THE INFLUENCE OF AN ENDOMYCORRHIZAL SYMBIOSIS ON NITROGEN MOVEMENT THROUGH SOIL COLUMNS UNDER REGIMES OF ARTIFICIAL THROUGHFALL AND ARTIFICIAL ACID RAIN

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ABSTRACT

The effect of artificial acid rain on nitrogen uptake by a fungus [*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe] endomycorrhizal with roots of sweetgum tree seedlings (*Liquidambar styraciflua* L.) was investigated by applying 3 kinds of test solutions to the surfaces of soil profiles planted with five combinations of sweetgum seedlings and fungus. Soil profiles were taken from a deciduous forest and were reconstituted in plastic pipes. Test solutions applied in different months were 1) artificial throughfall, 2) high ionic strength solutions first of pH 5.9 and later of pH 3.0, and 3) artificial eastern U.S. acid rain. The five combinations of soil and plants used were: 1) sterilized soil, 2) sterilized soil + sweetgum seedlings, 3) sterilized soil + mycorrhizal fungus on pruned sweetgum seedlings, 4) sterilized soil + sweetgum seedlings + mycorrhizal fungus, and 5) sterilized soil + sweetgum seedlings + mycorrhizal fungus + indigenous microorganisms. Nitrogen concentrations in soil solution samples collected by filter candles from 5 and 25 cm depths within the soil columns were determined colorimetrically.

Differences in the soil solution nutrient concentrations among the five soil-tree-fungus treatments are interpreted as nutrient uptake or loss by plants. Following application of artificial throughfall solutions, mycorrhizal roots significantly decreased the NO_3-N and NH_4-N concentrations of soil solutions. The application of high ionic strength solutions of pH 5.9 and 3.0 produced greater NO_3-N and NH_4-N concentrations in the soil solution in columns containing the mycorrhizae than in columns containing either soil alone or in columns containing soil + sweetgum seedlings. Mycorrhizal roots appeared to loose nitrogen. When artificial eastern U. S. acid rainfall was used to acidify the top 5 cm of soil to a soil solution

pH of 2.0, NO_3-N concentrations were unaffected by soil-tree-fungus treatments while ammonia appeared to be excluded from soil exchange sites, apparently by H^+ ions. Ammonia uptake by mycorrhizal roots was not detectable and may have been masked by high NH_4-N concentrations in the soil solution or inhibited by competition with H^+ ions for the cation carrier sites at the nutrient "uptake surfaces" of the plants. Because soil solution nutrients not taken up by plants or by exchange sites in soil can be lost by leaching, it appears that acid rain will promote the leaching of NH_4-N from soil profiles but will have little influence on NO_3-N loss.

INTRODUCTION

Mycorrhizae often increase the uptake of nutrients by host plants. This study was designed to determine the effect of solutions having pH values of 3.0 and 2.0 on the nitrogen uptake capacity of mycorrhizal roots. The pattern of soil solution nitrogen concentrations in soil columns having five combinations of soil, tree seedlings, and endomycorrhizal fungus are reported here for three regimes in which test solutions were applied. The test solutions were: 1) artificial throughfall, 2) high ionic strength solutions of pH 5.9 and pH 3.0, and 3) artificial eastern U. S. acid rainfall. These solutions were applied to the surfaces of soil columns in plastic pipes. The effect of the type of solution applied and of the seedling and fungus treatment combinations on nutrient concentration were determined by monitoring changes in soil solution chemistry. Decreased concentration of nitrogen in the soil solutions in columns containing seedlings and seedlings + fungus compared to concentrations in columns containing soil alone is assumed to reflect nitrogen uptake by plants.

METHODS

The soil profiles were reconstituted in 15 cm diameter polyvinyl chloride pipes from A, B, and C horizon soil material taken from the Coweeta Experimental Watershed in Franklin, North Carolina. Soil filled pipes were steam heated to 90°C for three 8-hour periods to kill indigenous mycorrhizae. Ceramic water collecting candles were inserted through pipe walls into the soil at 5, 25 and 45 cm depths below the soil surface. Each water collecting candle was attached to a sample collecting bottle which could be evacuated to between -.1 and -.2 bars by use of a cartesian diver vacuum regulator and water pump aspirator system. Bottles at 5 and 25 cm were evacuated when water samples were needed while bottles at the 45 cm depth were evacuated continuously in order to prevent accumulation of water and development of anaerobic conditions at the bottoms of the soil columns. The soil columns were therefore flow through systems in which depletion of soil solution nutrients by plants could be interpreted as a reduction in the loss of nutrients by leaching.

Soils were planted with seeds of sweetgum, (*Liquidambar styraciflua*), a deciduous forest tree, which had been sterilized for 30 seconds in 30% H_2O_2. Seedlings were thinned to three per pipe. Spores of the endomycorrhizal fungus, *Glomus mosseae*, were placed in certain soils as shown in Figure 1. At three months the tree seedlings were pruned back in some treatments.

The resulting five soil-fungus-tree treatments, each replicated 8 times were: 1) sterile soil with tree seedlings pruned back, leaving only roots, 2) sterile soil + tree seedlings, 3) sterile soil + fungus supported by pruned back seedlings, 4) sterile soil + tree seedlings + fungus, and 5) sterile soil + tree seedlings + fungus + indigenous microorganisms. Indigenous microorganisms were reinoculated by preparing a slurry of distilled water and forest soil, then filtering the slurry and applying the filtrate to the tops of the appropriate soil columns. Application of test solutions was begun when seedlings were 6 months old. Concentrations of NO_3-N and NH_4-N in samples of soil solution were determined colorimetrically (Strickland and Parsons, 1960, Solorzano, 1969). When solution pH was less than 5, samples were titrated with NaOH to pH 7 before nitrogen determinations were made. Reported values were adjusted for volume change.

RESPONSE TO ARTIFICIAL THROUGHFALL SOLUTIONS

Artificial throughfall solutions containing major cations at concentrations mimicking those in the hardwood forest throughfall (Best and Monk, 1975) and either NH_4-N or NO_3-N at concentrations of 8.7 mg nitrogen per liter were applied, 500 ml to each pipe. Different solutions were applied on different days. All soil columns simultaneously received the same solution. The sequence of solution additions was distilled water, NH_4-N containing artificial throughfall, distilled water, NO_3-N containing artificial throughfall, and distilled water. One day after each solution addition, the soil solution samples were collected from 5 and 25 cm depths for analysis. Overall average soil solution nitrogen concentrations for the 5 solution additions for each of the 5 and 25 cm depths are shown in Figures 1A and 1B.

Concentrations of NO_3-N in soil solutions at the 5 cm depth were significantly depleted in soil columns containing mycorrhizae compared to soil columns containing either soil alone or containing soil + tree seedlings. The concentrations of NO_3-N in soil solutions of the latter two treatments ranged from 3 to 15 times greater than concentrations of NO_3-N applied, showing that considerable NO_3-N was present in the soil before artificial throughfall and distilled water solutions were added.

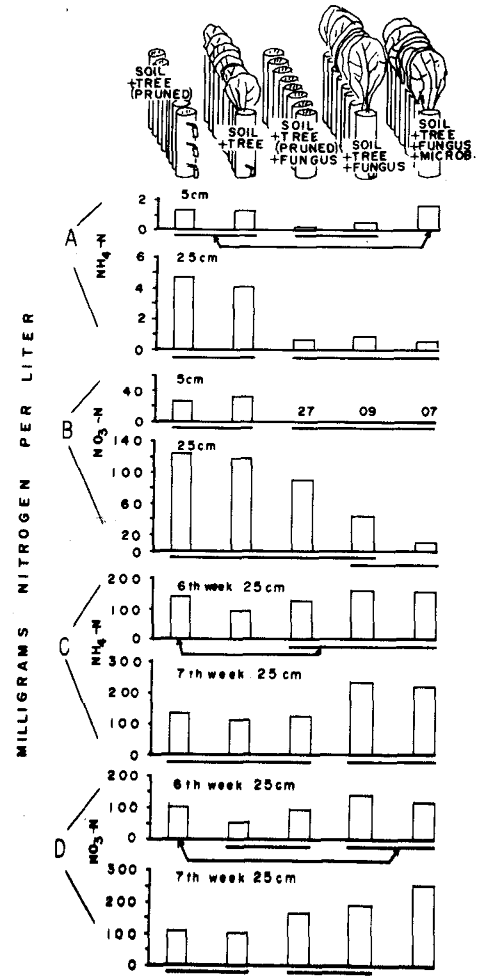


Figure 1. Mean NH_4-N and NO_3-N concentrations in soil solutions collected from 5 soil-tree-fungus treatments each replicated 8 times and subject to artificial throughfall (A and B) and to high ionic strength (C and D) nutrient additions. Horizontal bars connect means not significantly different ($p < .05$ level) as determined by Student-Newman-Keuls multiple range test (Sokal and Rohlf, 1969).

Concentrations of NH_4-N in soil solutions from the 5 and 25 cm depths were significantly depleted by mycorrhizae.

These results show that mycorrhizal roots significantly decreased the nitrogen concentrations in the soil solution. Nutrient flow past a particular depth can be estimated as the soil solution nutrient concentration multiplied by the water flow rate. Leaks at the bottoms of some columns prevented accurate water flow estimation. However, because the water flow rates through columns of soil alone are greater than through columns containing transpiring plants, we can infer that in columns where plants reduced the soil solution nutrient concentrations they also reduced the nutrient loss from that depth in the column by leaching. Mycorrhizal roots therefore decreased nitrogen movement in solution through the soil columns. These data provide a baseline for interpretation of the next experiments.

RESPONSE TO HIGH ION LOAD TREATMENTS

Solutions containing 1.2 g HCl, 6.8 g H_2SO_4, and 2.4 g HNO_3 per liter were adjusted with NH_4OH to produce solutions of pH 5.9 for the first 500 ml application and pH 3.0 for the second application. This was done to evaluate the effect of the added ions on K, Mg, Ca, and Na movement at pH values corresponding to the Coweeta soil solution pH values and to acid rain pH values. Because these solutions contained high concentrations of nitrogen, they were not particularly helpful in evaluating the effect of the mycorrhizae on nitrogen movement. However, during the 6th and 7th weeks after solution applications, soil solution NO_3-N and NH_4-N concentrations at the 25 cm depth were greatest in columns containing mycorrhizal roots. In these columns, soil solution NO_3-N and NH_4-N concentrations increased from the 6th to the 7th week. Increased exudation by mycorrhizal roots and oxidation of the exudates may explain increased soil solution nitrogen concentrations.

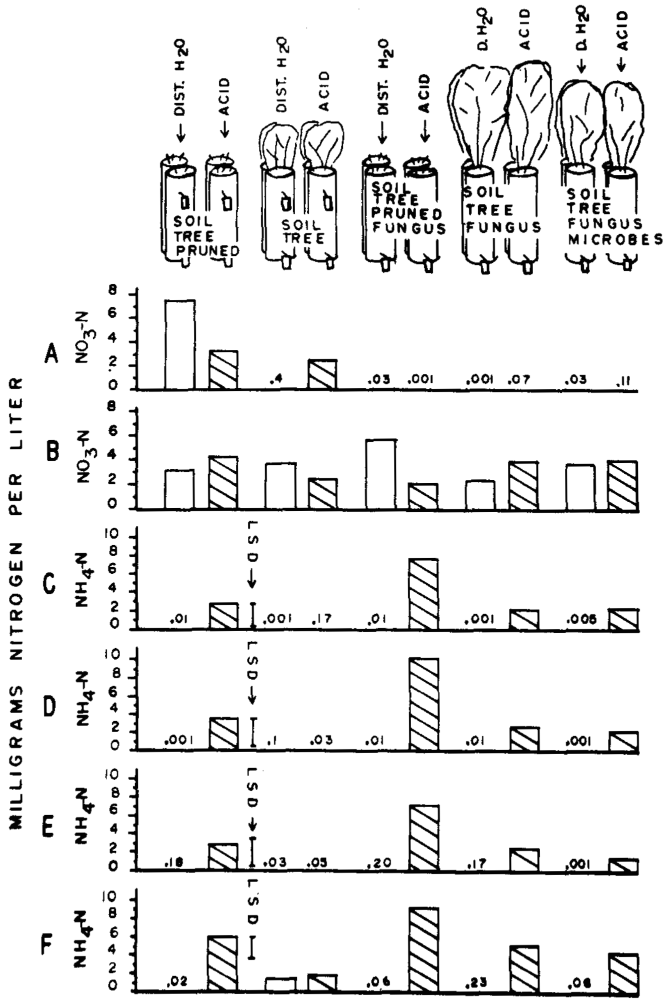
At this point four of the eight replicate soil columns in each of the five soil-tree-fungus treatments were taken apart to assess the extent of mycorrhizal infestation in the tree roots and to determine the plant weights. According to D. Marx (personal communication), U.S.D.A. Plant Pathologist, there was no evidence of damage to either the roots or to the mycorrhizal hyphae by any of the high ionic strength solution treatments.

RESPONSE TO EASTERN UNITED STATES ARTIFICIAL RAINFALL

Artificial acid rain was made from laboratory reagents and deionized water following the SO_4:NO_3:Cl ratio of about 14:7:1 reported by Cogbill and Likens (1974) for precipitation over the eastern United States. These solutions were used in a modified experimental design. The null hypothesis was that soil + tree + fungus treatments had no effect on the amounts of nitrogen in the soil solution of the top 5 cm of soil following acidification of the soil solution to pH 2.0.

The four remaining replicate pipes of each of the five soil + tree + fungus treatments, Figure 2, were used for the experiments. For each of the five soil + tree + fungus treatment types, the first two pipes received distilled water, 500 ml per application, as a control. The second pair of pipes received 500 ml of artificial acid rain. The pH of the soil solution samples from the 5 cm depth was determined 24 hours after solution applications. Because the soil solution pH changed very slowly, solutions of 0.001, 0.01, and finally of 0.1 molar H^+ were applied in efforts to reduce the soil solution pH to 2.0. A total of 0.33 moles of H^+ per pipe was required. After acidification to pH 3.0, HNO_3 was deleted; thus, subsequently applied acid solutions contained H_2SO and HCl only.

When the soil solutions reached pH 2.0, samples were retained for nitrogen analysis. This established the background concentrations. The abilities of the plants and of the soils to take up nitrogen were then tested by application of nitrogen in distilled water and in acid solutions of pH 2.0 to appropriate soil columns. Based on the few published values for nitrogen concentrations at which maximum nitrate uptake occurs in maize (Becking 1956 ; Epstein 1972), a concentration of 10 mg



Figures 2A-2F. Mean concentrations of NO_3-N and NH_4-N in soil solutions at a depth of 5 cm from 5 soil-tree-fungus treatment combinations (S-T-F). Within each S-T-F treatment, the first pair of soil columns received solutions of distilled water (Blank Bars) while the second pair received artificial acid rain (Hatched Bars) until a pH of 2 was attained at the 5 cm depth. Background concentrations of NO_3-N and NH_4-N were then determined. Abilities of plants to take up nitrogen were evaluated by adding nitrogen at concentrations of 10 mg N/liter in solutions of distilled water or in acid, applied 500 ml at a time to the surfaces of appropriate soil columns. Soil solution samples were collected after 24 hours. Least significant difference (LSD) values for ($P<.05$) relate only to columns which received acid. Fig. 2A. NO_3-N background concentrations on 18 June before nitrogen addition. Fig. 2B. NO_3-N concentrations following NO3-N additions averaged for the samples from 26 June and 7 December. Fig. 2C. NH_4-N concentrations on 18 June before NH_4-N additions. NH_4-N concentrations following NH_4-N additions are shown for 25 June in Fig. 2D, for 7 July in Fig. 2E, and for 11 July in Fig. 2F.

nitrogen per liter was chosen for application. Carrier solutions of acid or of distilled water containing nitrogen were applied to appropriate soil columns. Soil solution samples were collected 24 hours later for analysis. Additions of NO_3-N were made first as $NaNO_3$ on 25 June and with KNO_3 in December. The NH_4-N applications were made with NH_4Cl on 24 June and on 6 and 10 July. Data on nitrogen concentrations in the soil solutions were subjected to the SAS REGR analysis of variance procedures (Service, 1972) with a computer. When significant interaction effects were found in the resulting 3-way and 2-way analysis of variance tables they were broken down into 1-way tables with the SAS ANOVA program. In this way, the possible significance of differences among soil-tree-fungus treatment combinations receiving distilled water could be tested separately from those receiving acid. Results from the analysis of variance procedures are summarized in Table 1. Least significant difference (LSD) values generated by the SAS ANOVA program are given in Table 1 for each the distilled water and the acid receiving soil columns, but to avoid confusion, they are shown graphically in Figure 2 ONLY for columns which received acid.

Before NO_3-N addition to soil columns, background NO_3-N concentrations in the soil solutions were lowest (Figure 2A) in columns containing mycorrhizae. This is similar to the pattern observed in the experiments with artificial throughfall reported above and shown in Figure 1. However, with the diminished numbers of replicate soil columns, analysis of variance showed that NO_3-N concentrations were not significantly (Table 1, Trial 2A) affected by the soil-tree-fungus treatments or by the type of carrier solution. After NO_3-N addition,

Table 1. Summary of probabilities that differences among soil solution concentrations of NO_3-N and of NH_4-N occurred by chance alone as determined by 3-way, 2-way, and 1-way analysis of variance. Experimental conditions consisted of five soil-tree-fungus (S-T-F) combinations and of solution additions (SOL). Solutions were either distilled water or artificial acid rain. Solutions were applied first without, then with either NO_3-N or NH_4-N added on different dates. The effect of sampling date in trial 2B was not significant thus it is not tabulated. Least significant difference (LSD) values are indicated. Mean NO_3-N and NH_4-N concentrations from trials A through F are shown in Figures 2A through 2F.

TRIAL	ION	ION SOURCE	SAMPLE DATE	ANOVA	SOURCES OF EFFECTS S-T-F	SOL	S-T-F by SOL	LSD ($p < .05$)
2A	NO_3-N	background	18 June	2-way	.2	.7	.7	-
2B	NO_3-N	added	26 June 7 Dec	3-way	.6	.4	.009	-
2C	NH_4-N	background	18 June	2-way 1-way, water 1-way, acid	.0004 .6 .0051	.0001	.0004	- - 2.5
2D	NH_4-N	added	25 June	2-way 1-way, water 1-way, acid	.0003 .04 .0045	.0001	.0003	- .06 3.19
2E	NH_4-N	added	7 July	2-way 1-way, water 1-way, acid	.03 .7 .08	.001	.04	- - 4.9
2F	NH_4-N	added	11 July	2-way 1-way, water 1-way, acid	.0026 .052 .006	.0001	.0003	- 1.06 2.4

concentrations of NO_3-N in the soil solution increased (Figure 2B) but they were not significantly (Table 1, Trial 2B) affected by application date, by soil-tree-fungus treatments or by the type of carrier solution. The movement of NO_3-N through soil columns was affected by the amount of NO_3-N added, but not by the other experimental conditions.

Before NH_4-N addition, concentrations of soil solution NH_4-N (Figure 2C) were significantly greater (Table 1, Trial 2C) in the acidified soil columns than in those receiving distilled water. Concentrations also differed significantly among the soil-tree-fungus treatments which received acid. Soil solution NH_4-N concentrations following the application of NH_4-Cl either in distilled water or in artificial acid rain are shown in Figures 2D, 2E and 2F. The NH_4-N concentrations in the soil solutions were significantly greater (Table 1, Trials 2D, 2E, 2F; Figures 2D, 2E, 2F) in columns which had received NH_4-N in artificial acid rain. Soil solution NH_4-N concentrations also differed significantly among soil-tree-fungus treatments. According to 1-way analysis of variance, NH_4-N concentrations differed significantly among soil-tree-fungus treatments receiving NH_4Cl in distilled water during only one of three trials while they always differed significantly among soil-tree-fungus treatments which received artificial acid rain (Table 1). Among soil columns receiving acid rain, taking the NH_4-N concentrations in the pipe containing soil as the reference level, several features can be seen. First, concentrations of NH_4-N were consistently highest in columns in which the fungus had been supported by pruned down seedlings. At the time of NH_4-N additions in acid rain the seedlings were dead, thus the high concentrations may result from their decay. Second, compared to the reference level, concentrations were slightly lower in columns containing soil + tree + fungus and in columns containing soil + tree + fungus + microbes. These mycorrhizal roots did not significantly deplete the soil solution NH_4-N. Third, NH_4-N concentrations in soil solutions from columns containing non-mycorrhizal seedlings were between 3 and 119 times lower than in columns containing soil alone. While this difference varies greatly among trials, according to LSD interval statistics, the non-mycorrhizal seedlings significantly depleted the soil solution NH_4-N. Concentrations were decreased to about the same levels found in columns containing the non-mycorrhizal seedlings which received NH_4-N in distilled water.

DISCUSSION

Under regimes of artificial throughfall, the fungus-tree-root symbiosis clearly decreased the NH_4-N and NO_3-N concentrations in the soil solutions. When solutions having high concentrations of NH_4^+, NO_3^- Cl^- and SO_4^{-2} and lower concentrations of Na^+, K^+, Mg^{+2}, and Ca^{+2} were added in solutions of pH 3.0, nitrogen losses appeared to increase in treatments where mycorrhizae were present. Increased rates of root exudation and subsequent oxidation of exudates might explain this observation.

In experiments where nitrogen was applied in either artificial eastern U.S. acid rain or in distilled water to pairs of soil columns in each of the five soil-tree-fungus treatment combinations, there was no evidence of NO_3-N uptake by plants. The addition of NO_3-N increased concentrations of NO_3-N in the soil solutions of all columns about equally. The NH_4-N concentrations were significantly greater in columns which has received acid than in columns which had received distilled water. This was true of NH_4-N concentrations before and after NH_4-N additions. In soil columns to which NH_4-N was added in distilled water, most of the NH_4-N apparently went onto cation exchange sites since the average NH_4-N concentrations in solution sampled at the 5 cm depth did not attain even 1 mg N per liter although solutions of 10 mg N per liter had been applied to the soil surfaces. The purpose of applying NH_4-N in distilled water was to determine if, in fact, plants not receiving acid were actually capable of taking up nitrogen at the time NH_4-N was being applied to the corresponding acid-receiving soil columns. Uptake was to be estimated as the depletion of the NH_4-N concentration of the soil solution below concentrations observed in columns containing soil alone. The problem of nutrient uptake by soils such that possible uptake by plants can be measured might be solved in future studies by use of solid support materials having a lower exchange capacity than forest soils used in this study.

Among soil columns receiving NH_4-N in acid, concentrations of NH_4-N in soil solutions were not significantly depleted by mycorrhizal roots but were significantly depleted by non-mycorrhizal roots when compared to the NH_4-N concentrations in columns containing soil alone. Differences in the behaviors of mycorrhizal and non-mycorrhizal roots might have several causes. Cation carriers of mycorrhizal roots might be more subject to inhibition by hydrogen ions than are carriers in non-mycorrhizal roots. Negative relations between cation uptake and hydrogen ion concentrations have been reported. The uptake rates of the monovalent cations K, Cs, Rb, and Na (Jacobson et al 1960) and of the divalent cations Mn, Mg, and Ca (Maas et al 1969) by barley roots were found to decrease with decreasing pH. Further, the uptake of Rb by barley roots (Rains et al 1964) and of Ca, Zn, and Mn by sugarcane leaf tissue (Bowen 1969) were shown to have been inhibited by hydrogen which competed with these ions for binding sites on the cation carriers. Alternatively, instead of possible differences in their susceptibility to the inhibition of cation transport by hydrogen ions, the mycorrhizal and non-mycorrhizal root may have been exploiting different nitrogen sources in the experimental soil columns. It is possible that the non-mycorrhizal seedlings were exploiting nitrogen supplies mostly in the upper parts of the soil columns while the mycorrhizal seedlings were exploiting nitrogen supplies in regions beneath those which had been acidified and sampled for analysis. This needs to be tested by acidifying entire soil columns.

If mycorrhizal roots are more subject to the competitive inhibition of cation uptake by hydrogen ions than are non-mycorrhizal roots, then acidification of soil solutions, as might occur under long term acid rain conditions, would result in decreased cation uptake by mycorrhizal roots. In ecosystems where mycorrhizal roots are prevalent, decreased ion uptake could lead to decreased plant productivity. Decreased uptake of cations by plants and the displacement of cations off of exchange sites into soil solution could result in losses of nutrient capital from ecosystems by leaching.

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