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THE ROLE OF ECTOMYCORRHIZAL FUNGI ON FERTILIZED AND UNFERTILIZED NURSERY GROWN WHITE SPRUCE

Alistair James Henry Smith II
Michigan Technological University

THE ROLE OF ECTOMYCORRHIZAL FUNGI ON FERTILIZED AND UNFERTILIZED NURSERY GROWN WHITE SPRUCE

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Alistair J. H. Smith II

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School of Forest Resources and Environmental Science

Thesis Co-Advisor: *Erik Lilleskov*

Thesis Co-Advisor: *Helja-Sisko Helmisaari*

Committee Member: *Dana Richter*

Committee Member: *Amy Marcarelli*

School Dean: *Terry Sharik*

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Preface

Ectomycorrhizal fungi are a common occurrence in many ecosystems. However, for how prevalent they may be, very little is known about plant/fungus interactions. It was the aim of Dr. Erik Lilleskov (Advising, protocol development, photographs of plant collection), Lynette Potvin (Sample processing), and myself (Sample collection, protocol development, sample processing, DNA extraction, DNA analysis, statistical work, and written paper) to attempt to fill in the knowledge gaps pertaining to ectomycorrhiza. We hope that this paper is an adequate first step.

0- Abstract

Nursery grown seedlings are an essential part of the forestry industry. These seedlings are grown under high nutrient conditions caused by fertilization. Though grown in a controlled environment, symbionts such as ectomycorrhizal fungi (EcMF) are often found in these conditions. To examine the effects of EcMF in these conditions, colonized *Picea glauca* seedlings were collected from Toumey Nursery in Watersmeet, MI. After collection, the EcMF present were morphotyped, and seedlings with different morphotypes were divided equally into two treatment types- fertilized and unfertilized. Seedlings received treatment for one growing season. After that time, seedlings were collected, ectomycorrhizas identified using [1]morphotyping and DNA sequencing, and seedlings were analyzed for differences in leaf nutrient concentration, content, root to shoot ratio, total biomass, and EcMF community structure.

DNA sequencing identified 5 unique species groups- *Amphinema* sp. 1, *Amphinema* sp. 5, *Thelephora terrestris, Sphaerosporella brunnea*, and *Boletus variipes*. In the unfertilized treatment it was found that *Amphinema* sp. 1 strongly negatively impacted foliar N concentration. In fertilized seedlings, *Thelephora terrestris* had a strong negative impact on foliar phosphorus concentration, while *Amphinema sp. 1* positively impacted foliar boron, magnesium, manganese, and phosphorus concentration. In terms of content, *Amphinema sp. 1* led to significantly higher content of manganese and boron in fertilized treatments, as well as elevated phosphorus in unfertilized seedlings. *Amphinema* sp. 5 had a significant negative effect on phosphorus content. When examining root to shoot ratio and biomass, those seedlings with more non-mycorrhizal tips had a higher root to shoot ratio.

Findings from the study shed light on the interactions of the species. *Amphinema* sp. 5 shows very different functionality than *Amphinema* sp. 1. *Amphinema* sp. 1 appears to have the highest positive effect on seedling nutrition when in both fertilized and unfertilized environments. *Amphinema* sp. 5 and *T. terrestris* appear to behave parasitically in both fertilized and unfertilized conditions.

[1] The material contained in this chapter has been submitted to Forest Ecology and Management

1- Introduction

The use of nursery stock seedlings in the forestry industry has been common practice for decades. The advantages of nursery seedlings are numerous, though one quality in particular makes them favorable over natural regeneration- their initial growing conditions. One critical contributor to the initial growth of these seedlings is fertilization. In white spruce (*Picea glauca;* A. Voss), fertilized seedlings showed higher levels of nutrient uptake, initial growth, and production of biomass after planting than unfertilized seedlings (McAlister & Timmer 1998).

Despite the abundant nutrients present in nursery soil, trees form relationships with ectomycorrhizal fungi (EcMF). Ectomycorrhizal fungi have been known to alter the nutrient status of seedlings, and are a well-documented occurrence in nursery settings for many years (Crohgan 1984; Richter & Bruhn 1993). These fungi often aid in the accumulation of beneficial nutrients such as nitrogen (N), phosphorus (P), magnesium (Mg), and other elements (Smith & Read 2008). Separately or in combination fertilization and ectomycorrhizal fungi can serve to provide the host seedling with many nutrients that may otherwise be limiting, but all EcMF species are not identical in their effects on plant nutrition.

Whether through intentional or unintentional inoculation, EcMF can be found growing on the roots of many nursery grown plants (Menkis et al 2005). Although many EcMF often experience declines in areas with too high nutrient availability, in particular N (Arnolds 1991; Wallenda & Kottke 1998), some EcMF are less impacted by such conditions (Lilleskov 2001; Lilleskov 2002; Lilleskov et al. 2012 and references therein). Additionally, the frequent disturbances of the soil associated with nurseries favor pioneer EcMF (Kranabetter 2004; Danielson & Visser, 1990). The combination of these factors creates favorable growing conditions for common greenhouse species of fungi such as *Thelephora terrestris, Amphinema byssoides,* and *Paxillus involutus* (Brunner & Brodbeck 2001).

The communities present during development in the nursery may be adapted to the high nutrient conditions that constant fertilization provides (Flykt et al. 2008). However, it is unclear whether these fungi are acting as mutualists. It has been

suggested that under certain conditions, EcMF can actually behave parasitically (Kummel & Salant 2006; Karst et al 2008; Johnson et al 1997). Therefore, it is important to examine if fungi colonizing seedlings are providing any benefit.

Once the seedling and fungi are moved to the field, the nutrient conditions under which the community exists are highly altered; available inorganic N and P decrease (Kranabetter 2004; Danielson & Visser 1990; and Krasowski, 1999). The process of adaptation has potential implications for both the fungal community and the host. Changes in the dominant EcMF symbionts are likely to occur (Flykt et al 2008). Such a shift carries the potential to alter the types of nutrients being absorbed, the rate at which they are being absorbed, and in what quantity the nutrients are being stored in the host organism (Krasowski 1999), and the carbon cost of nutrient uptake (Smith & Reed 2008). It may be beneficial for the seedling to accumulate excess nutrients in the nursery setting, in order to ease the transition. However, too high nutrient content may leave seedlings vulnerable to frost damage or insect attack (Holopainen et al 1995).

In order to further examine the potential stoichiometric, growth, and allocation impacts of the EcMF community under fertilized and unfertilized conditions, white spruce seedlings were collected from the Toumey Nursery in Watersmeet, Michigan. The USFS nursery grows many species of trees for planting on federal lands. With this information in mind, we were able to develop four questions for investigation. First, do ectomycorrhizal fungi have an effect on the foliar nutrient concentration of both fertilized and unfertilized *Picea glauca* seedlings? Similarly, we investigated to see if the ectomycorrhizal fungi have an effect on the foliar nutrient content of both fertilized and unfertilized *Picea glauca* seedlings? Third, do ectomycorrhizal fungi have an effect on growth and root to shoot ratios of *Picea glauca* seedlings? Finally, how does fertilization interact with EcMF communities to affect seedling foliar nutrient concentration and content, growth, and root to shoot ratios?

2- Methods

2.1- Sample Collection

One-year-old white spruce seedlings for this study were collected from the Toumey nursery in Watersmeet, Michigan on the April 29th, 2011 (Fig 1.). During their first growing season the seedlings had received a 9-45-15 starter fertilizer, followed by treatment with a high N fertilizer (Scott's Champion 21-8-18), and finally a 4-25-35 finisher fertilizer. Seedlings were chosen by not only their dominant morphotype, but also in order to equally represent the fungal diversity present on the roots, as evident by fungal mycelium visible at the bottom of the tree tube (Fig 2, 3, & 4.). The seedlings were brought to the Northern Research Station Forestry Sciences Laboratory in Houghton, Michigan. Seedlings were removed from their original planting containers and given an individual identifier.

Once at the laboratory, EcMF were morphotyped over the next several days under a dissecting microscope. Ectomycorrhizas on the surface of the intact peat plug were morphotyped by rhizomorph presence and type, mantle color and texture, and hyphal anatomy (Agerer *1987 – 2008*). This morphotyping was not intended to be exhaustive, but simply to ensure that a diversity of morphotypes could be represented in both of the fertilization treatments.

2.2- Outplanting

Once initial morphotyping was completed, 73 trees were repotted in Stuewe and Sons 60 Deepots with 1050 cm^3 of pure peat moss (Sunshine Organic Genuine Canadian Sphagnum) added surrounding the original plug. Initial morphotypes were evenly distributed between the treatments. To minimize contamination by airborne fungal spores, the pots were capped with an approximately 2.5 cm thick layer of fibrous synthetic filler (Poly-Fil 100% Polyester Fiberfill). Additionally, the outside of the Deepot container rack was covered with insulating foam to keep roots cool.

The 73 trees were then placed outdoors in full sunlight, and assigned to one of two treatments- fertilized or unfertilized. Fertilized seedlings received an amount of 100ml of water and fertilizer mix (Scott's Champion 21-8-18, trace elements included [Table 1.]) diluted to achieve 100 ppm of N. This was the same fertilizer and fertilization rate that had been applied at the nursery. Those trees in the "no fertilizer added" category received 100 ml of DI water. Trees were watered every day for the first week of establishment, then as needed for the remainder of the growing season. All watering treatments applied to the fertilization group occurred with the same dilute fertilizer solution.

2.3- Sample harvest and chemical analysis

Trees were harvested on October 3rd, 2011. Due to logistical limitations, 21 of the original 73 seedlings were processed. Root tip colonization was estimated by using the methods of Giovannetti & Mosse (1980) with some modifications. Roots were laid on a 1 cm gridded tray. At every intersection with a root tip, the tip was morphotyped and recorded. This was done for 300 root tips from outside the original peat plug, and 300 root tips from within the original peat plug (See Table 2.). These regions could be easily distinguished morphologically based on peat characteristics and root tracking of container walls. Ten root tips per morphotype were then collected separately from both the old and new roots of the tree for DNA analysis. The root tips were freeze-dried and stored in 1.5 mL eppendorf tubes. Upon completion of root tip sampling, remaining roots were divided into fine and coarse roots, to be stored for analysis. The stem, foliage, and roots were placed into a 45°C oven for drying. Once dry, all tissues were weighed, and the foliage was collected to be ground and analyzed. To determine carbon and N concentration, 1.5 mg of the ground foliar tissue was weighed out and analyzed by running the sample through an elemental analyzer (Fisons NA1500). An additional 300 mg of foliar tissue was weighed out for analysis for K, Ca, Mg, Mn, Fe, Cu, B, Al, Zn, Na, and S concentration, which was carried out by the Penn State Agricultural Sciences

laboratory using acid digestion on an Autoblock digester (Environmental Express, Charleston, SC, USA) and inductively coupled plasma (ICP) analysis (Huang & Schulte 1985). Elemental content was estimated by multiplying biomass by concentration.

2.4- DNA Fungal Identification

To identify the mycorrhizae present on the sample root tips, PCR DNA amplifications were carried out on a subset of the root tips collected from the different morphotypes. Individual root tips were selected from the subset of 10 to undergo DNA extraction. The DNA extraction was done using a REDExtract-N-Amp Plant PCR kit (Sigma-Aldrich Corp., St. Louis, MO, USA). We followed manufacturer's instructions with the following exceptions: root tips were digested in 10 μL of extraction solution, and suspended with 10 μL of dilution solution.

For DNA amplification, 5.68 μL of water was combined with 10 μL of the REDExtract-N-Amp PCR Readymix, as well as 0.16 μL of primer ITS1F and 0.16 μL of primer ITS4. To this cocktail was added 4 μL of the extracted DNA solution. After a brief centrifuge, the samples were then placed in a Mastercycler thermocycler (Eppendorf North America, Hauppague, NY, USA) using program DS35 (Table) 3. Using gel electrophoresis, PCR products were visualized and analyzed for successful PCR amplification. After ethidium bromide staining, bands were imaged using Kodak EDAS 290 (Kodak, Rochester, NY, USA). Those samples displaying single, clearly defined bands of appropriate size were deemed successful, and cleaned according to the protocol laid out in the QIAquick PCR Purification Protocol (Qiagen, Venlo, Netherlands). Samples were washed with buffer PBI at a ratio of 5:1 (buffer:PCR product) in 1.5ml eppendorf tubes, before being transferred to QIAquick spin columns. These columns were centrifuged for 60 seconds to remove cleaning

agent, then centrifuged for an additional 30 seconds to remove any residual cleaning material. Samples were then washed in an 8:2 (buffer: 100% ethanol) buffer PE mix.

Samples were then centrifuged for 30 seconds. Once through flow was discarded, samples were eluted in 50 μL of DI H_2O. DNA concentration was determined by placing a 0.5 μL sample onto a NanoDrop3300 (Thermo-scientific, Waltham, MA, USA).

Cleaned and quantitated samples were sent to the Nevada Genomics lab of University of Nevada, Reno, for sequencing on an ABI3730 (Applied Biosystems, Foster City, CA, USA). Sequences were then run through DNA BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for identification.

Two species of *Amphinema* were common on tree roots. To better understand their relationship to known *Amphinema* species we developed phylogenetic trees comparing our isolates to previously sequenced *Amphinema* species. Using Unipro Ugene, DNA sequences were aligned with knowns, and both percent similarity matrices and a neighbor-joining tree generated.

2.5- Morphological identification

In a few samples, DNA sequencing was not successful. To identify these samples, other successfully sequenced samples were examined under a microscope. Notes on rhizomorph structure, mantle structure, clamp connections, hyphal anastomoses, and emanating hyphal abundance were taken. These identified standards were then used to morphologically identify the unknown root tips.

2.6- Statistical analysis

In order to properly account for the effects of communities present on the white spruce seedlings, multivariate methods were carried out using the R package vegan (Oksanen et al 2012). Data were first transformed using Wisconsin double

standardization. Next dissimilarities were calculated using the default Bray's dissimilarity index. Next, these data were ordinated using non-metric multidimensional scaling (NMDS). Once ordinated, EnvFit was used to relate plant growth and nutritional variables to the NMDS ordination (Oskanen 2013). Biplots were generated to help visualize the comparison. To test the relationship between individual fungal species and specific nutrients, regression analyses were carried out in Sigmaplot 9. These models were often linear, though in some cases, non-linear fits were better. Additionally, Welch's two tailed T-tests were conducted in order to compare fertilized and unfertilized treatments.

3- Results

3.1- Fungal community composition

Results of the DNA analysis revealed three species of fungi on multiple seedlings, as well as two other species that appeared only on a single seedling. Of the three dominant fungi, two species were of the genus *Amphinema*. The third dominant species was identified as *Thelephora terrestris*. Of the two *Amphinema* species, both matched to as yet uncharacterized species. Therefore, the species were given the provisional identifications applied by Kõljalg: *Amphinema* sp. 1 (*sensu* Urmas Kõljalg, JN943919.1), and *Amphinema* sp. 5, (*sensu* Urmas Kõljalg, JN943909.1) as described in the original DNAblast results.

Amphinema sp. 1, although phylogenetically and morphologically similar to *Amphinema byssoides*, was identifiable as a unique species at the DNA level (Fig 5). *Amphinema* sp. 1 is characterized by a plectenchymatous mantle with many emanating hyphae ([Fig 6.]). Hyphae are covered by small protuberences, giving the hyphae a warty appearance (Fig 7.). H anastomoses act as connections between the emanating hyphae (Fig 8.). Clamp connections were present. Additionally, somewhat loose rhizomorphs are sometimes present (Fig 9.).

Amphinema sp. 5 is relatively distinct from *Amphinema* sp. 1. As with *Amphinema* sp. 1, the mantle is plectenchymatous and is characterized by loose emanating hyphae (Agerer 1987 [Fig 10.]). However, the hyphae of *Amphinema* sp. 5 are smooth. H anastomoses are also present acting as connections between the hyphae (Fig 11.). Clamp connections were present. Rhizomorphs were not observed.

Thelephora terrestris was readily identifiable, due both to its strong DNA matches, as well as distinct morphotype (Agerer 1987). The mantle of the fungus was tight to the root tip with few to no emanating hyphae (Fig 12.). Cystidia were clear visible extending from the mantle (Fig 13.). Clamp connections were present. Rhizomorphs were well organized into tight bundles (Fig 14.).

Two additional types of mycorrhizal fungi were also found to be growing on single seedlings within the sample group. *Boletus variipes* is a basidiomycete and the fungus is more commonly associated with the family *Quercus*. The second species was identified as the ascomycete *Sphaerosporella brunnea*, which is commonly found as EcMF in greenhouses and known to associate with evergreen species (Danielson 1984).

3.2- Foliar chemistry

The effects of fertilization on the white spruce foliar nutrients were clearly evident (Table 4.). As one might expect, the mean concentration of nutrients were higher in all cases except for Na and K (Fig 15 & 16.). The most notable difference between treatments in the plant chemistry was in foliar N, which was the only element to show a statistically significant difference between the two treatments (p value < 0.0001). N was present in the fertilized trees compared to unfertilized at a ratio of 2.84:1. P showed a marginally significant positive effect of fertilization (p value$= 0.051$).

3.3- Fungal community effects on foliar nutrient concentration (NMDS)

The fungal community, as a whole, caused significant changes amongst the nutrient statuses of the seedlings (Fig 17 & 18). NMDS analysis revealed that in the unfertilized seedlings, the fungal community affected foliar concentrations of N (R^2=0.56, p=0.03), with a marginal effect on K (R^2=0.47, p=0.08). Results of nutrient analysis of the fertilized seedlings revealed significant fungal community effects on Mg (R^2 = 0.57, p = 0.04), Mn (R^2 = 0.80, p = 0.003), and B (R^2 = 0.80, p = 0.003), with marginally significant changes to P (R^2= 0.50, p = 0.08), Ca (R^2 = 0.50, p= 0.09), S (R^2 = 0.53, p = 0.08), Cu (R^2 = 0.54, p = 0.07), and Zn (R^2 = 0.58, p = 0.05).

3.4- Fungal species effects on foliar nutrient concentration

Individual fungal species differed in their correlation with many nutrients in both unfertilized and unfertilized seedlings. Regressions indicated unfertilized seedling N concentration was significantly negatively related to abundance of *Amphinema* sp. 1 (R^2=0.57, p=0.01 [Fig 19.]).

In fertilized seedlings *Amphinema* sp. 1 and *T. terrestris* demonstrated opposite trends. In contrast with the unfertilized treatments, *Amphinema* sp. 1 positively affected many nutrient concentrations. *Amphinema* sp. 1 was positively correlated with P concentration (R^2=0.52, p=0.07 [Fig 20.]), B (R^2=0.44, p=0.03 [Fig 21.]), Mg (R^2=0.56, p=0.01 [Fig 22.]), and Mn (R^2=0.78, p=0.0006 [Fig 23.]). The concentration of these nutrients well surpassed those in the seedlings with high numbers of non-mycorrhizal tips.

In fertilized seedlings *T. terrestris* was associated with generally non-significant negative trends, and a significant negative correlation with P (R^2=0.54, p=0.01 [Fig 20.]). At high *T. terrestris* abundance, concentrations of P were observed to be even less than those in seedling with high numbers of non-mycorrhizal tips.

While *Amphinema* sp. 5 was present in both treatments, there were an insufficient number of samples to draw strong conclusions about the effects of that

species on foliar chemistry. However, a potential strong decrease in B and Mg was seen in fertilized seedlings.

Ratios of all the elements in the different fertilization treatments were calculated (Table 6). To understand EcMF effects on stoichiometry, we examined species effects on N to P ratios. In unfertilized seedlings, *Amphinema* sp. 1 demonstrated a strong negative trend ($R^2 = 0.90$, $p = 0.0002$ [Fig 24.]). *Amphinema* sp. 5 and *T. terrestris* both demonstrated marginally significant positive trends- *Amphinema* sp. 5 ($R^2 = 0.52$, $p = 0.10$), *T. terrestris* ($R^2 = 0.54$, $p = 0.09$ [Fig 24.]). No significant trends were observed in fertilized seedlings (Fig. 25). No significance tests could be performed for the effect of *Boletus variipes* and *Sphaerosporella brunnea,* as each of these species was only present on one unfertilized seedling.

3.5- Fungal community effects on foliar nutrient content (NMDS)

When examining the NMDS results for the fungal community's effect on nutrient content, few significant trends emerged (Fig 26 & 27.). In unfertilized trees, a significant trend was observed in K content ($R^2 = 0.54$, $p = 0.05$). Additionally, a marginally significant trend was observed in P content ($R^2 = 0.54$, $p = 0.06$). In fertilized seedlings, no significant trend was observed. However, two marginally significant trends were observed; one in Mn ($R^2 = 0.50$, $p = 0.07$) and one in Cu ($R^2 = 0.54$, $p = 0.099$).

3.6- Fungal species effects on foliar nutrient content

The greatest species effect on nutrient content can be observed in fertilized seedlings, within *Amphinema* sp. 1, which showed a significant positive effect on B ($R^2=0.44$, $p=0.04$ [Fig 28.]), and Mn ($R^2=0.62$, $p=0.01$ [Fig 29.]). Additionally, in the fertilized treatment a strongly negative relationship was observed between Mg content and number of non-mycorrhizal root tips ($R^2=0.42$, $p=0.04$ [Fig 30.]).

For unfertilized seedlings, only P was significantly affected, exhibiting a strongly positive relationship with abundance of *Amphinema* sp. 1 (R^2=0.75, p=0.0006 [Fig 31.]) and a strong negative relationship with abundance of *Amphinema* sp. 5 (R^2=0.40, p=0.03 [Fig 31.]). Although K was found to be significant in the NMDS, no significant species effects were found.

3.7- Treatment effects on biomass and root:shoot ratios

As with foliar chemistry, fertilization treatment affected overall growth and root to shoot ratios. Those seedlings receiving fertilization had higher mean above ground biomass, higher mean below ground biomass, and a higher mean root to shoot ratio than those unfertilized seedlings (Table 6).

3.8- Community effects on biomass and root:shoot ratios (NMDS)

To determine effects of fungal community on foliar biomass, stem biomass, below ground biomass, total biomass, and root:shoot ratio, NMDS was conducted (Fig 32 & 33). No significant trends were observed in unfertilized. A marginally significant relationship was observed in fertilized seedlings only for root:shoot ratio (R^2=0.52, p=0.06).

3.9- Fungal species effects on root:shoot ratios.

To understand the origin of the community effect on root:shoot ratios we examined species-level effects. In the unfertilized treatment, there were no significant trends associated with individual species of fungus for root:shoot ratio (Fig 34.). Within the fertilized treatments, non-mycorrhizal tips demonstrated a strong positive relationship to root:shoot ratio (R^2=0.82 , p<0.001 [Fig 35.]).

4- Discussion

4.1- Individual species effects on seedling chemistry and stoichiometry

When examining growth limiting nutrients, the trends were intuitive (Table 7 & 8). N was the common limiting nutrient in unfertilized seedlings. The average concentration for N in these trees was 6 g/kg, well below the estimated threshold value for deficiency of 10.5 g/kg (Binkley & Fisher 2013). In fertilized seedlings, growth limiting nutrients were not an issue. All macronutrients and micronutrients for which we had threshold data were found in non-limiting concentrations (Binkley & Fisher 2013; Lehto et al 2010; Polle et al, 1992). It should also be noted that the micronutrients in the fertilized seedlings were present in higher mean concentrations that those in unfertilized seedlings

Nutrient ratios in the seedlings also responded to fertilization treatment (See Table 4). N was present in much higher ratios in fertilized seedlings than in unfertilized. P and K, though macronutrients also present in the fertilizer, were much more variable. Possibly the most interesting comparisons was the ratios of N:P in unfertilized treatments. In fertilized treatments, the N:P ratio was fairly balanced (mean- 9.38:1). However, in unfertilized seedlings this ratio was considerably lower (4.14:1). Interestingly, all fungal species exhibited both significant and marginally significant trends. In *Amphinema* sp. 1, the trend was strongly negative, suggesting that the availability of N in the peat substrate is extremely low, or all the EcMF are keeping the N for their own processes. It should also be noted there was considerable variability in the concentrations of micronutrients in the seedlings.

One of the more interesting components of this experiment is the interaction of individual EcMF with fertilization (Table 7.). In unfertilized treatments *Amphinema* sp. 5 and *T. terrestris* showed signs of positive trends in their effect on N concentration. This may suggest that *Amphinema* sp. 5 and *T. terrestris'* primary contribution to the host is the supply of N. However, when examining foliar content, these species display weak negative trends. This may potentially be an indicator that

the fungi are not actually providing the tree with increased N, but instead reducing the concentration of available nutrients. This may in turn impact the overall growth of the seedling, as the nutrients needed to support valuable cellular components may be less available.

When examining *Amphinema* sp. 5 and *T. terrestris* impacts in fertilized seedlings, a different trend is noticed. Increased presence of *Amphinema* sp. 5 and *T. terrestris* lead to general decreases in nutrient concentration. This trend is very noticeable in the effect of *T. terrestris* on the concentration of P in fertilized seedlings. The decrease in P concentration is accompanied by no change in the P content of the seedlings. This relationship suggests that although the concentration of P is decreasing in the seedlings, P is still likely being supplied in level sufficient to continue development of P containing plant structures.

These trends give us valuable insight to the functionality of *T. terrestris*. Many past studies have revealed that *T. terrestris* is more tolerant of high N sites (Lilleskov et al 2002b; Chalot & Brun 1998; Arnolds 1991). However, the data suggests that *T. terrestris* contributes the most under low N conditions. This relationship between *T. terrestris* and the study seedlings suggest that *T. terrestris* may actually be a poor symbiotic partner under high N conditions, and not make significant positive or negative contributions under low N conditions, due to it's minimalistic contributions to the host seedling (Johnson et al 1997).

Amphinema sp. 1 displayed trends quite different from those observed in *Amphinema* sp. 5 and *T. terrestris*. *Amphinema* sp. 1 demonstrated a significant ability to beneficially supply P, B, and other valuable nutrients to host seedlings receiving fertilization. Especially interesting was an increase in the concentration of Mg in fertilized seedlings. Mg is critical for photosynthesis. Previous studies have tied Mg concentration to increased photosynthetic C gain (Ericsson & Kähr 1995). Therefore, one might speculate that *Amphinema* sp. 1 supplies Mg in elevated quantities to receive more C compounds from the host seedling. In unfertilized seedlings, *Amphinema* sp. 1 was tied to a decrease in concentration of foliar N, as well as a decrease in N:P ratio. *Amphinema* sp. 1's lack of N supply may in fact alter

biomass production. This would be expected to stimulate belowground C allocation. Additionally, the increase in Mg may also help to boost the levels of carbon being sent to the fungus. It is possible that in unfertilized plants, *Amphinema* sp. 1 is a poor mutualist, not supplying the nutrients needed to grow under those conditions. However, when one looks at the general effects of *Amphinema* sp. 1, total biomass is positively affected. Additionally, content of N is not decreased significantly, while P is significantly increased. It appears that *Amphinema* sp. 1 is not negatively affecting the overall content of N within the host plant, but instead boosting the levels of other nutrients and hence diluting the N pool.

In addition to the differences in concentration between the fungal species in each treatment, some differences also exist in the total content of the foliage. Interestingly, concentration and content of nutrients in seedlings do not necessarily coincide with one another. In unfertilized treatments, no significant differences existed between any fungal species.

Ectomycorrhizas on fertilized seedlings affected content of some nutrients. Those seedlings with elevated counts of *Amphinema* sp. 1 demonstrated increased content of B. There has been uncertainty as to the purpose of this micronutrient in plants (Blevins & Lukaszweski 1994; Bolaños et al 2004). It is hypothesized that B may play a role in plant cell membranes (Blevins & Lukaszewski 1998; Lehto et al 2010). Relevant to the present study, it has been shown that B fertilization leads to increased EcMF colonization (Mitchell et al 1987; Lehto et al 2004; Lehto et al 2010). This increase in EcMF could heighten the ability of plants to secure more critical limiting nutrients. Thus overcoming B limitation is clearly beneficial to both host and EcMF.

Amphinema sp. 1 also produces a similar increase in Mn content. Mn has been shown to be a critical component of chloroplasts, specifically benefiting photosystem II (Teichler-Zallen 1969). It is possible that EcMF may increase the supply of Mn in order to boost the supply of carbon compounds.

4.2- Individual species effects on plant biomass and allocation

Despite having obvious effects on plant nutrition, effects on plant biomass and root:shoot allocation were much less pronounced. No significant differences were observed between biomass as a function of EcMF species. Additionally, there were no significant effects of EcMF species on root:shoot ratio. These trends suggest that any differences between the seedlings were caused by the fertilization treatment and presence of ectomycorrhizal fungi, not the particular fungus.

When examining the effects of the EcMF on both treatments, it is uncommon for the nutrient concentration in the leaf tissue to vary in seedlings not supporting EcMF (Ericsson & Kähr 1995). In the experimental seedlings, a diverse range of foliar concentrations were observed. Despite the variety of foliar nutrient concentrations in the seedlings, there were no significant differences in above ground biomass amongst seedlings in the same treatment.

However, one significant difference was seen in root: shoot ratio. Not surprisingly, there was a significant positive correlation between root:shoot ratio and abundance of non-mycorrhizal root tips. As the seedling does not have a large surface area created by mycorrhiza, it must increase its total root area. This corresponds with evidence that EcMF aid in expansion of surface area for nutrient uptake (Smith & Read 2008). However, an alternative explanation for the change in ratio may be that when not supporting EcMF, the carbon that was supporting the fungus may then be available for root production.

4.3 Practical applications of fungi in field versus nursery settings

In the past, many studies have been conducted to determine the effects of EcMF on nursery grown seedlings. Many of these studies have examined the effects of EcMF directly in the nursery, or immediately after out-planting (Rudawska et al 2006; Rincón et al 2005; Quoreshi & Timmer 1998; Trappe 1977). In these studies, *T. terrestris* is commonly considered a green-house parasite (Trappe 1977; Quoreshi

& Timmer 1998). *T. terrestris* has been shown to inhibit growth in inoculated seedlings in both nursery and out planted settings (Trappe 1997). In this study, no significant trends affecting growth could be attributed to abundance of *T. terrestris*. However, some conclusions may be able to be drawn based on *T. terrestris'* nutrient contributions. In fertilized settings, *T. terrestris* demonstrated significant negative trends in concentration of both P and Mn. This reduction limits pools of essential nutrients for future growth. Additionally, in unfertilized seedlings, no significant trends were observed, either positive or negatively, in all aspects of plant growth and nutrient concentration. Based on this observation, we can draw no strong conclusions as to the fungus' contributions in this environment.

Amphinema sp. 1 and *Amphinema* sp. 5 have no literature on effects of host nutrition or growth relating to their species. However, as this paper has shown, various species of *Amphinema* are often lumped under *A. byssoides*. As we have demonstrated, this may be problematic since species of *Amphinema* appear to behave differently under similar nutrient conditions. Still, while examining the data, it is possible to formulate some possible hypotheses as to the benefit and function of these fungi.

Amphinema sp. 1 appears largely to be a mutualist in both fertilized and unfertilized conditions. Positive trends are seen in fertilized treatments for above ground biomass, as well as in unfertilized treatments for total biomass. In addition, *Amphinema* sp. 1 demonstrates an ability to increase both concentration and content in its host seedling (N concentration being the exception). This increase in nutrient supply allows *Amphinema* sp. 1 to boost the potential growth of its host, making it a favorable mutualist.

Although we must be cautious given the low sample number, the effects of *Amphinema* sp. 5 on seedlings were in stark contrast to those of *Amphinema* sp. 1. *Amphinema* sp. 5 not only was associated with trends for decreasing concentration of most nutrients in both fertilized and unfertilized treatments, but also decreasing content in unfertilized seedlings. These trends are reflected in seedling growth, where aboveground biomass and total biomass exhibited negative trends in unfertilized

seedlings colonized by *Amphinema* sp. 5. Clearly this species requires greater study as a potentially strong conditional parasite under unfertilized conditions.

5- Conclusions

Molecular methods allowed us to distinguish the species of fungi colonizing nursery seedlings, including two unidentified species of *Amphinema* with apparently divergent effects on seedling nutrition under varying fertilization regimes. This is the first time that these species have been identified as nursery colonists, having

previously been lumped under the closely related *A. byssoides*. Furthermore, our statistical approach allowed us to test for community and species effects on plant nutrition in a more realistic multispecies community setting. In the nursery setting, where seedlings are in high nutrient environments, *Amphinema* sp. 1 proves to be an efficient mutualist, elevating the concentration and content of many macronutrients and micronutrients. This potentially allows for the seedlings to shift C usage from below-ground growth to above-ground production. In the field, *Amphinema* sp. 1 continues to show mutualistic qualities.

Both *Amphinema* sp. 5 and *T. terrestris* demonstrate strong contrasts to *Amphinema* sp. 1, acting more parasitically in both conditions. These EcMF are often associated with negative or neutral trends in concentration, content, and elements of growth, which are detrimental for development of the seedling. Still more detrimental, for *Amphinema* sp. 5 the negative effects of the ectomycorrhizae seem to be most noticeable in unfertilized seedlings. By reducing the nutrient content in seedlings in both the fertilized and unfertilized setting *Amphinema* sp. 5 and *T. terrestris* demonstrate very little mutualism. It is in the best interest of the nurseries to reduce the abundance of these species in order to promote growth of their seedlings.

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Fig 1- Examining *Picea glauca* seedlings at the United States Forest Service Toumey nursery in Watersmeet, MI, USA for ectomycorrhizal presence *(Photo by Erik Lilleskov)*

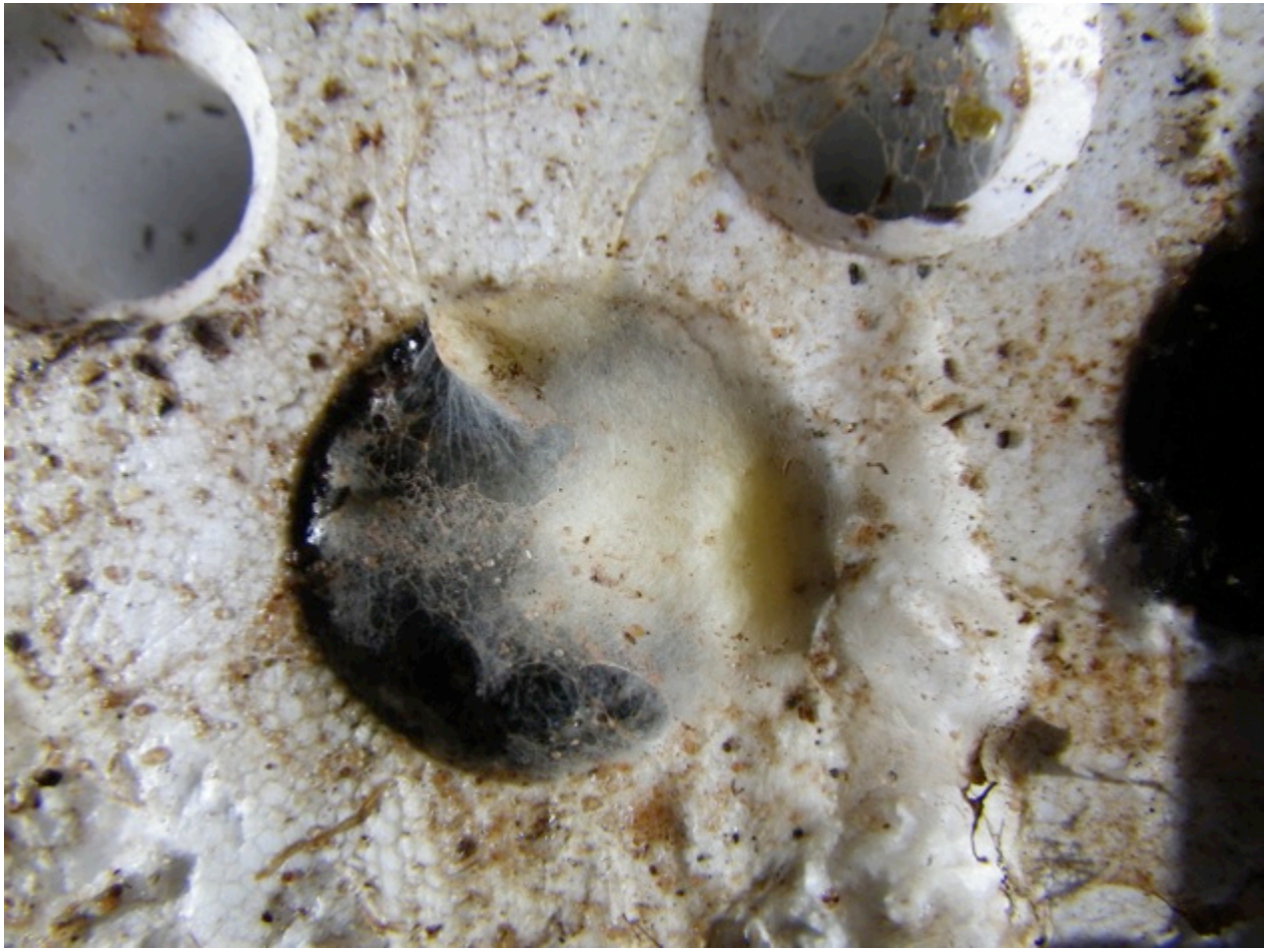

Fig 2- White morphotype as observed on Toumey Nursery seedling container. *(Photo by Erik Lilleskov)*

Fig 3- Brown morphotype as observed on Toumey Nursery seedling container. *(Photo credit- Erik Lilleskov)*

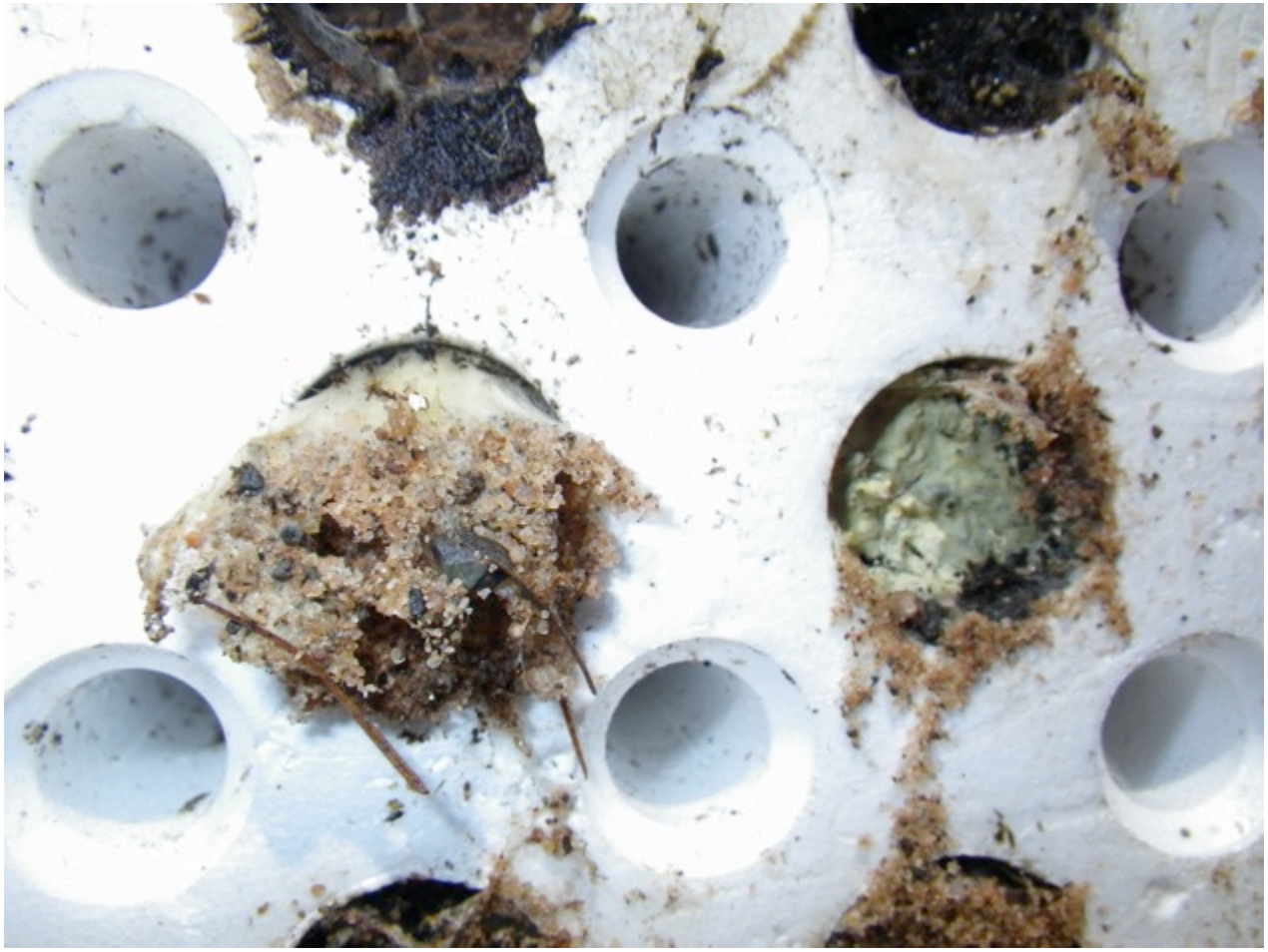

Fig 4- White (left), Yellow (right) and brown (top left, partial) morphotypes as observed on Toumey Nursery seedling container. *(Photo by Erik Lilleskov)*

Table 1.- Scott's Champion fertilizer

		Concentration
Nitrogen	*(Ammonium N)*	8.70%
	(Nitrate N)	12.30%
Phosphorus	*(Phosphate)*	8.00%
Potassium	*(Soluable potash)*	18.00%
Magnesium	*(Water soluble)*	0.15%
Boron		0.0262%
Copper	*(Water soluble)*	0.0262%
Iron	*(Chelated)*	0.1050%
Manganese	*(Water soluble)*	0.0105%
Molybdenum	*(Water soluble)*	0.0525%

Table 2.- Percentage of seedling root tips colonized by species of fungus

	Seedling	*Amphinema* sp. 1	*Amphinema* sp. 5	*Thelephora terrestris*	Non-mycorrhizal	Sphaerosporella Brunnea	*Boletus variipes*
Fertilized	1	0.00	49.17	47.17	3.67	0.00	0.00
	2	0.00	46.33	42.17	11.50	0.00	0.00
	3	0.00	0.00	87.17	12.83	0.00	0.00
	4	52.50	0.00	47.50	0.00	0.00	0.00
	5	5.67	0.00	76.83	17.50	0.00	0.00
	6	14.67	0.00	58.67	26.67	0.00	0.00
	7	0.00	0.00	86.00	14.00	0.00	0.00
	8	0.00	0.00	75.33	24.67	0.00	0.00
	9	22.00	0.00	25.50	52.50	0.00	0.00
	10	44.00	0.00	49.50	6.50	0.00	0.00
Unfertilized	11	47.83	0.00	0.00	2.17	50.00	0.00
	12	49.67	0.00	45.50	4.83	0.00	0.00
	13	35.33	0.00	25.67	39.00	0.00	0.00
	14	0.00	0.00	90.33	9.67	0.00	0.00
	15	80.00	0.00	0.00	20.00	0.00	0.00
	16	3.50	0.00	88.67	7.83	0.00	0.00
	17	0.00	65.83	31.17	3.00	0.00	0.00
	18	48.17	0.00	14.50	2.17	0.00	35.17
	19	0.00	36.17	46.00	17.83	0.00	0.00
	20	27.17	59.00	0.00	13.83	0.00	0.00
	21	50.00	0.00	45.83	4.17	0.00	0.00

Table 3.- Specifications for thermocycler program DS35

	Temp (C)	Time	Cycles
Initial Denaturation	94	1.25 min	1
Denaturation	95	35 sec	
Annealing	44	55 sec	35
Extension	72	42 sec	
Final Extension	72	10 min	1
Hold	10	Indefinitely	

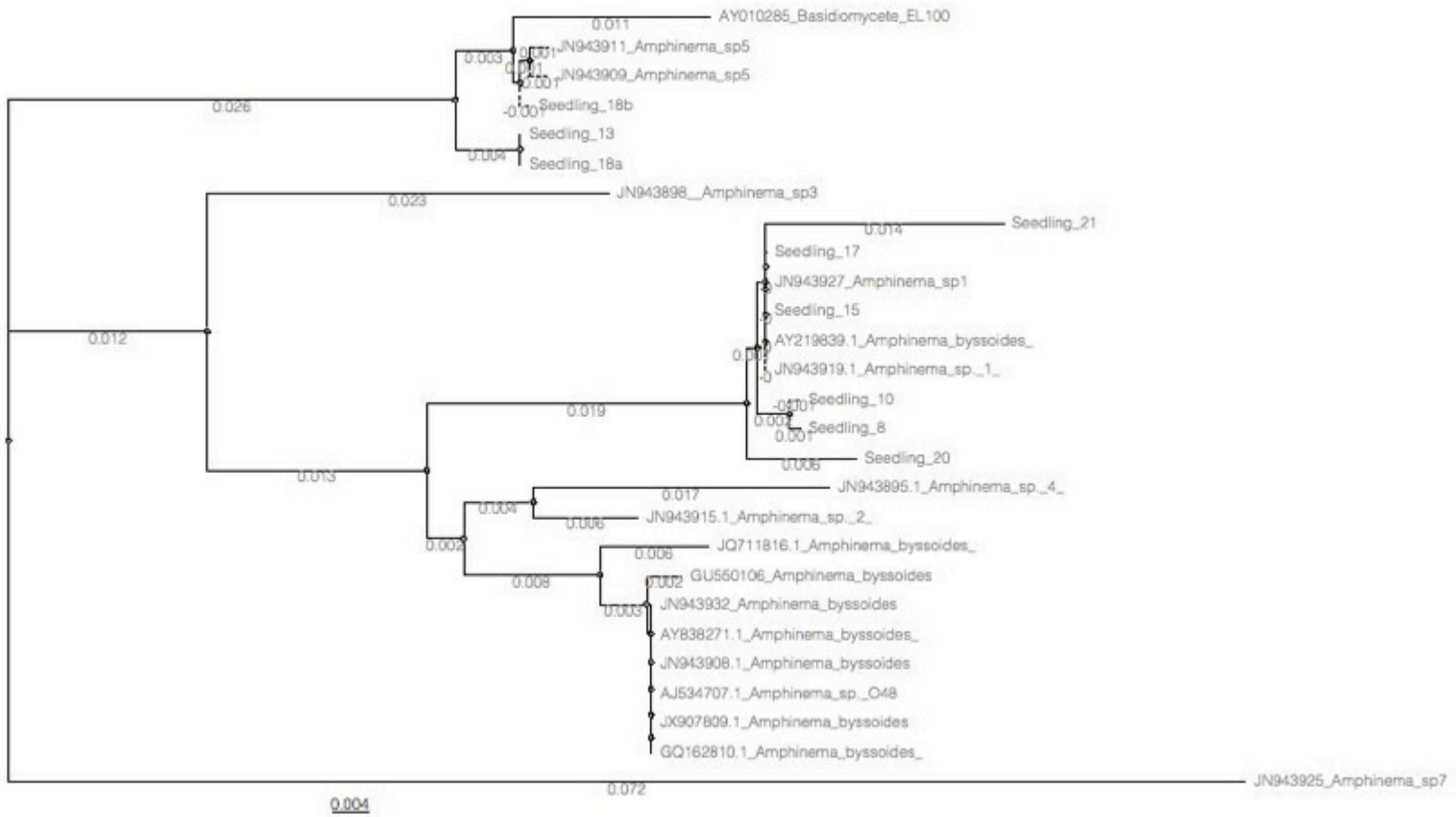

Fig 5.- Phylogenetic tree showing relatedness of various *Amphinema* spp. isolates, as well as sequenced *Amphinema* sp. 1 and *Amphinema* sp. 5 isolates from the experiment.

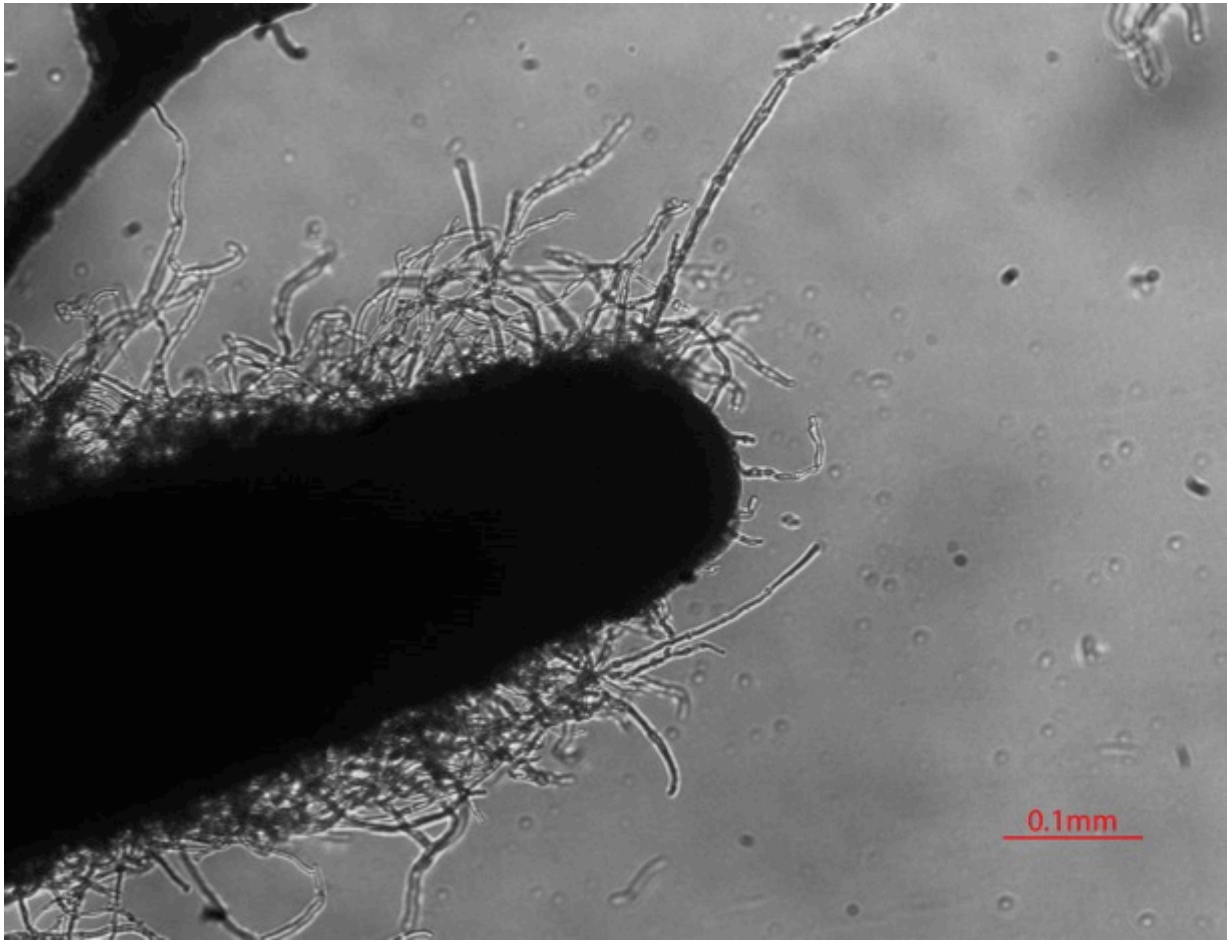

Fig 6.- *Amphinema* sp. 1 root tip showing loose emanating hyphae protruding at 100X magnification

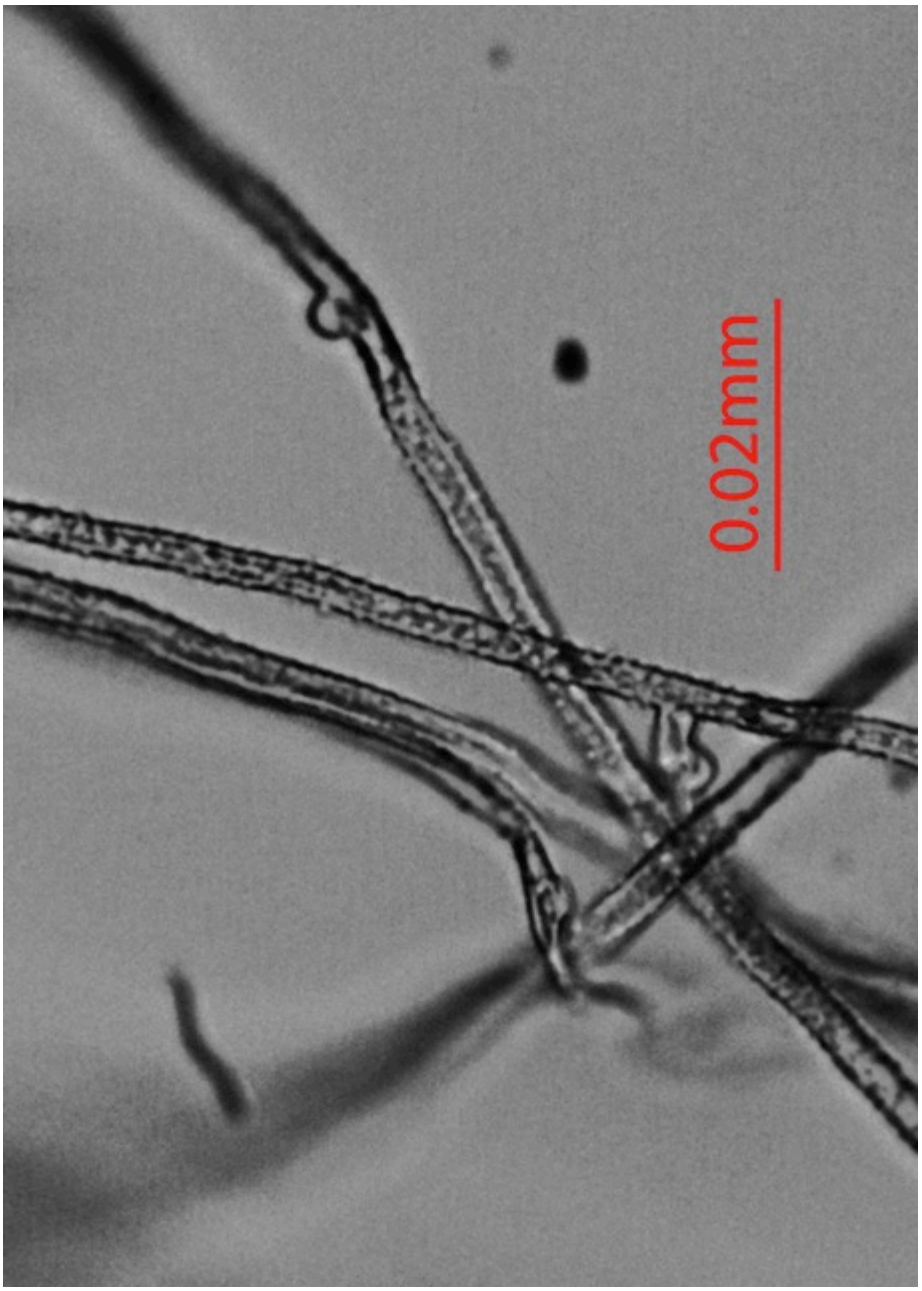

Fig 7.- *Amphinema* sp. 1 rough surface texture 400X magnification

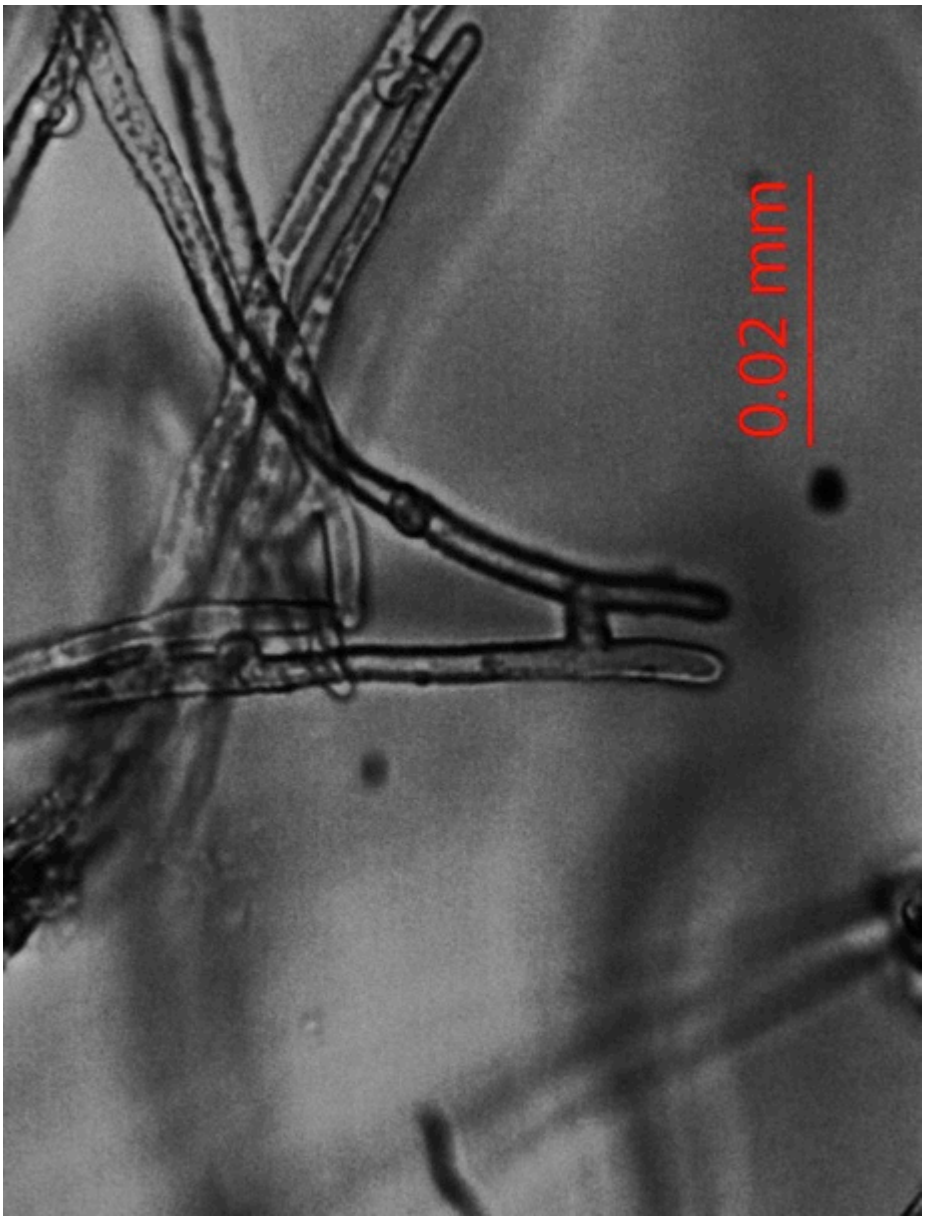

Fig 8.- *Amphinema* sp. 1 H anastamosis 400X magnification

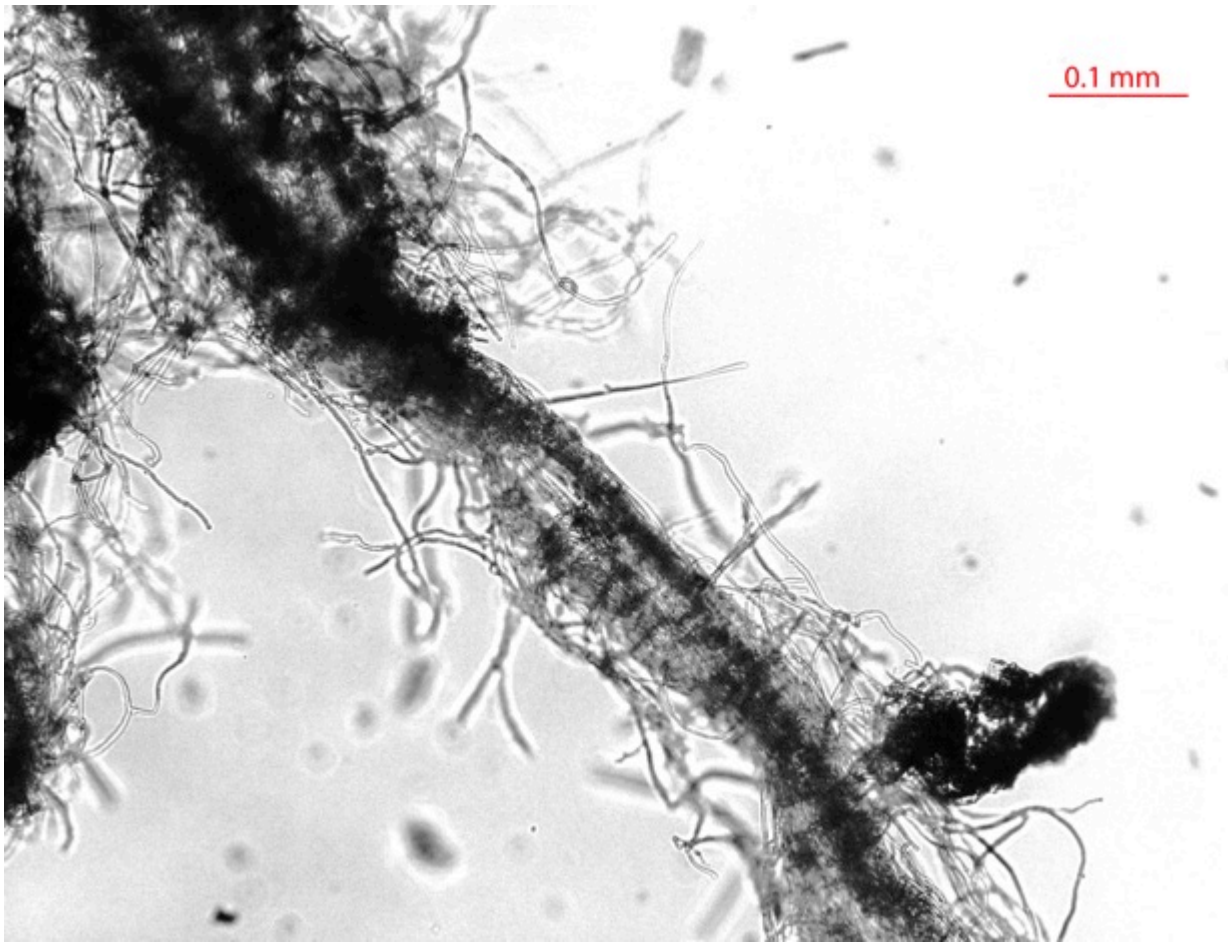

Fig 9.- *Amphinema* sp. 1 rhizomorph at 100X magnification

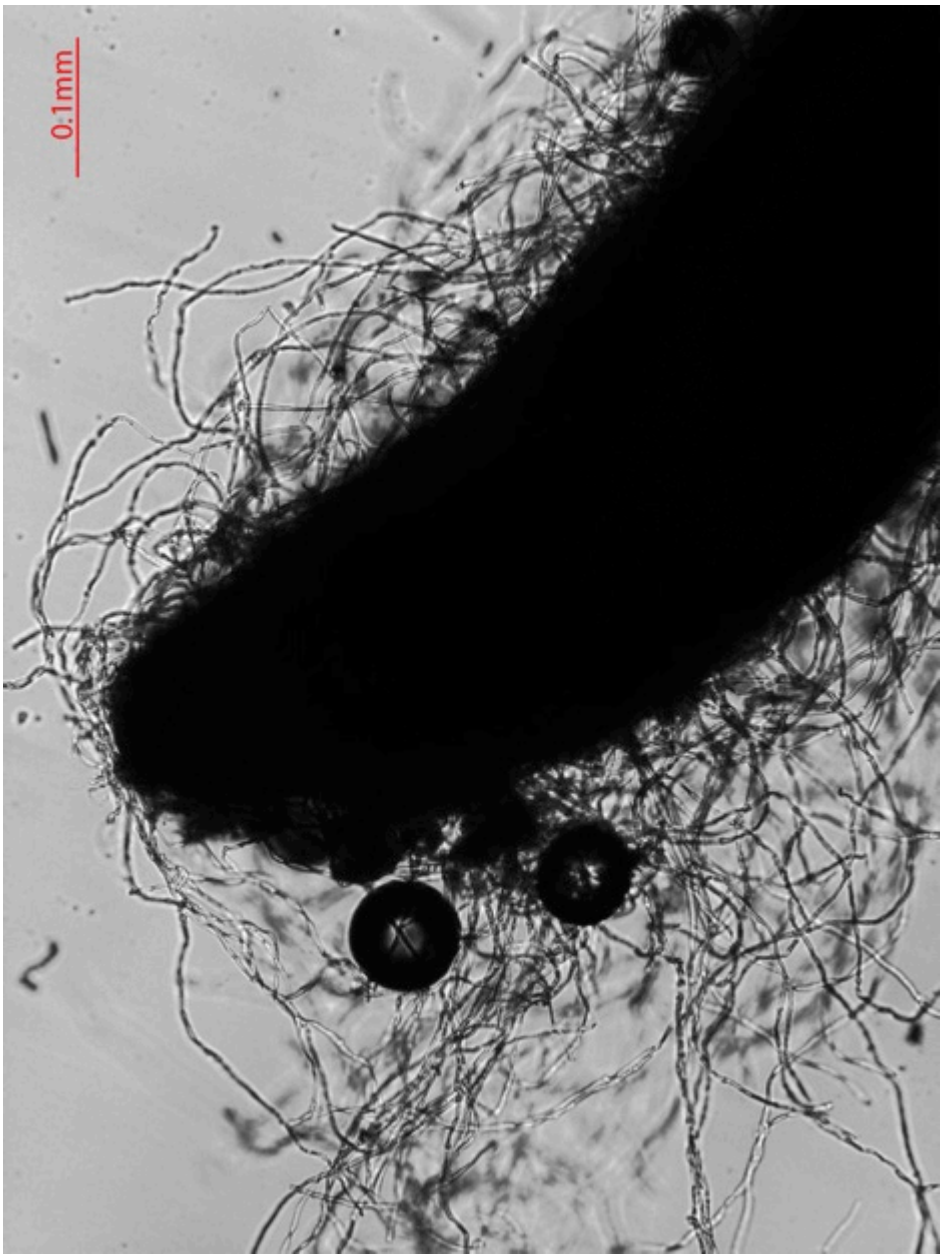

Fig 10.- *Amphinema* sp. 5 root tip with loose emanating hyphae at 100X magnification

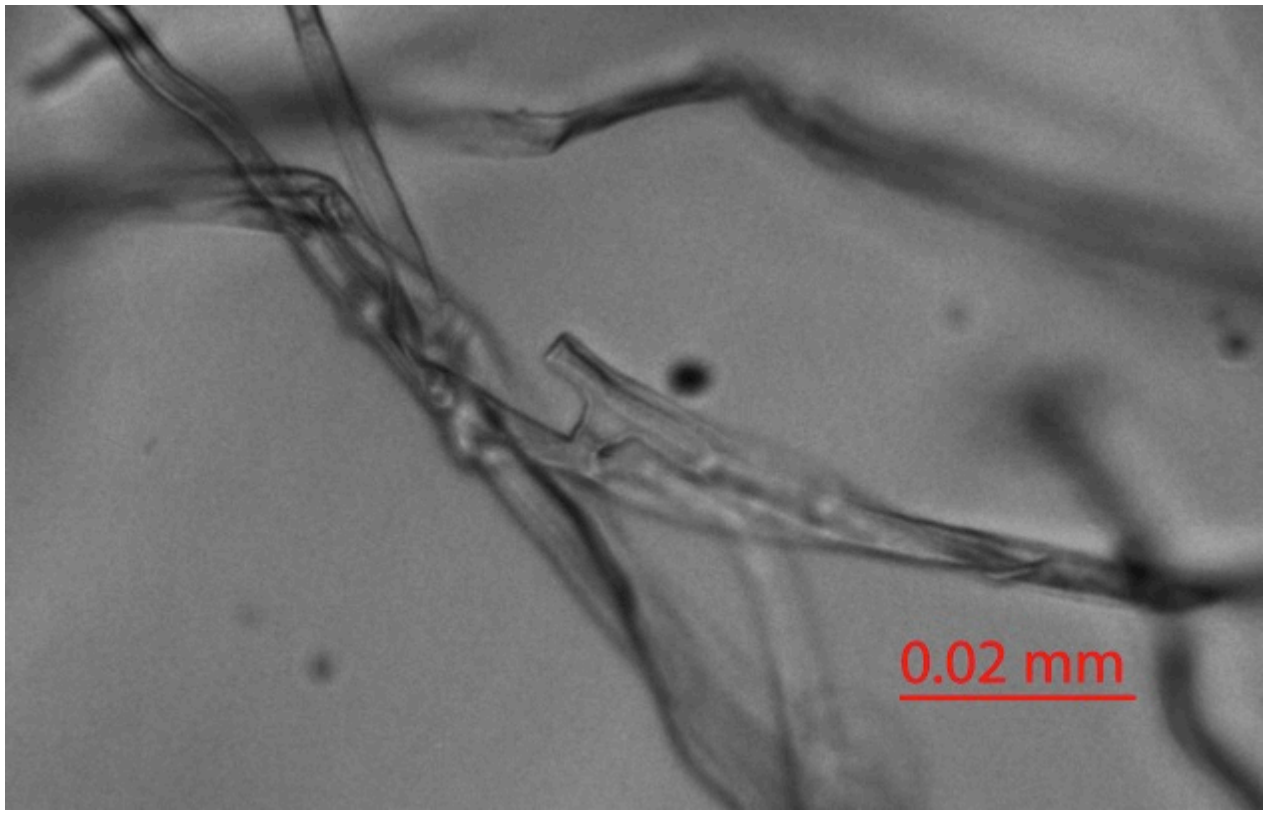

Fig 11.- *Amphinema* sp. 5 H anastamosis 400X magnification

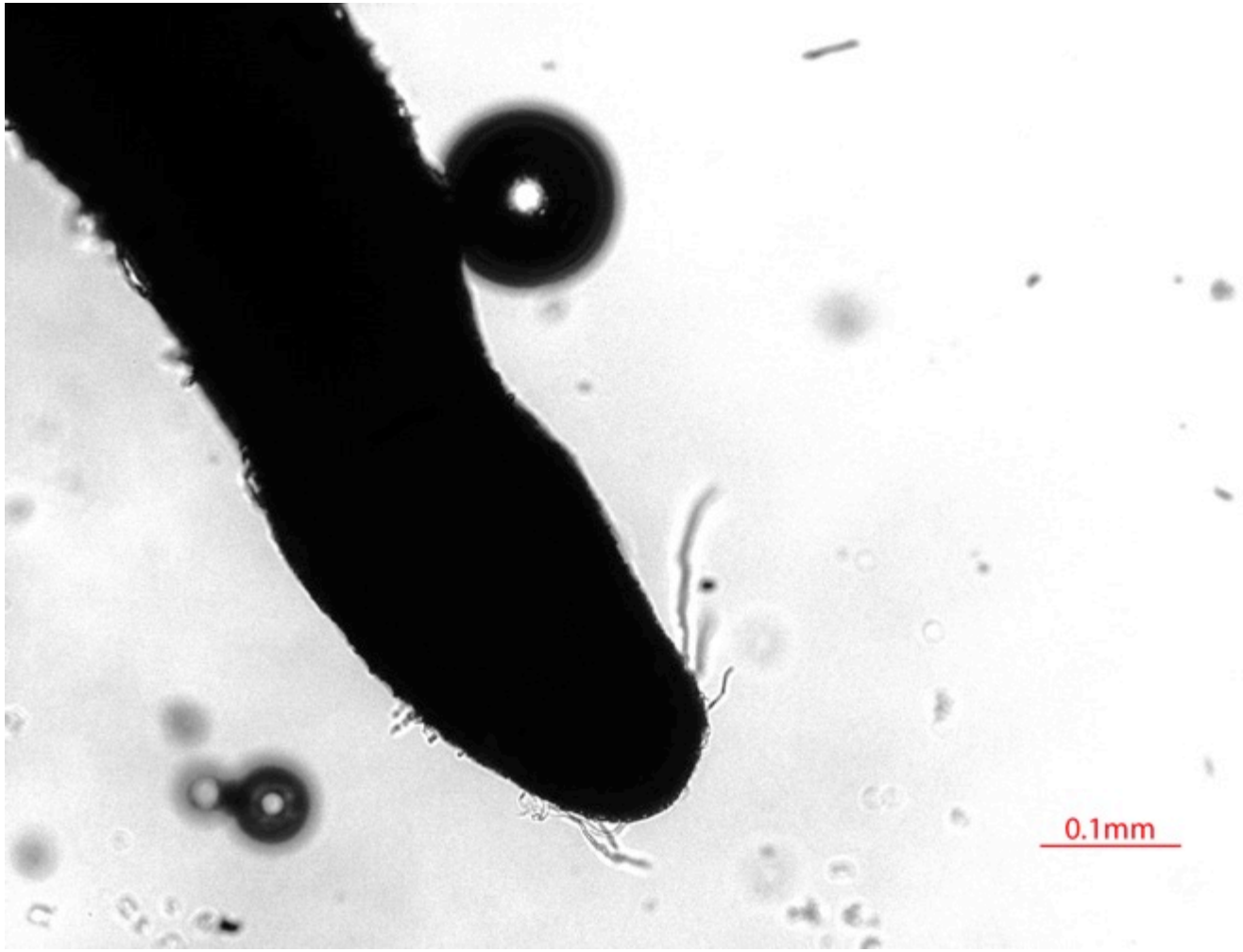

Fig 12.- *Thelephora terrestris* root tip with emanating cystidia at 100X magnification

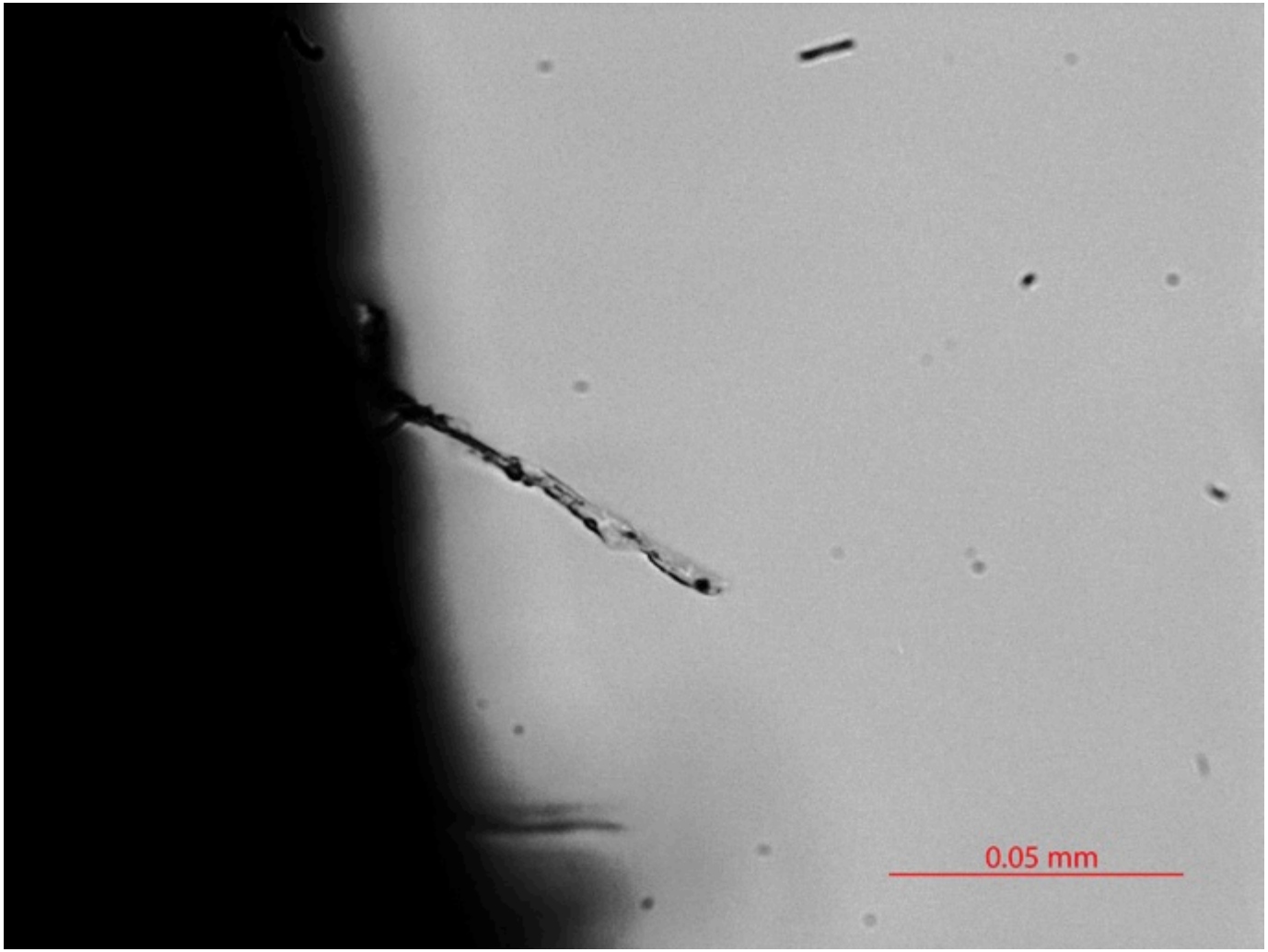

Fig 13.- *Thelephora terrestris* cystidia at 400X magnification

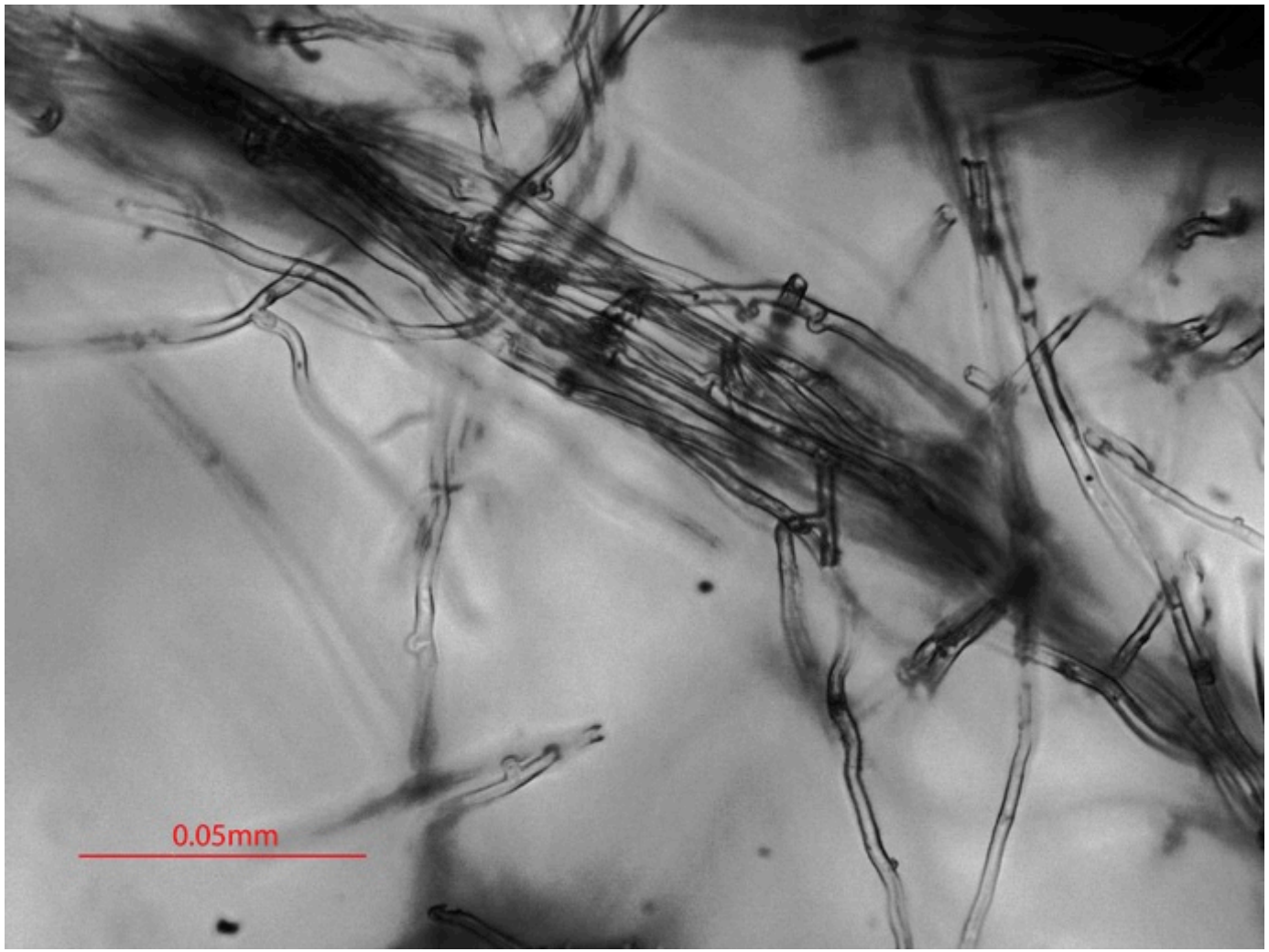

Fig 14.- *Thelephora terrestris* rhizomorph 400X magnification

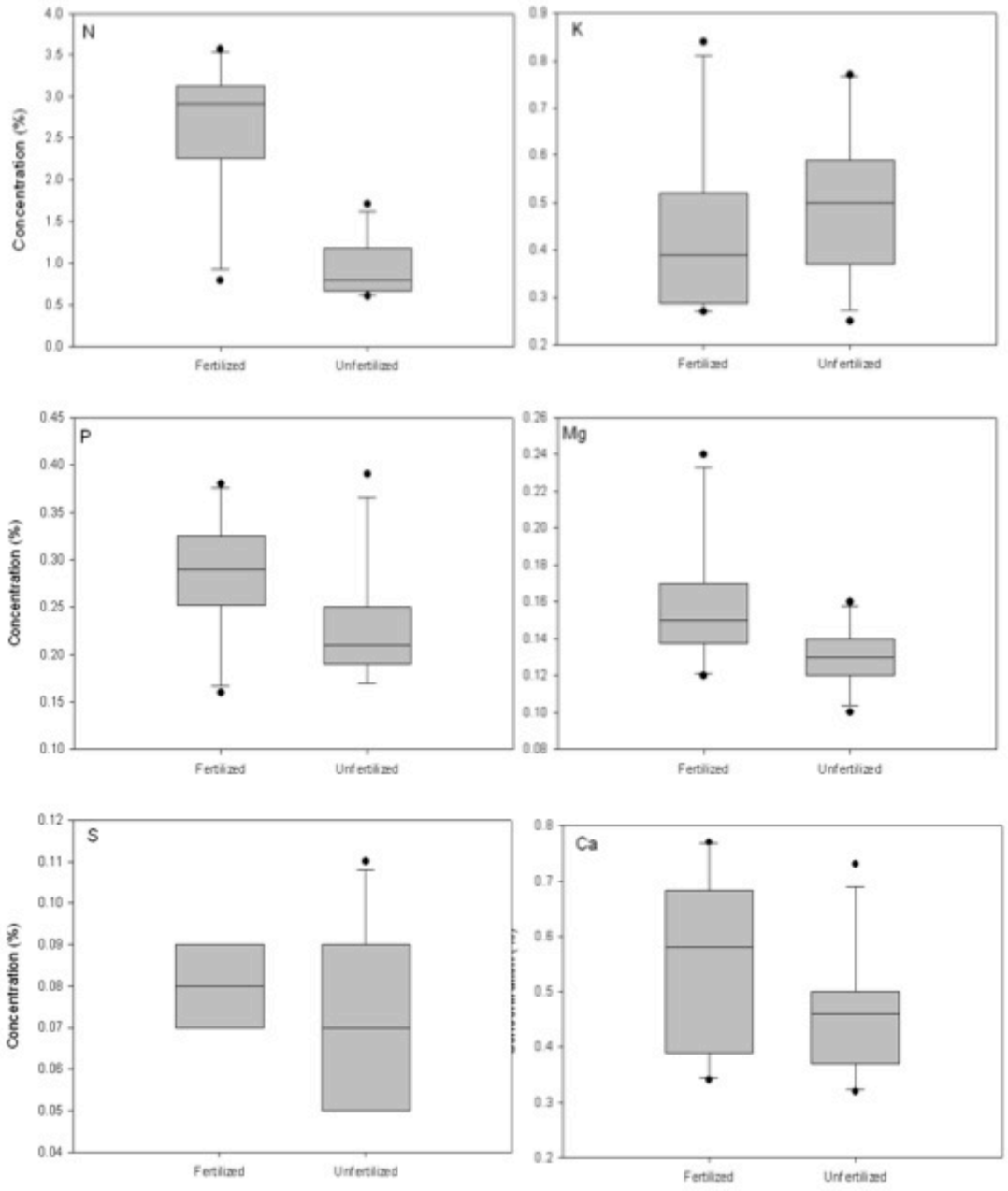

Fig 15.- Comparison of nutrient content for two treatment types (N, P, K, Ca, Mg, S)

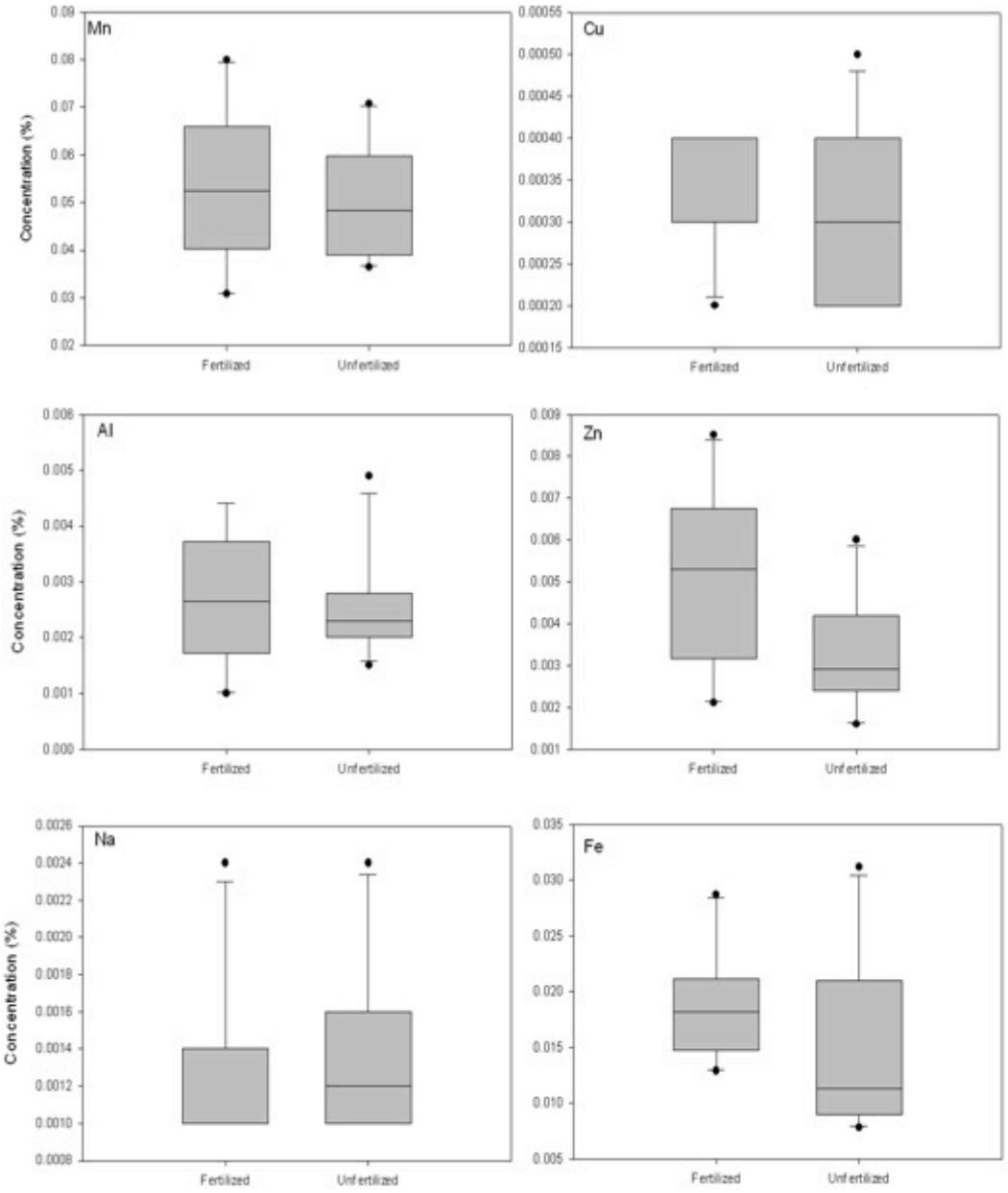

Fig 16.- Comparison of nutrient content for two treatment types (Mn, Cu, Al, Zn, Na, Fe)

Table 4.- Nutrient concentrations of all sample trees expressed as a percentage.

	#	N	P	K	Ca	Mg	S	Mn	Fe	Cu	B	Al	Zn	Na
					(g/Kg)					(mg/Kg)				
Unfertilized	11	9.4 ± 1.0	2.3 ± 0.2	5.1 ± 0.5	4.6 ± 0.3	1.3 ± 0.05	0.7 ± 0.07	0.5 ± 0.03	0.2 ± 0.02	3 ± 0.3	26 ± 2.7	65.5 ± 8.2	32.8 ± 4.1	13.5 ± 1.5
Fertilized	10	26.6 ± 2.5	2.8 ± 0.2	4.4 ± 0.5	5.5 ± 0.5	1.6 ± 0.1	0.8 ± 0.03	0.5 ± 0.05	0.2 ± 0.01	3.3 ± 0.2	27 ± 3.8	60.9 ± 5.2	51.7 ± 6.6	12.2 ± 1.4

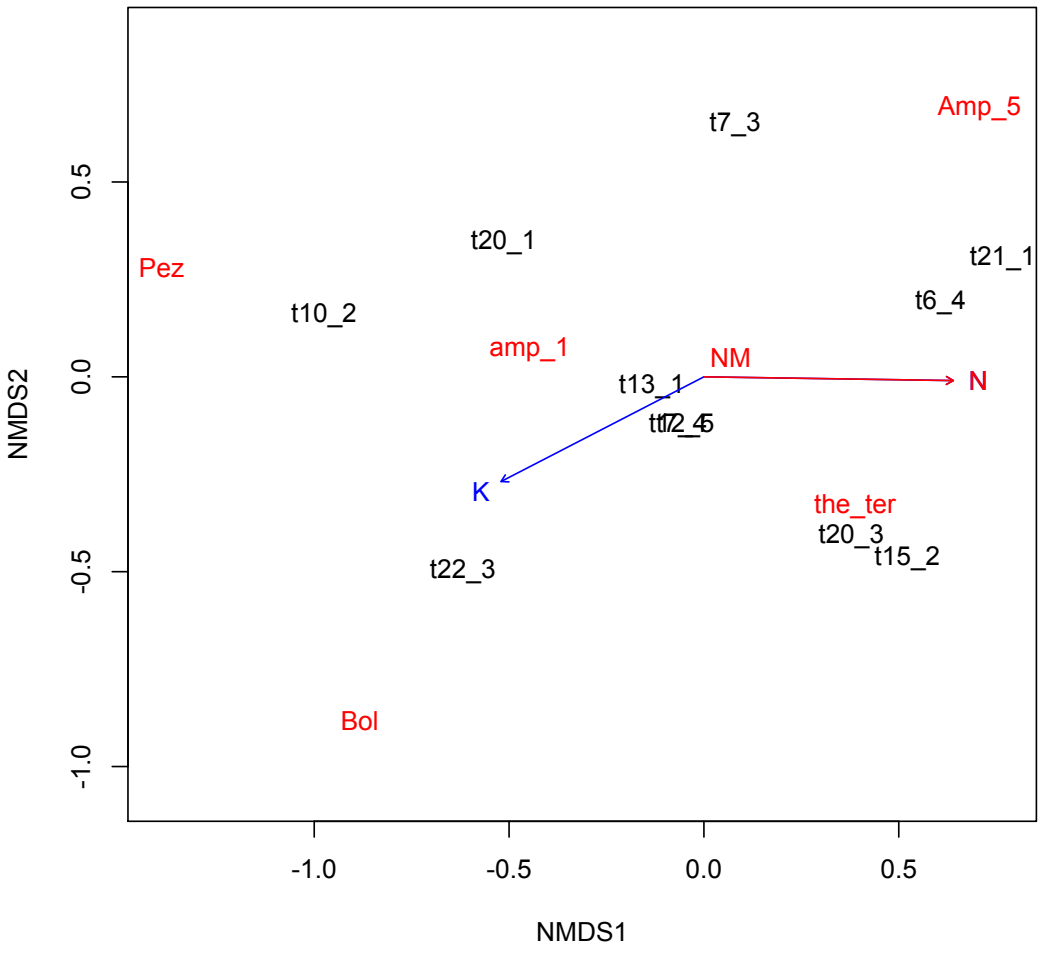

Fig 17.- Non-metric multidimensional scaling (NMDS) ordination analysis of EcMF communities on seedlings with EnvFit correlations of foliar nutrient concentrations to the ordination axes for unfertilized seedlings. Bol represents *Boletus variipes*, Pez represents *Sphaerosporella brunnea*, amp_1 represents *Amphinema* sp. 1, Amp_5 represents *Amphinema* sp. 5, the_ter represents *Thelephora terrestris*, and t#_# represent seedlings. Red arrows indicate signicant trends ($p < 0.05$), while blue arrows represent marginally significant trends ($p<0.1$).

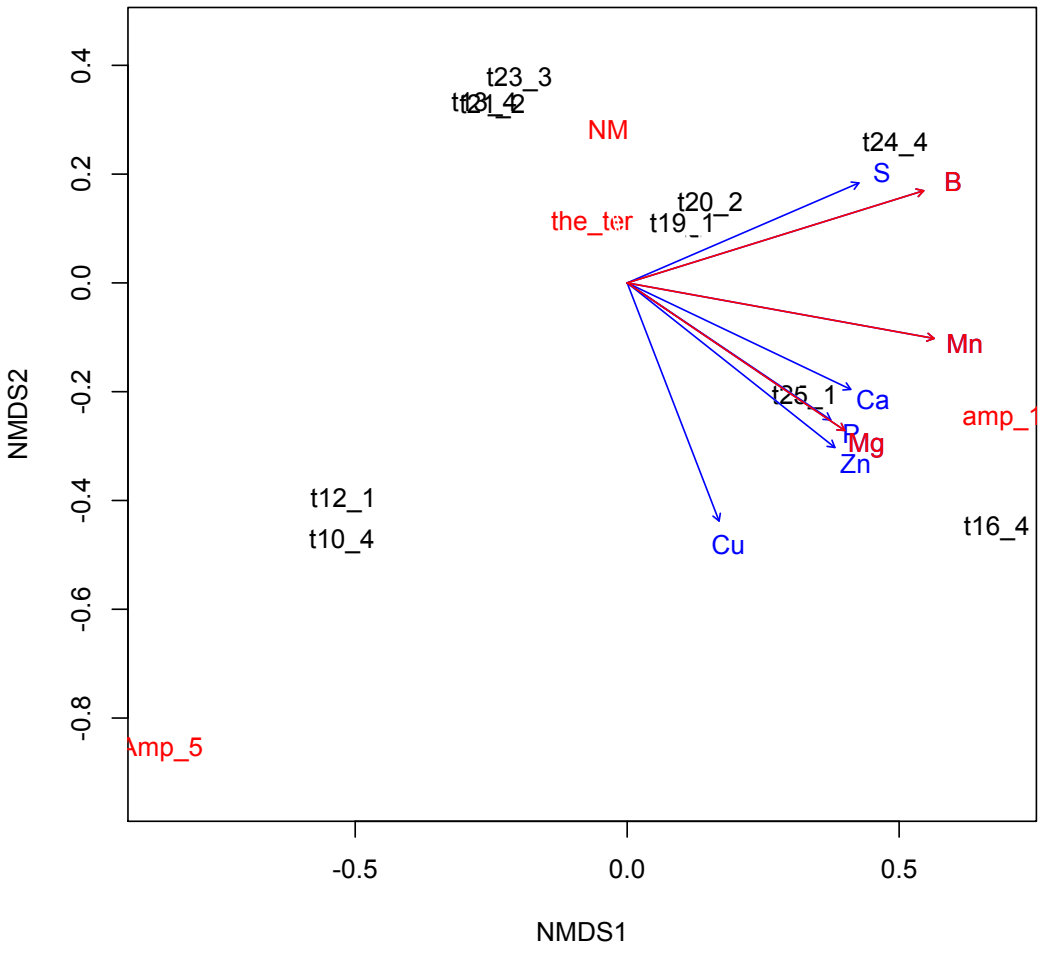

Fig 18.- Non-metric multidimensional scaling (NMDS) ordination analysis of EcMF communities on seedlings with EnvFit correlations of foliar nutrient concentrations to the ordination axes for fertilized seedlings. Bol represents *Boletus variipes*, Pez represents *Sphaerosporella brunnea*, amp_1 represents *Amphinema* sp. 1, Amp_5 represents *Amphinema* sp. 5, the_ter represents *Thelephora terrestris*, and t#_# represent seedlings Red arrows indicate signicant trends ($p < 0.05$), while blue arrows represent marginally significant trends ($p<0.1$).

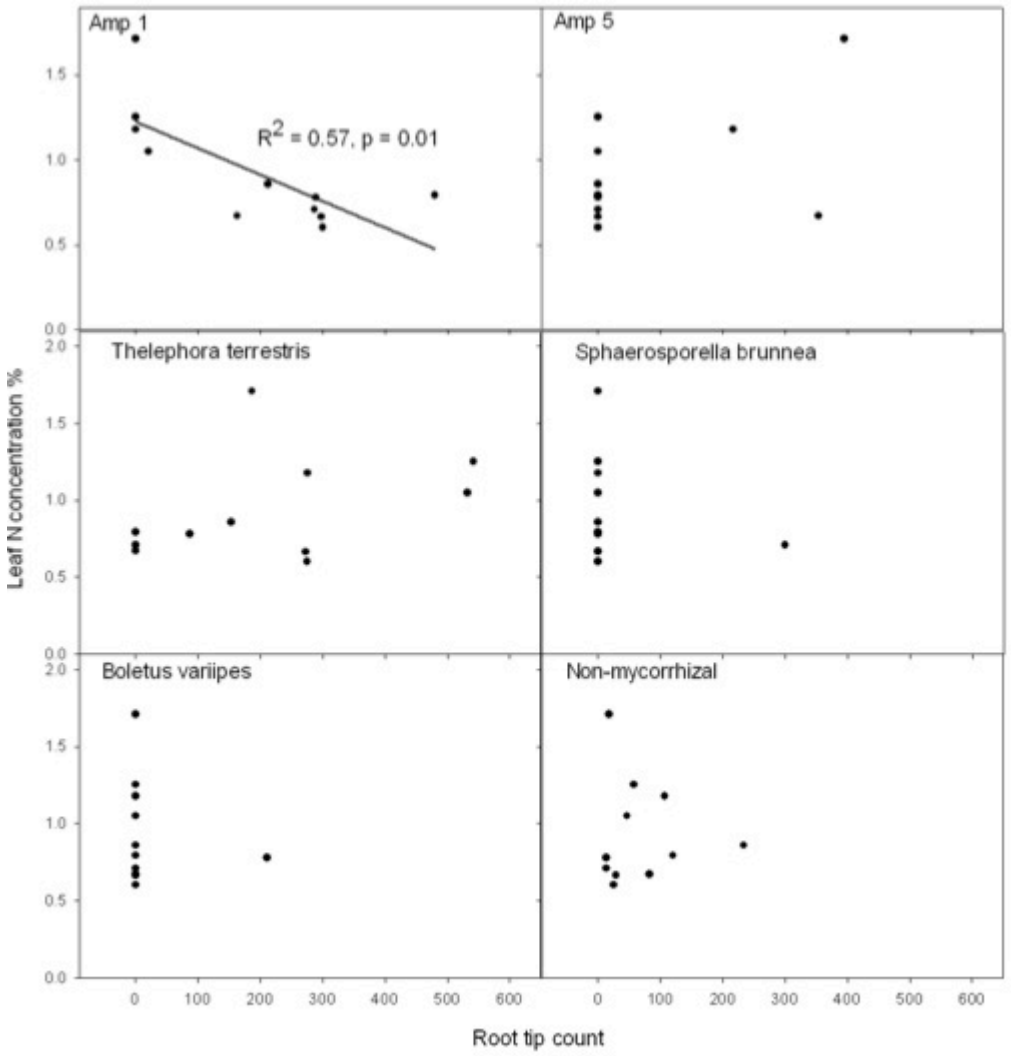

Fig 19.- Relationships between fungal species and foliar nitrogen concentration of unfertilized seedlings.

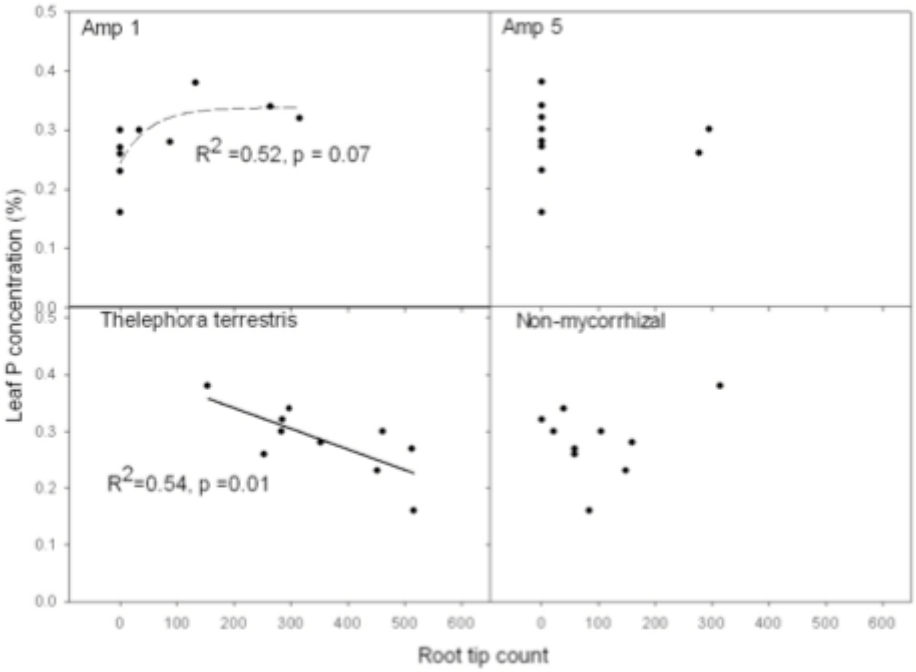

Fig 20.- Relationships between fungal species and foliar phosphorus concentration of fertilized seedlings.

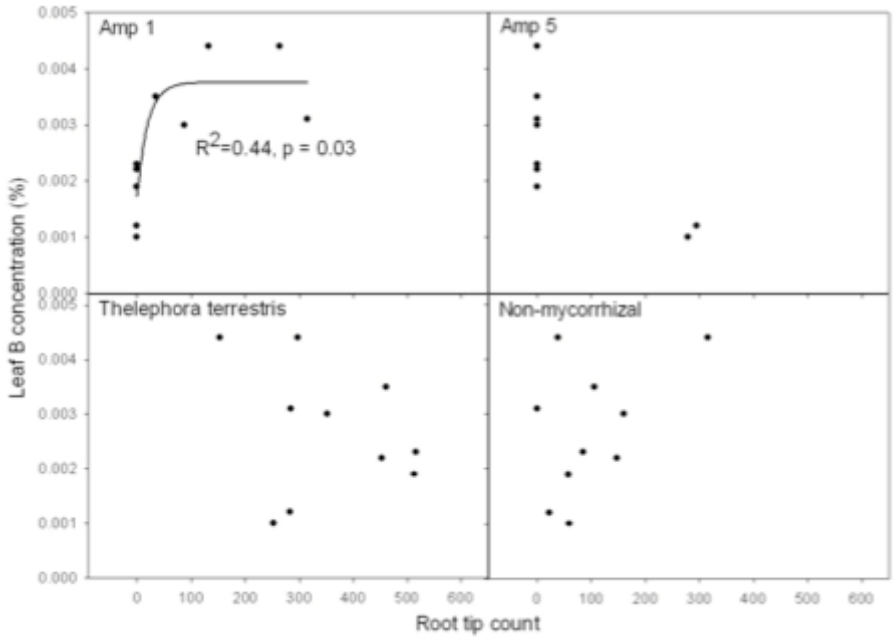

Fig 21.- Relationships between fungal species and foliar boron concentration of fertilized seedlings.

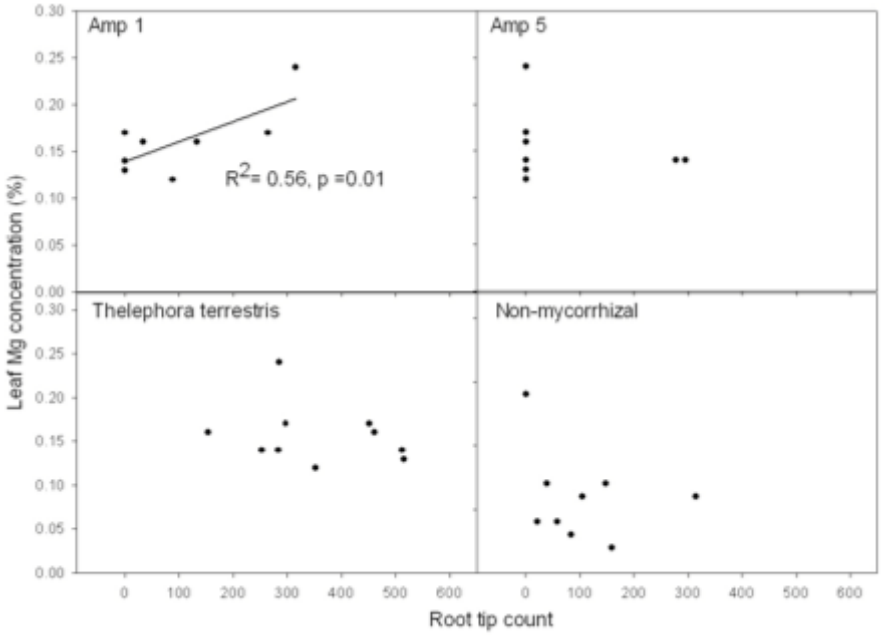

Fig 22.- Relationships between fungal species and foliar magnesium concentration of fertilized seedlings.

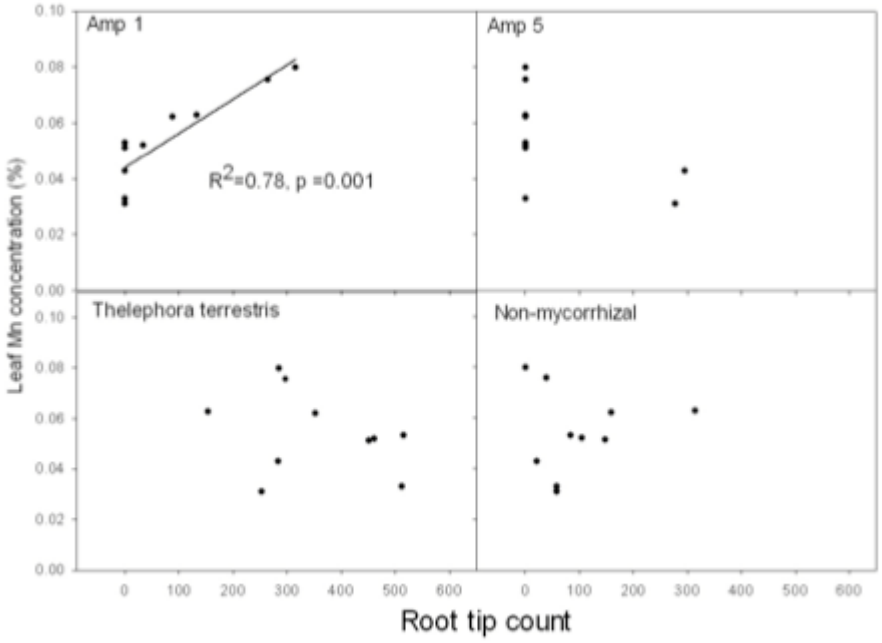

Fig 23.- Relationships between fungal species and foliar manganese concentration of fertilized seedlings.

Table 5.- Nutrient ratios based on concentration within seedlings.

		N	P	K	Ca	Mg	S	Mn	Fe	Cu	B	Al	Zn	Na
Fertilized	**N**	-	9.40	6.11	4.82	17.01	33.80	49.20	142.10	8090.91	988.89	438.42	516.44	2188.52
	P	0.11	-	0.64	0.51	1.78	3.54	5.16	14.90	848.48	103.70	45.98	54.16	229.51
	K	0.17	1.55	-	0.79	2.80	5.57	8.11	23.42	1333.33	162.96	72.25	85.11	360.66
	Ca	0.21	1.94	1.26	-	3.50	6.96	10.13	29.27	1666.67	203.70	90.31	106.38	450.82
	Mg	0.06	0.56	0.37	0.29	-	2.03	2.95	8.52	484.85	59.26	26.27	30.95	131.15
	S	0.03	0.28	0.18	0.14	0.51	-	1.47	4.26	242.42	29.63	13.14	15.47	65.57
	Mn	0.02	0.18	0.11	0.09	0.32	0.63	-	2.66	151.52	18.52	8.21	9.67	40.98
	Fe	0.01	0.07	0.04	0.03	0.12	0.24	0.35	-	56.97	6.96	3.09	3.64	15.41
	Cu	1.1E-04	1.1E-03	6.9E-04	5.4E-04	1.9E-03	3.8E-03	5.5E-03	0.02	-	0.11	0.05	0.06	0.25
	B	1.0E-03	9.5E-03	6.2E-03	4.9E-03	1.7E-02	3.4E-02	5.0E-02	0.14	8.18	-	0.44	0.52	2.21
	Al	2.3E-03	2.1E-02	1.4E-02	1.1E-02	3.9E-02	7.7E-02	1.1E-01	0.32	18.48	2.26	-	1.18	5.00
	Zn	2.0E-03	1.8E-02	1.2E-02	9.4E-03	3.3E-02	6.6E-02	9.6E-02	0.28	15.76	1.93	0.85	-	4.26
	Na	4.5E-04	4.2E-03	2.7E-03	2.2E-03	7.6E-03	1.5E-02	2.2E-02	0.06	3.64	0.44	0.20	0.23	-
Unfertilized	**N**	-	4.16	1.86	2.06	7.16	13.06	18.89	61.65	3166.67	371.89	145.14	289.47	701.34
	P	0.24	-	0.45	0.50	1.73	3.16	4.57	14.93	766.67	90.04	35.14	70.08	169.80
	K	0.54	2.24	-	1.10	3.84	7.01	10.14	33.10	1700.00	199.64	77.92	155.40	376.51
	Ca	0.49	2.02	0.90	-	3.47	6.33	9.15	29.85	1533.33	180.07	70.28	140.17	339.60
	Mg	0.14	0.57	0.25	0.28	-	1.79	2.59	8.44	433.33	50.89	19.86	39.61	95.97
	S	0.07	0.31	0.14	0.15	0.53	-	1.39	4.54	233.33	27.40	10.69	21.33	51.68
	Mn	0.05	0.22	0.10	0.11	0.38	0.69	-	3.24	166.67	19.57	7.64	15.24	36.91
	Fe	0.02	0.07	0.03	0.03	0.12	0.21	0.31	-	51.33	6.03	2.35	4.69	11.37
	Cu	3.2E-04	1.3E-03	5.9E-04	6.5E-04	2.3E-03	4.1E-03	6.0E-03	0.02	-	0.12	0.05	0.09	0.22
	B	2.8E-03	1.1E-02	5.1E-03	5.6E-03	2.0E-02	3.6E-02	5.2E-02	0.17	8.67	-	0.40	0.79	1.92
	Al	6.9E-03	2.8E-02	1.3E-02	1.4E-02	4.9E-02	8.9E-02	1.3E-01	0.42	21.67	2.54	-	1.98	4.80
	Zn	3.5E-03	1.4E-02	6.4E-03	7.1E-03	2.5E-02	4.5E-02	6.6E-02	0.21	11.00	1.29	0.50	-	2.44
	Na	1.5E-03	6.1E-03	2.7E-03	3.0E-03	1.1E-02	1.9E-02	2.8E-02	0.09	4.67	0.55	0.21	0.43	-

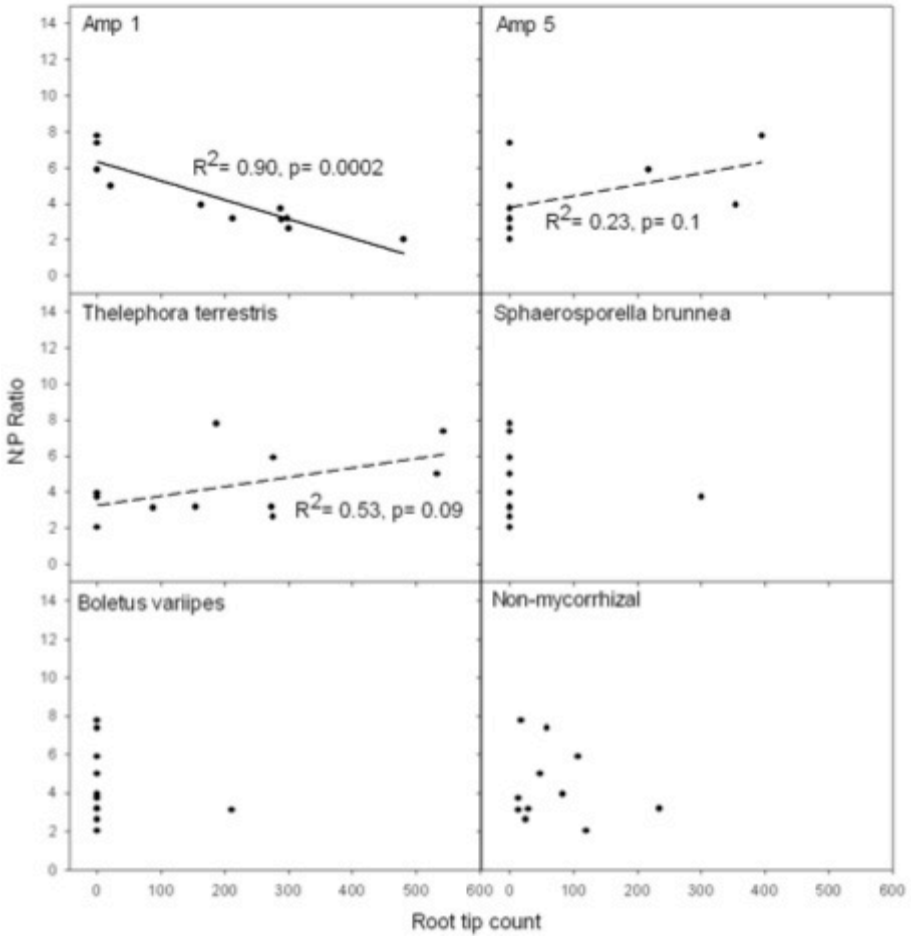

Figure 24.- Ratio of N:P in unfertilized seedlings.

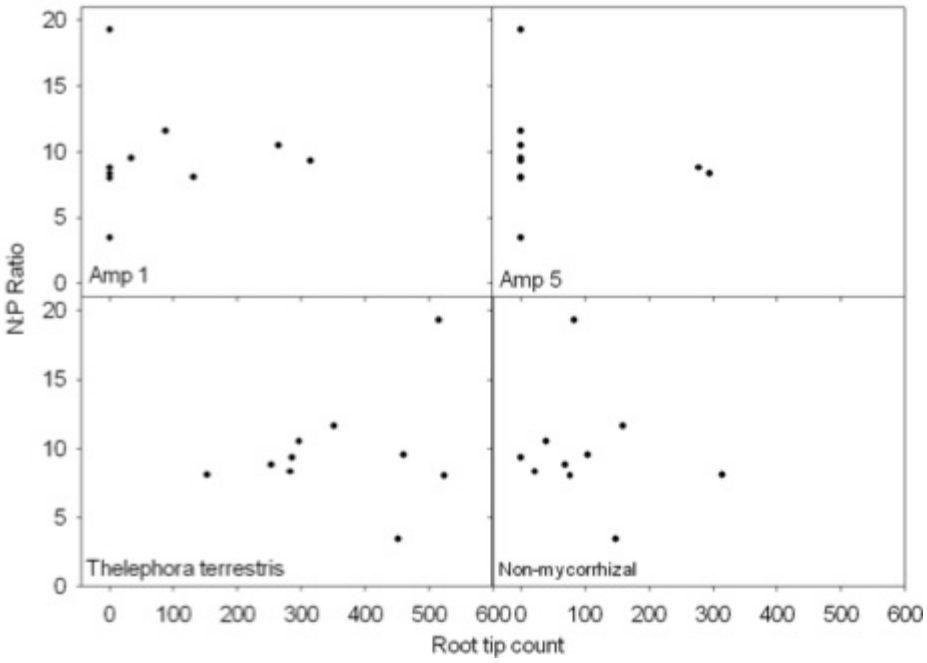

Figure 25.- Ratio of N:P in fertilized seedlings

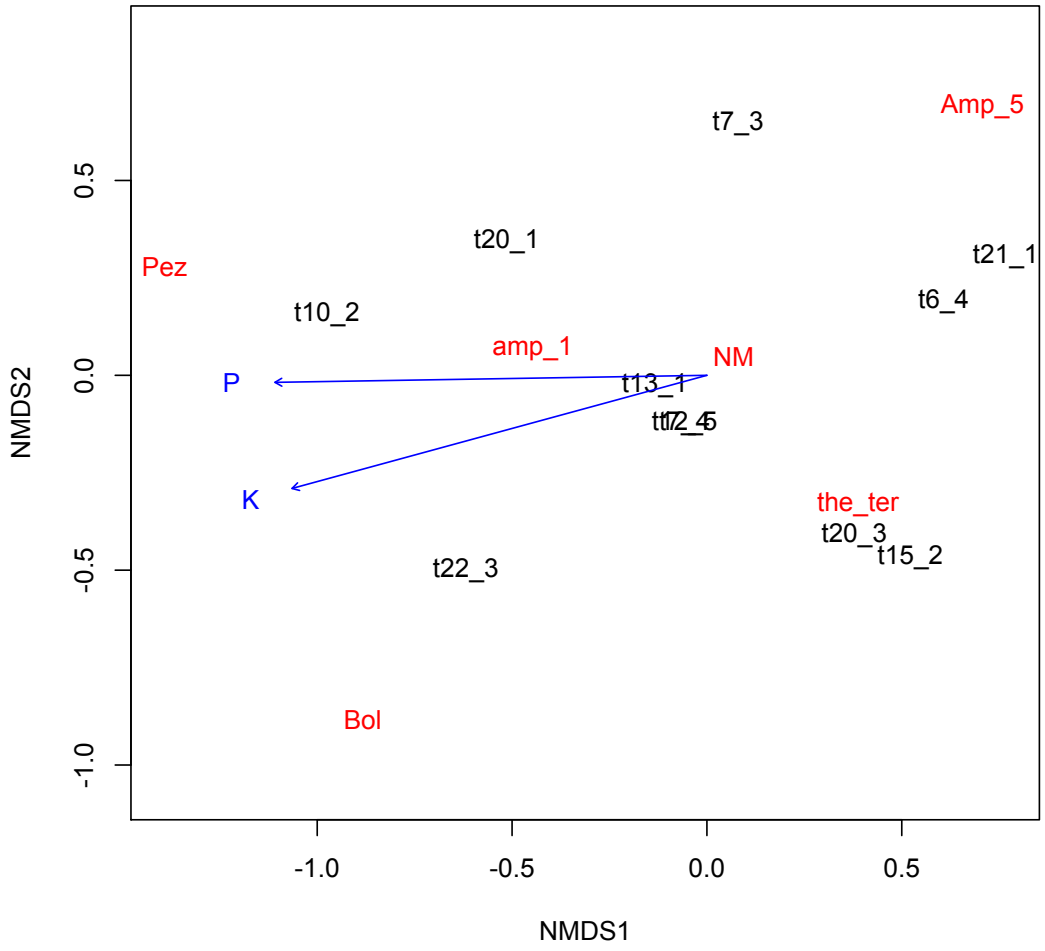

Fig 26.- Non-metric multidimensional scaling (NMDS) ordination analysis of EcMF communities on seedlings with EnvFit correlations of foliar nutrient content to the ordination axes for unfertilized seedlings. Bol represents *Boletus variipes*, Pez represents *Sphaerosporella brunnea*, amp_1 represents *Amphinema* sp. 1, Amp_5 represents *Amphinema* sp. 5, the_ter represents *Thelephora terrestris*, and t#_# represent seedlings. Red arrows indicate signicant trends ($p < 0.05$), while blue arrows represent marginally significant trends ($p<0.1$).

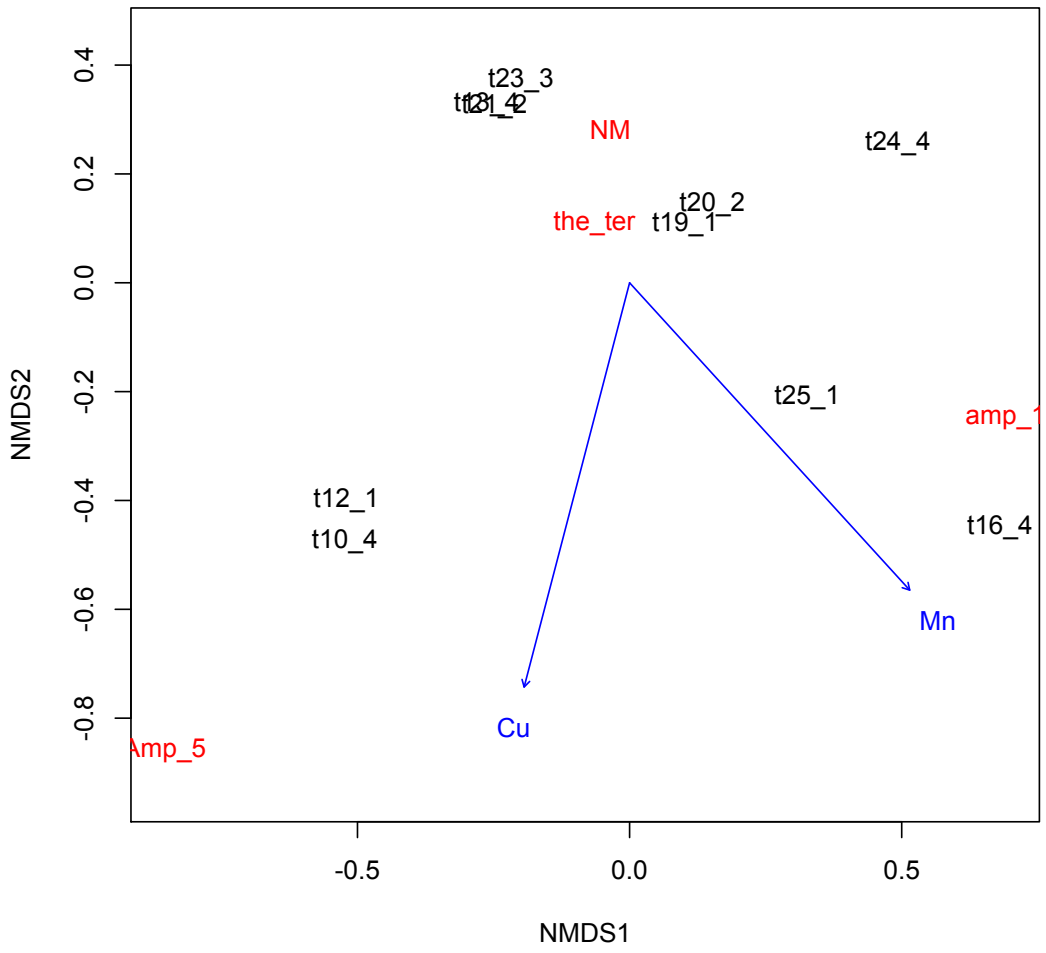

Fig 27.- Non-metric multidimensional scaling (NMDS) ordination analysis of EcMF communities on seedlings with EnvFit correlations of foliar nutrient content to the ordination axes for fertilized seedlings. Bol represents *Boletus variipes*, Pez represents *Sphaerosporella brunnea*, amp_1 represents *Amphinema* sp. 1, Amp_5 represents *Amphinema* sp. 5, the_ter represents *Thelephora terrestris*, and t#_# represent seedlings. Red arrows indicate signicant trends ($p < 0.05$), while blue arrows represent marginally significant trends ($p<0.1$).

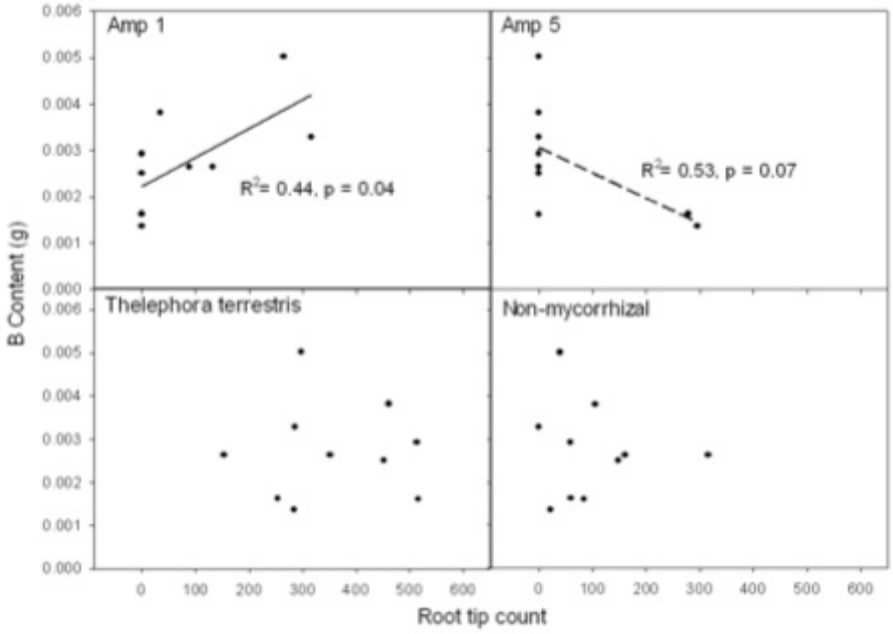

Fig 28.- Relationships between fungal species and foliar boron content of fertilized seedlings. A significant positive trend was observed in *Amphinema* sp. 1.

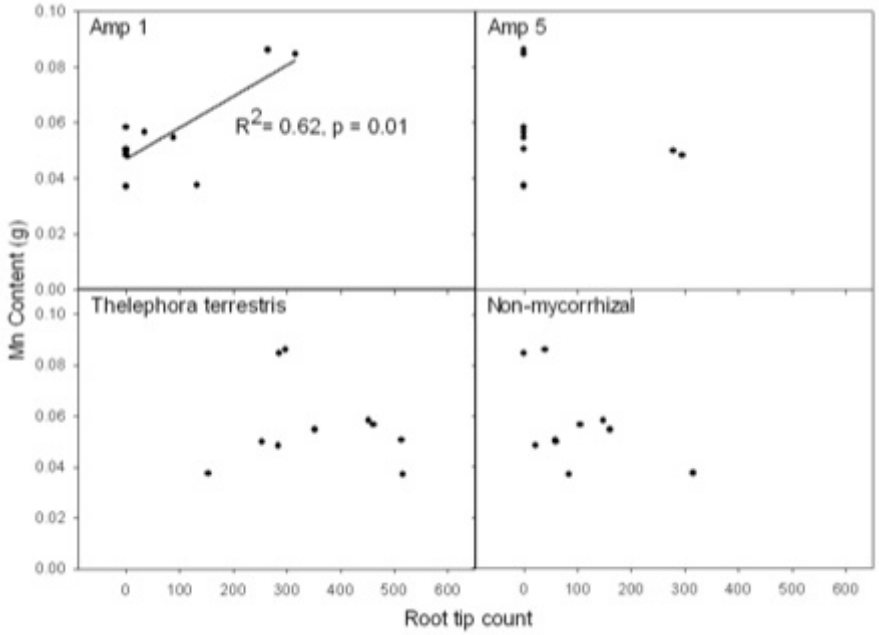

Fig 29.- Relationships between fungal species and foliar manganese content of fertilized seedlings. A significant positive trend was observed in *Amphinema* sp. 1.

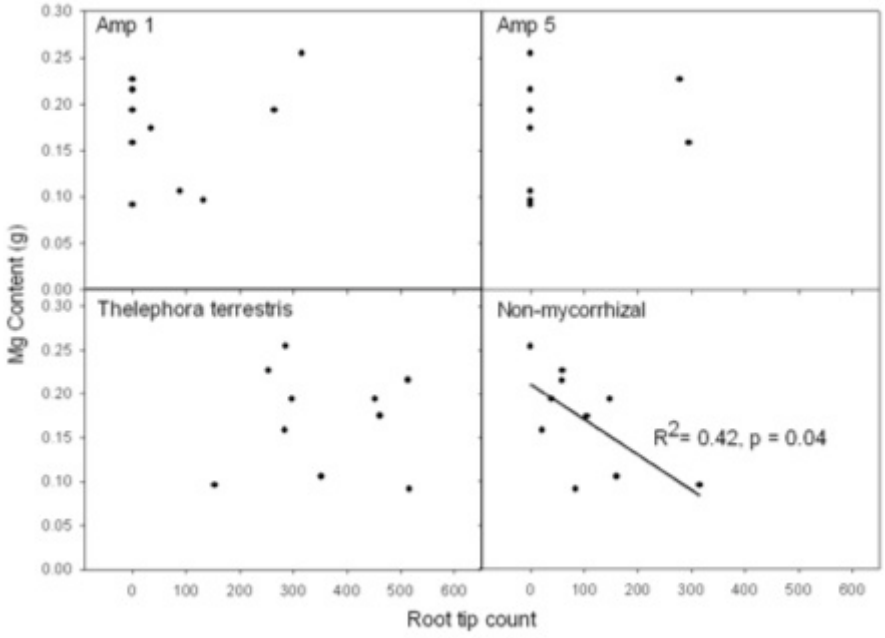

Fig 30.- Relationships between fungal species and foliar magnesium content of fertilized seedlings. A significant negative trend was observed in non-mycorrhizal root tips.

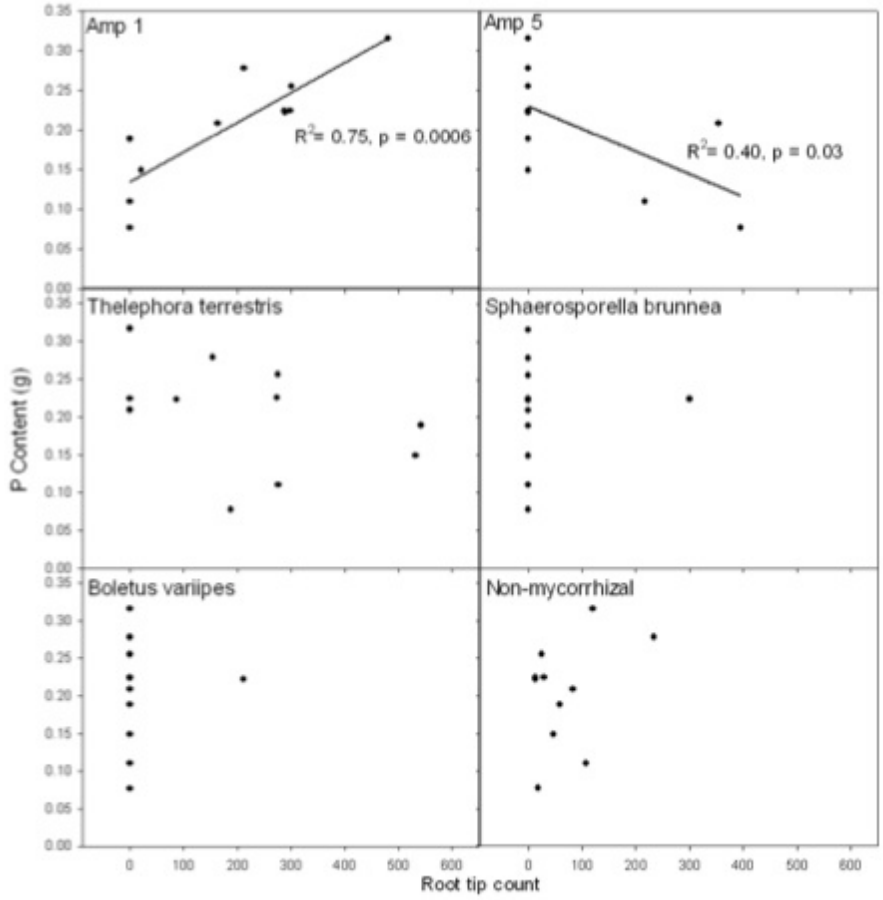

Fig 31.- Relationships between fungal species and foliar phosphorus content of unfertilized seedlings. A significant positive trend was observed in *Amphinema* sp. 1. A significant negative trend was observed in *Amphinema* sp. 5.

Table 6.- Individual measures of foliar biomass (g), stem biomass (g), below ground biomass (g), total above ground biomass (g), and total biomass (g), as well as standard error and P value.

Treatment	Needle (g)	Stem (g)	Roots (g)	R:S ratio	Above Ground Biomass (g)	Total biomass (g)
Fertilized	1.15 ± 0.02	1.27 ± 0.05	1.71 ± 0.05	1.43 ± 0.7	2.43 ± 0.07	4.13 ± 0.11
Un-fertilized	0.91 ± 0.03	0.72 ± 0.02	1.32 ± 0.04	1.23 ± 0.4	1.63 ± 0.04	2.95 ± 0.09
P value	0.05	0.003	0.01	0.1	0.01	0.02

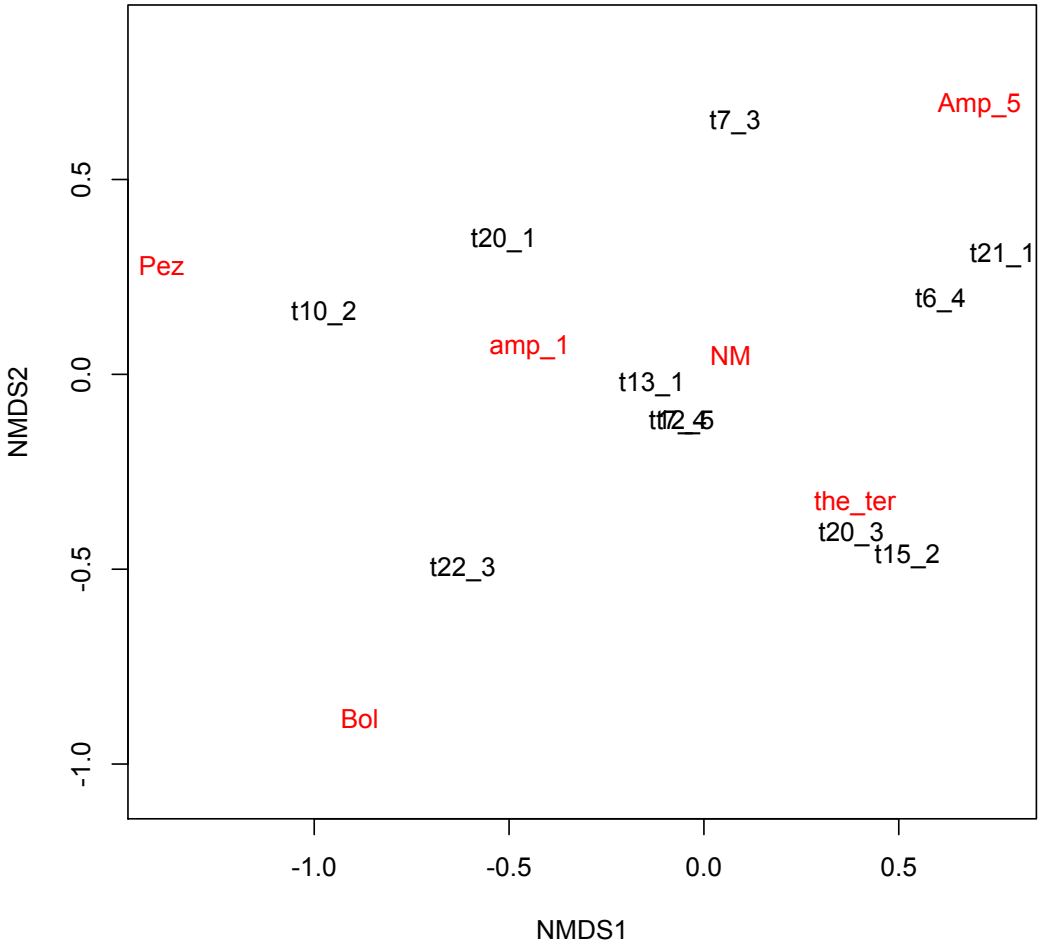

Fig 32.- Non-metric dimensional analysis using the R package VEGAN to determine significance of difference amongst total biomass, stem biomass, root biomass, needle biomass, and root to shoot ratio in unfertilized seedlings. Bol represents *Boletus variipes*, Pez represents *Sphaerosporella brunnea*, amp_1 represents *Amphinema* sp. 1, Amp_5 represents *Amphinema* sp. 5, the_ter represents *Thelephora terrestris*, and t#_# represent seedlings. There were no significant trends in the Envfit analysis.

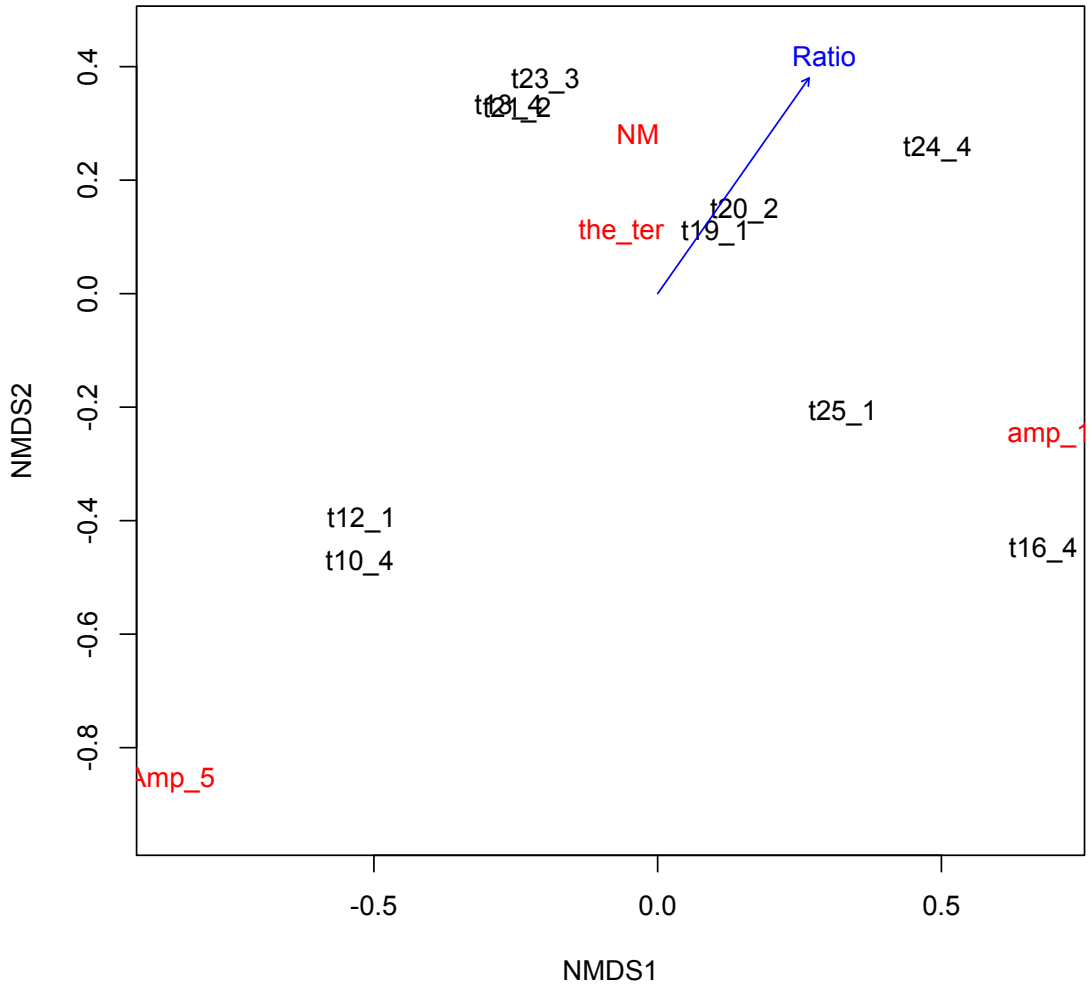

Fig 33.- Non-metric dimensional analysis using the R package VEGAN to determine significance of difference amongst total biomass, stem biomass, root biomass, needle biomass, and root to shoot ratio in fertilized seedlings. Bol represents *Boletus variipes*, Pez represents *Sphaerosporella brunnea*, amp_1 represents *Amphinema* sp. 1, Amp_5 represents *Amphinema* sp. 5, the_ter represents *Thelephora terrestris*, and t#_# represent seedlings. For the Envfit analysis, red arrows indicate signicant trends ($p < 0.05$), while blue arrows represent marginally significant trends ($p<0.1$).

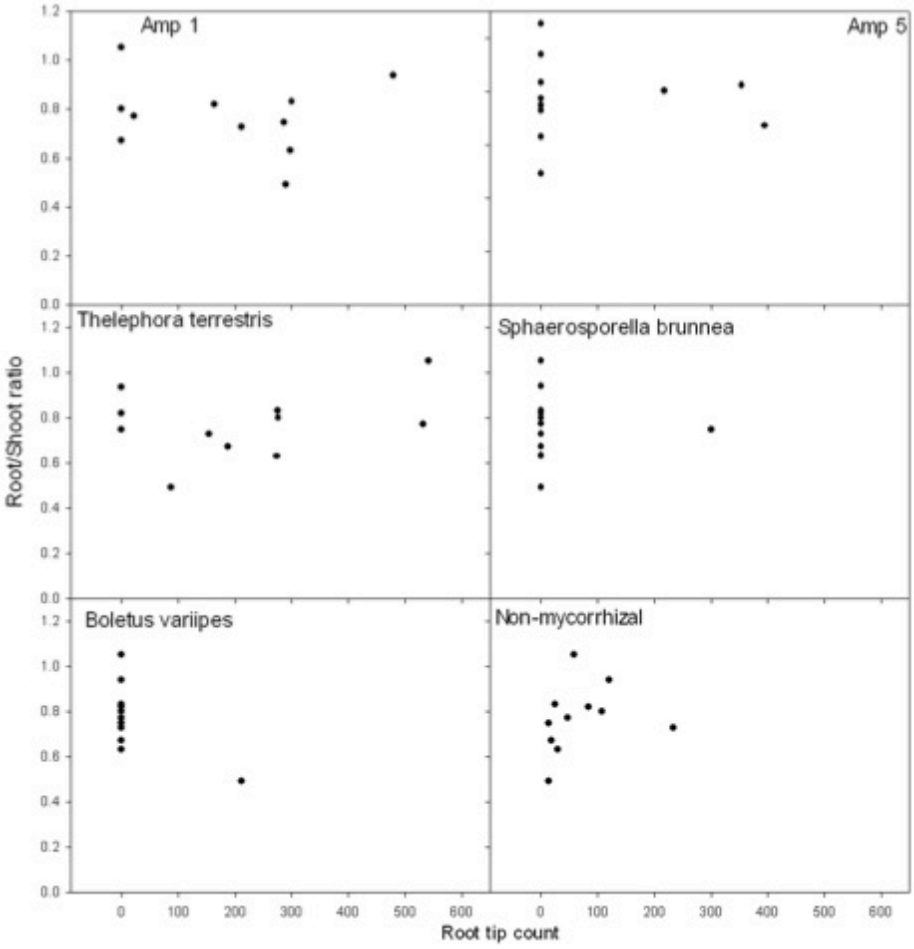

Fig 34.- Root to shoot ratios for unfertilized seedlings..

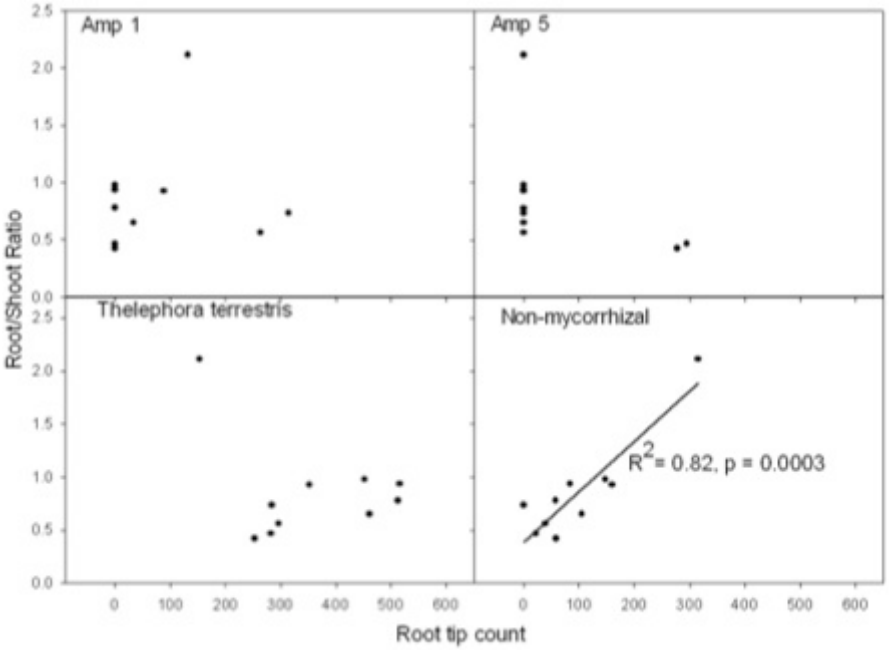

Fig 35.- Root to shoot ratios for fertilized seedlings.

Table 7.- Limiting nutrients quantities

Nutrient	mg/kg
N	10500
P	1000
K	2500
Ca	1000
Mg	500
B	2.5
Mn	10

Table 8.- Symbiotic functionality based on content (C1) and concentration (C2) of all fungal types in unfertilized seedlings. Those trends that were tested and did not provide significant results are in parentheses. Those trends that were significant are displayed without parentheses.

	Fertilized								Unfertilized							
Nutrient	***Amphinema sp. 1***		***Amphinema sp. 5***		***Thelephora terrestris***		**Non-mycorrhizal**		***Amphinema sp. 1***		***Amphinema sp. 5***		***Thelephora terrestris***		**Non-mycorrhizal**	
	Ct	***Cn***	***Ct***	***Cn***	***Ct***	***Cn***	***Ct***	***Cn***	***Ct***	***Cn***	***Ct***	***Cn***	***Ct***	***Cn***	***Ct***	***Cn***
Nitrogen	(+)	(+)	(+)	(-)	0	(-)	(-)	0	(+)	-	(-)	(+)	(-)	(+)	(+)	(-)
Phosphorus	(+)	(+)	(+)	0	0	-	(-)	(+)	+	(+)	-	(-)	(-)	(-)	(+)	(+)
Potassium	(-)	(-)	(+)	(-)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Calcium	(+)	(+)	0	(-)	0	0	(-)	0	(+)	(+)	(-)	0	(-)	0	(+)	(+)
Magnesium	(+)	+	(+)	(-)	0	(-)	-	(-)	(-)	(+)	(-)	(+)	(+)	(-)	0	(+)
Sulfur	(-)	0	(+)	(-)	(+)	0	(-)	0	0	(-)	(-)	(+)	(+)	(+)	(+)	(+)
Manganese	+	+	(+)	(-)	0	-	(-)	0	(+)	0	(-)	0	(-)	0	(+)	0
Iron	(-)	(-)	(+)	(-)	0	(+)	(-)	(+)	0	0	(-)	0	0	0	0	(+)
Copper	(+)	(+)	(+)	(+)	0	0	-	(-)	0	(+)	(-)	(-)	(+)	(-)	0	0
Aluminum	(-)	0	(+)	(+)	(+)	(+)	(-)	(+)	0	(+)	(-)	(-)	(+)	(-)	(-)	0
Zinc	(+)	+	0	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(-)	(+)	(+)
Sodium	0	0	0	0	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	0	(-)	(-)	0
Boron	+	+	(-)	(-)	0	(-)	0	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(+)	(+)
Root:shoot	0		(-)		(-)		+		0		0		0		(+)	
Biomass	0		0		(+)		(-)		(+)		(-)		0		0	
Abv biomass	(+)		0		0		(-)		0		(-)		0		0	