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METHODOLOGY DEVELOPMENT FOR STUDY OF ROOT INFLUENCES ON NITROGEN MINERALIZATION

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METHODOLOGY DEVELOPMENT FOR STUDY OF ROOT INFLUENCES ON NITROGEN MINERALIZATION

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INTRODUCTION

In many cropping systems the crop's nitrogen (N) needs are met either entirely or partially with organic sources of N fertilizer. The use of organic amendments to supply N to crops is common practice in Maine for several reasons. Animals are an important part of many agricultural systems in the state. Farmers with animals will continue to find it important to avoid disposal problems and gain the economic benefit of recycling N from manure instead of buying inorganic N. Soils will also continue to serve as sites for disposal for municipal sewage sludges which can serve as sources of N for crop growth.

National, as well as local, factors contribute to the reasons that additions of organic N to soils will continue to be an important practice. The sustainability of current agricultural systems is being questioned in Maine and throughout the country as the public and agricultural scientists alike become increasingly aware of the environmental and economic impacts of various agricultural practices. An important premise underlying the practice of sustainable agriculture is that, wherever possible, nutrients should be recycled on a farm and purchased inputs, such as inorganic N fertilizer, should be minimized (Doran et al. 1987).

Accurately predicting the rate at which N is made available to a growing crop from an organic amendment or soil organic matter depends on a clear understanding of the various factors that influence this rate. More accurate predictions of this rate have the potential to improve the efficiency with which farmers use both organic and inorganic N fertilizer. It is important that farmers do not apply excess fertilizer N for both economic and environmental reasons.

Predicting the amount of organic N needed to supply crop needs involves predicting the rate at which organic N is microbially transformed or 'mineralized' into inorganic forms that plant roots absorb. Because mineralization is a microbial transformation, the rate is influenced by environmental factors such as soil temperature and moisture content. The influence of these factors on N mineralization has been the subject of a number of studies (e.g., Honeycutt et al. 1988; Gale and Gilmour 1988; Fisher et al. 1987; Marion and Black 1987).

Another factor that may affect N mineralization has been studied very little. This factor is the carbon added to the soil by plant roots during their growth. The area of soil within a few millimeters of an active plant root is referred to as the rhizosphere. There has been an increasing awareness in recent years by soil microbiologists and soil chemists that the rhizosphere is biologically and chemically different from soil located farther away from plant roots, which is often called "bulk soil" (Rovira 1969; Merckx et al. 1985; Merckx et al. 1986). Most soil studies, however, including those on N mineralization, are carried out using bulk soil.

The carbon exuded and cells sloughed off by roots tend to stimulate microbial populations, which are higher around plant roots than in bulk soil (Dijkstra et al. 1987; Papavizas and Davey 1961). Since N mineralization is a microbial process, it is reasonable to question whether it is stimulated in the vicinity of plant roots. Studies that investigate this effect are scarce. The effect of root growth on N mineralization rates has probably received more attention in forest ecosystems than in agricultural systems. This is understandable given that, since most forests are unfertilized, all the N available to trees comes from mineralization of soil organic matter. Several studies have suggested that N mineralization rates are affected by the growth of some tree species (Carlyle and Malcolm 1986a&b; Fisher and Gosz 1986; Hendrickson and Robinson 1984).

Work on plants' effects on N mineralization in agricultural systems may have been inhibited by the conclusions of early workers that crop growth depresses net N mineralization (Goring and Clark 1948). Goring and Clark compared the N mineralized in pots planted with eight different crop plants to the N mineralized in unplanted pots after 5 weeks, 9 weeks, and 13 weeks of growth. Because they found more N mineralized at 13 weeks in the unplanted pots than in the planted pots, they concluded that plant growth depressed net N mineralization. This conclusion is cited frequently in the literature with reference to their work.

Interpretation of the results of Goring and Clark's work, however, is complicated by several factors. One is that they sampled at only three times during the growth period. At the first sampling time (5 weeks), soil in several of the planted pots had mineralized more N than in the unplanted control. This suggests that plant roots may be stimulating N mineralization during the crucial early stages of growth. Another complicating factor is that they added inorganic N (nitrate) to their pots as well as organic N (manure). This addition of nitrate may have stimulated another microbial process, denitrification, which is the reduction of nitrate to gaseous N_2. Denitrification results in a net loss of N in the system and occurs in wet, anaerobic soils. If denitrification was higher in planted pots than unplanted pots, the net loss of N in the planted pots may be interpreted as a decrease in N mineralization.

Despite the lack of research on this question, recent literature suggests that concluding that plant growth depresses N mineralization on the basis of Goring and Clark's early paper is premature. In 1987 Haider et al. published work that was designed to look at the effect of corn growth on denitrification. They carefully monitored N transformations in their pots and were surprised to discover a two- to five-fold increase in N mineralization in the planted pots. The magnitude of the effect found by Haider and colleagues warrants attention to this area of research.

The current study was designed to explore a particular set of methodologies for addressing the question of whether plant root growth affects the rate of N mineralization. Specifically, the experimental design involved supplying plants with only organic N as manure and no nitrate. A highly sandy growing medium was used in an attempt to prevent overly wet conditions in the pots. In addition, instead of only comparing planted and unplanted pots, four different planting densities (0, 1, 2, 3 plants per pot) were used as treatments.

MATERIALS AND METHODS

Potting Medium

The pots used were 32 oz (900 ml) white plastic cups each with four drainage holes. The potting medium consisted of a bottom layer of approximately 1 g of glass wool overlain by 150 g of sand and above the sand layer a mix of 700 g sand, 100 g Pure Tex manure, 25 g Witter Center manure and 50 g field moist soil. The mix was wet with 200 ml of deionized water and 100 g sand was added to the surface. The soil was from the Madawaska series (coarse loamy, mixed frigid, Aquic Haplorthod) collected from Rogers Farm about one week prior to potting. It was sieved through a 2 mm sieve and stored moist in a covered plastic bin. The Pure Tex manure was a commercial brand containing 2.31% N which was sieved through a 2 mm sieve before use, but was not dried or ground. The Witter Center manure was collected fresh from the Witter Center barns. It was oven dried and ground in a Wiley Mill before use; the N content was 2.51%. All pots were incubated moist in the greenhouse for 12 days after which the corn seeds were planted. During the incubation period, all pots were leached with approximately 500 ml of 1/4-strength nutrient solution on days 1, 3, 4, 5, 7. Foil covers were placed loosely over pots to reduce evaporation.

Experimental Design

The design was a randomized complete block with four treatments (0, 1, 2, or 3 plants per plot) and four replicates. Each block consisted of 16 pots, all of which were harvested at once. There were three harvest dates, 16, 23, and 28 days after planting.

Watering

The pots were watered with nutrient solution which was diluted from stock solutions as needed. The full strength nutrient solution was adapted from Warncke and Barber (1974) and had the following composition: $CaCl_2$, 1 mM; KH_2PO_4, 0.5 mM; $MgSO_4$, 1 mM; K_2SO_4, 0.5 mM; $FeSO_4$, 100 μM; H_3BO_4, 48 μM; $MnCl_2$, 12 μM; $ZnSO_4$, 1 μM; NH_4MoO_4, 0.8 μM; $CuSO_4$, 0.4 μM.

During the first week after planting all pots were watered with 1/4-strength nutrient solution; thereafter the unplanted pots received 1/4-strength solution, and the planted pots normally received full-strength solution. The plants had developed visual nutrient deficiency symptoms (chlorosis and purpling) by about three weeks after planting. In order to ensure that calcium phosphates were not precipitating in the nutrient solution and limiting phosphorus supply to the plants, phosphorus-containing and non-phosphorus-containing nutrient solutions were prepared separately. On day 23 the planted pots received 100 ml of solution that contained two times full-strength concentrations of all nutrients except P, and 150 ml of solution containing only P at a concentration of four times full strength. Both solutions contained higher concentrations of nutrients than the full-strength solution since nutrient deficiencies were suspected.

The need for watering was determined by weighing several pots and determining moisture loss. Water additions were made when it was determined that 50 ml could be added to the pots without causing leaching. The unplanted pots were watered somewhat less frequently than the planted pots in an attempt to keep moisture levels similar in planted and unplanted pots. All pots were watered to excess on days 14 and 23, and the amount of leachate from each pot was measured.

Harvest and Analysis

At harvest, plants were clipped off at the base of the stem, and the above ground biomass was weighed. Roots were removed from the potting medium by hand and washed briefly in two portions of deionized water. They were blotted dry and weighed. Roots and tops were dried separately in paper bags, weighed, and ground before analysis. The potting media were mixed thoroughly, sealed in plastic bags, and refrigerated. Before analysis, a subsample of each media was oven-dried and thoroughly mixed. The percentage N in the manures and potting media was determined by a macro-Kjeldahl method, and the percentage N in the corn tissue was determined by a micro-Kjeldahl method using a block digester.

RESULTS AND DISCUSSION

Table 1 shows the means of plant dry weight and N content, as well as percentage N remaining in the potting mixes, for each treatment at each harvest date. The standard deviations are given under each mean. As would be expected, root and shoot dry weights increased with increasing plants per pot and increasing period of growth. Plant N concentrations tended to decline with plant age. The effect of planting density on N remaining in the potting mixes at harvest was examined separately for each harvest date by analysis of variance. The effect was not significant at any harvest date at the 0.05 level.

Several problems with the experimental design may have contributed to the failure to detect significant treatment effects. The period of corn growth was in-

Table 1. The Influence of Planting Density on Loss of Nitrogen From Potting Media at Three Different Harvests.

Treatment	Harvest	Shoot Dry Wt.	Root Dry Wt.	Shoot N	Root N	Pot N
plants/pot	days	--------g-------		-----%-----		
0	16					0.23* (0.002)
1	16	0.49 (0.13)	0.32 (0.06)	1.52 (0.24)	1.31 (0.09)	0.22 (0.02)
2	16	0.68 (0.14)	0.48 (0.08)	1.60 (0.33)	1.25 (0.11)	0.25 (0.01)
3	16	0.89 (0.19)	0.68 (0.12)	1.50 (0.17)	1.15 (0.07)	0.25 (0.01)
0	23					0.25 (0.02)
1	23	0.95 (0.14)	0.47 (0.04)	1.40 (0.14)	1.15 (0.19)	0.22 (0.01)
2	23	1.34 (0.29)	0.78 (0.10)	1.19 (0.08)	1.02 (0.08)	0.25 (0.02)
3	23	1.68 (0.11)	1.14 (0.11)	1.15 (0.15)	1.02 (0.05)	0.23 (0.01)
0	28					0.24 (0.05)
1	28	1.92 (0.48)		1.10 (0.08)		0.14 (0.06)
2	28	2.57 (0.38)		1.06 (0.03)		0.21 (0.05)
3	28	2.72 (0.45)	1.42 (0.30)	1.03 (0.13)	0.97 (0.18)	0.21 (0.04)

*Data are presented as means of four replications. Standard deviations are shown in parenthesis.

tentionally short because inorganic N was not supplied to the plants, and vigorous, rapid growth could not be sustained by the minimal release of N from manure in the pots. The final harvest was completed by day 28. Although the period of most rapid N uptake by corn is roughly 30 to 70 days after emergence, (Larson and Hanway 1977) the 28-day-old plants appeared yellow, suggesting N deficiency. In addition, the relatively low percentage N in the above ground biomass of such young plants indicates N deficiency (Hanway 1962). The small amount of corn growth in the pots resulted in recovery by the corn of only 1-2% of the added manure N. Although some additional, undetermined amount of N was undoubtedly lost in the pot leachates, the total N lost from the manure was not enough to cause the N concentrations in the potting media to decrease measurably and significantly. Detecting significant changes in N concentrations was difficult because of the high variation in percentage N among pots. A large part of this variability was due to the physical nature of the potting mix, which was essentially a mixture of sand and finely divided manure. The difference in particle sizes and densities caused a great deal of stratification of the mix, despite careful mixing to obtain a representative sample.

An additional drawback of the experimental design chosen for this study was the water relations in the pots. Although the weight of manure added to each pot was much less than the weight of sand, the two components were nearly equal by volume. The large volume of manure caused pots to remain wet for several days after watering despite high greenhouse temperatures. The initial intent of the sandy potting mix was to create a well-drained growing medium requiring frequent watering and resulting in a greater similarity in the moisture contents of the planted and unplanted pots. The amount of manure required to ensure N mineralization for the corn, however, resulted in a rather poorly drained growing medium.

The added manure also resulted in a dark brown leachate from the pots, indicating high levels of soluble organic compounds. The pots were incubated moist and leached on 5 different days before planting in an attempt to reduce levels of soluble organic matter draining from the pots. Although, judging by color, this treatment reduced the concentration of soluble organics, leachates were still highly colored during the course of the experiment. The initial intent had been to determine nitrate and ammonium concentrations in the leachates; however, soluble organics prevented analysis of these ions due to interferences in the analytical methods used.

One final observation from this study may have contributed to difficulties in detecting a significant treatment effect. This was the tendency of the plant roots to grow along the side of the pots and form a coiled mat at the bottom of the pots. Thus, only a small amount of root biomass actually ramified throughout the potting medium, and much of the organic N was not in close contact with any roots, even in the planted pots.

CONCLUSIONS

The purpose of this study was to assess one specific experimental design for determining the effect of plant root growth on N mineralization in soils. Two major difficulties with the methodology employed were evident: the difficulty of supplying sufficient organic N to sustain vigorous corn growth; and the poorly drained nature of the potting medium. This poor drainage would a) promote denitrification in the pots, b) contribute to poor corn growth, and c) promote root proliferation along the sides of the pots rather than throughout the potting medium.

These experimental difficulties suggest several ways to improve the experimental approach in future studies on this topic. Better control and monitoring of water contents in the pots is needed. A continuous watering system, with tensiometers to measure moisture levels, would be the best approach to this problem. These watering systems generally import water to the pots through wicks leading from a large reservoir. The difference in elevation between the water reservior and the pots controls the moisture tension maintained in the pots.

In addition to water supply, N supply to the plants must be improved. One approach is split-root experiments where a portion of the root system of each plant is grown separately from the experimental pot and is well supplied with inorganic N. Another portion of the root system is grown in the experimental pot containing organic N, and N mineralization from this source is monitored. A longer period of corn growth is necessary in order to induce measurable changes in levels of organic N in this type of study. These suggested modifications in experimental approach have potential to improve our understanding of the mechanisms of N mineralization in the rhizosphere.

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