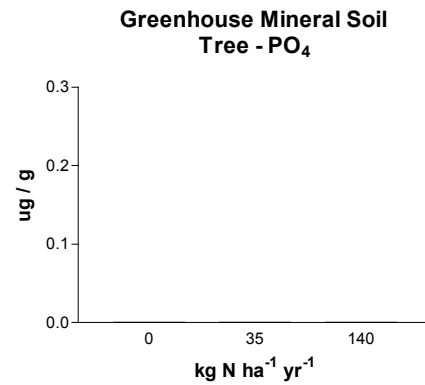
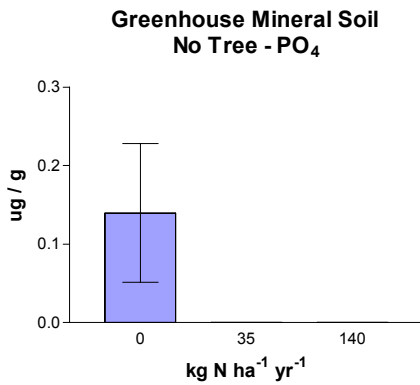
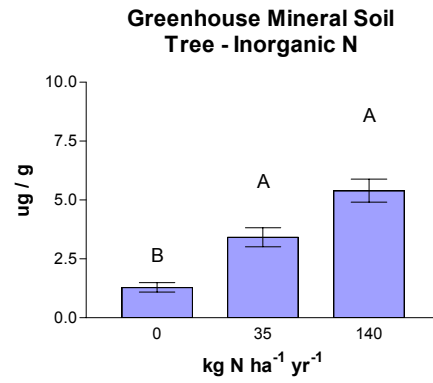
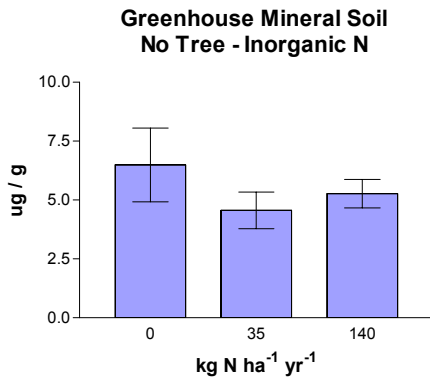
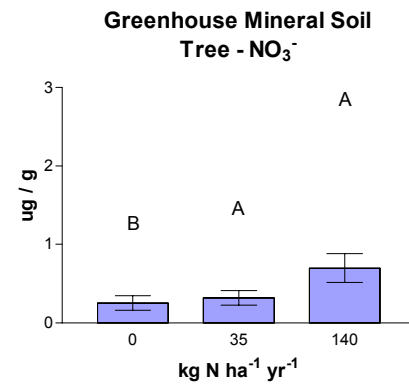
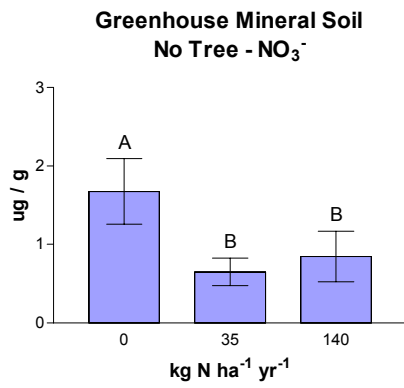
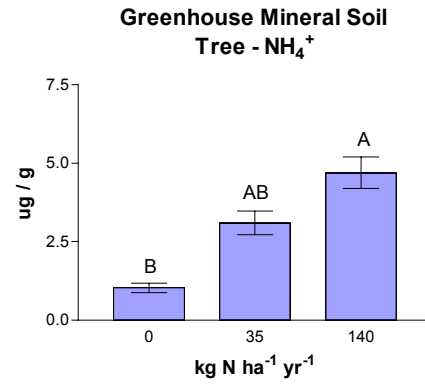
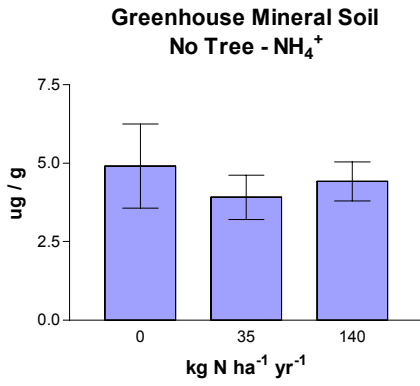


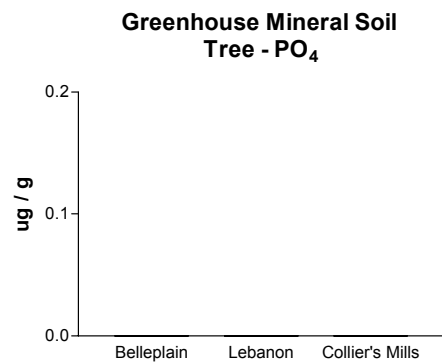
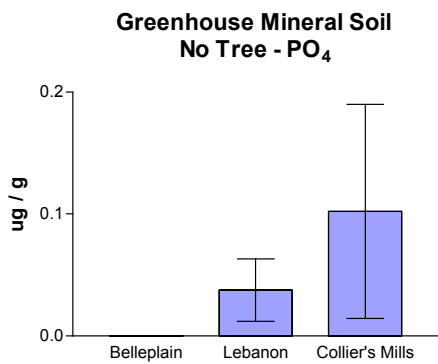
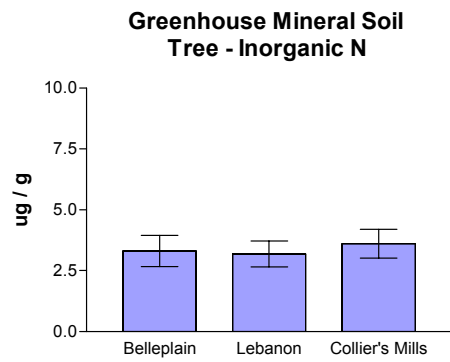
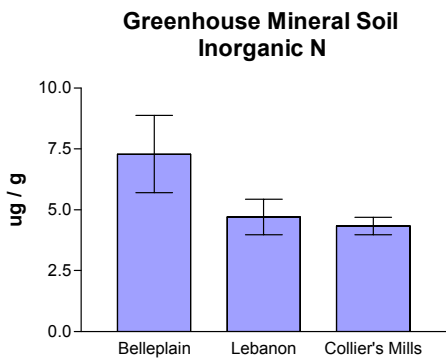
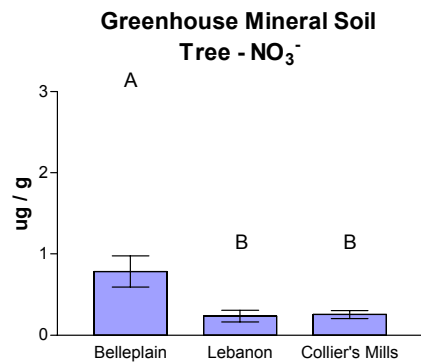
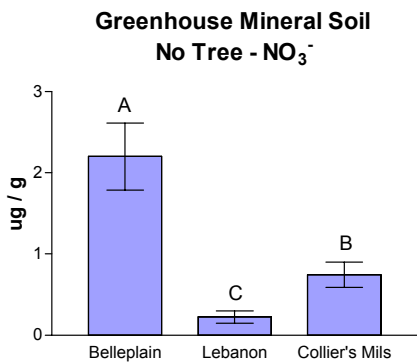
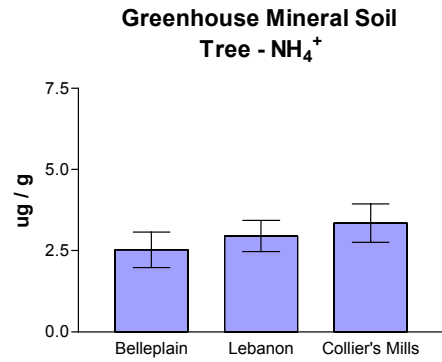
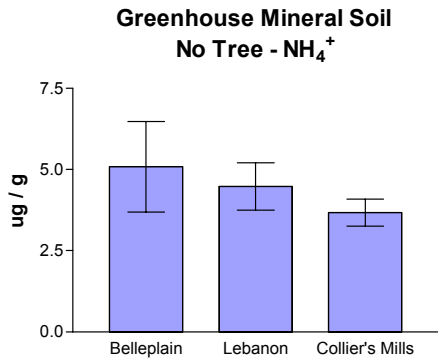
**Greenhouse Seedling Study 2002:
Total Richness**

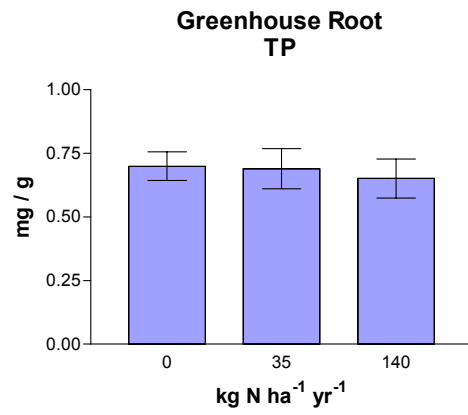
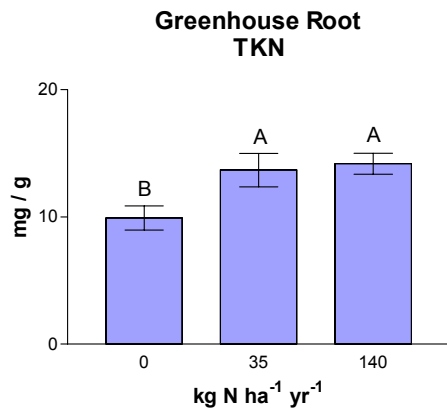
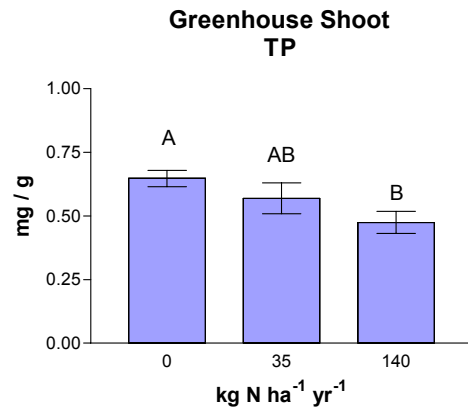
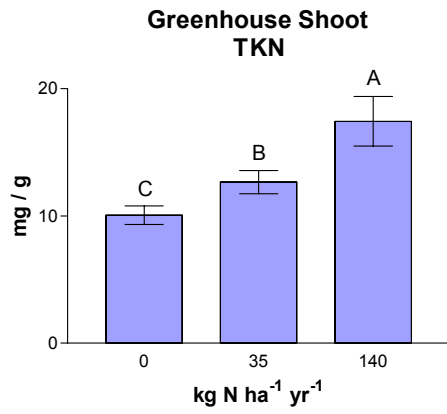




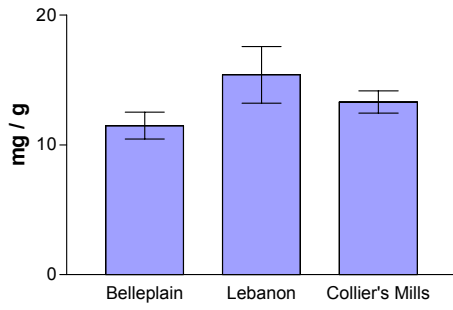




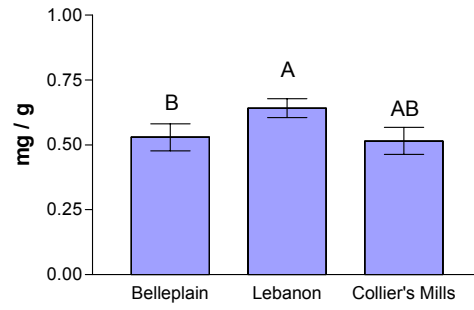




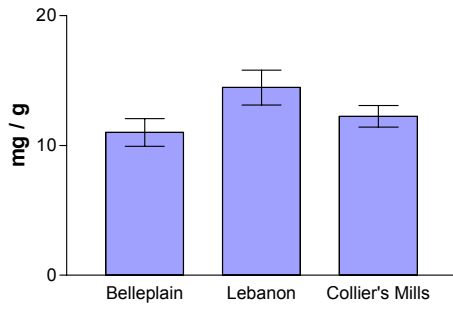
**Greenhouse Shoot
TKN**



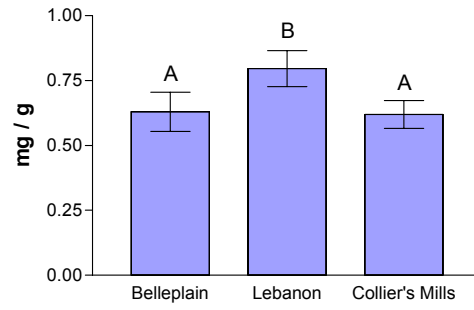
**Greenhouse Shoot
TP**



**Greenhouse Root
TKN**

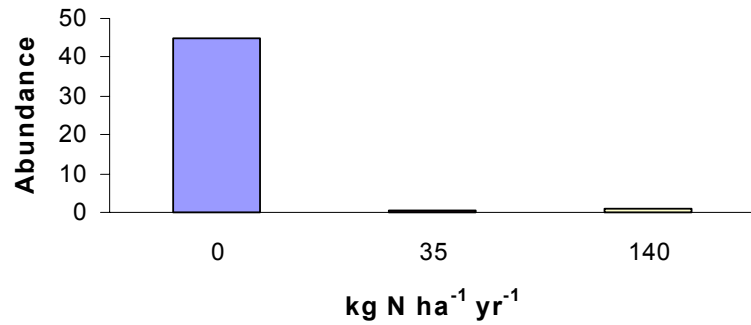


**Greenhouse Root
TP**

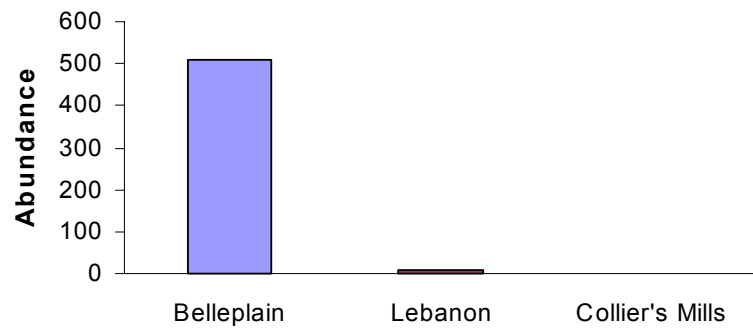


Appendix 4. Potential ectomycorrhizal indicator species for N deposition.

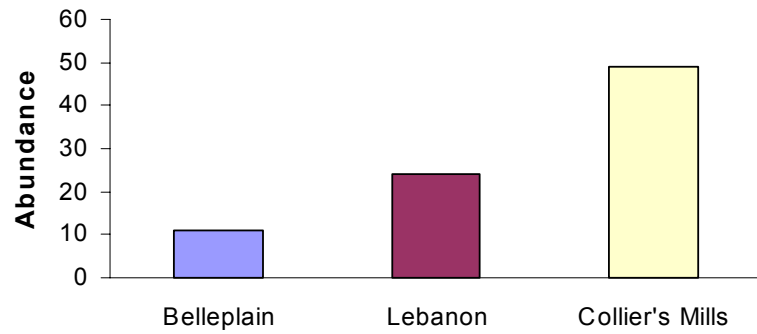
**Cortinarius-like Type:
Greenhouse Seedlings**



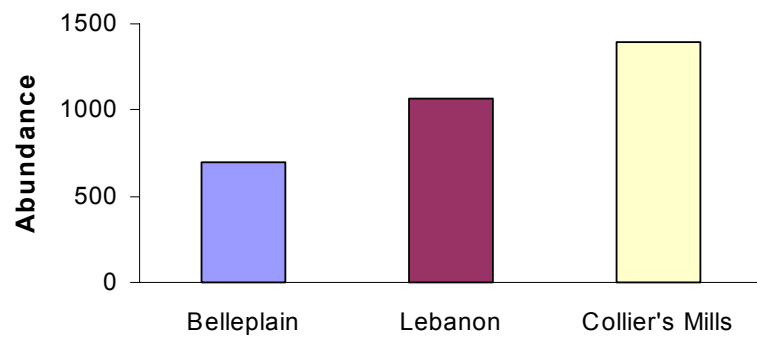
**Lactarius-like Type:
Field Cores**



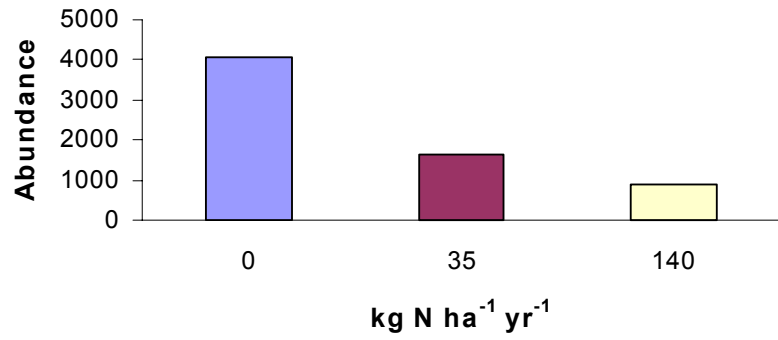
Suilloid-like Type: Field Cores



Ascomycete Type: Field Cores



Russula-like Type: Greenhouse Seedlings



Russula-like Type: Field Cores

