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Effect of amendment of manure and corn residues on soil N mineralization and enzyme activity

Nazila KHORSANDI, Farshid NOURBAKHS*^{*}

Department of Soil Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

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Abstract – Understanding the effect of manure and crop residue application on N mineralization in soil is crucial in order to minimize nitrate contamination of surface and groundwater. Besides, developing an alternative management practice for corn residue burning is of great importance and needs more knowledge about inorganic N dynamics. Here we conducted a laboratory experiment to study the effect of corn residue application on inorganic N dynamics and soil enzyme activities in soils with different manure fertilization histories. The laboratory experiment was a combination of 3 fertilization histories of: 0, 50 and 100 Mg cow manure ha⁻¹ year⁻¹ for five consecutive years; and 3 residue treatments including: no residue, corn shoot and corn root application. We monitored inorganic N periodically during 20 weeks of incubation. We also monitored the activities of urease, L-asparaginase, L-glutaminase and β -glucosidase before and after incubation. Our findings show that for residue-amended soils, inorganic N decreased in the first 2 weeks of incubation by 50 to 86%, followed by a gradual increase. In contrast, for control soils without residue amendment, the inorganic N concentration increased from 64 to 86% during the first week of incubation. Our results thus show that the application of corn residues can control the flush of inorganic nitrogen and reduce the potential of nitrate leaching in manure-applied soils. Furthermore, manure application at the highest rate increased the activity of urease by 47%, L-asparaginase by 70%, L-glutaminase by 60% and β -glucosidase by 78%. β -glucosidase increased due to corn shoot application by about 8% and was the most responsive enzyme to the treatments. Overall, the enzyme activities were more influenced by manure application history than corn residue application.

corn residue / manure application / N mineralization and immobilization / soil enzymes

1. INTRODUCTION

The central role of soil organic matter in maintaining soil function and plant productivity in agroecosystems has long been recognized (Puget and Drinkwater, 2001). Different sources of organic amendments including solid waste compost (Hachicha et al., 2006), municipal solid wastes (Montemurro et al., 2005) and green manure (Turgut et al., 2005) have been applied to enhance both crop yield and soil quality. Crop residue is the primary substrate for replenishment of soil organic matter and is a vital natural resource for conserving and sustaining soil productivity (Aulakh et al., 2001). In Asia, large amounts of crop residues are burned or removed after harvest. This results in loss of organic matter and nutrients and causes atmospheric pollution, which pose a threat to human and ecosystem health (Nguyen et al., 1994; Aulakh et al., 2001). Therefore, there is a need to develop agronomic strategies for efficient utilization of crop residues that replenish organic C and reducing nitrate leaching.

Application of farm wastes releases more N than can be retained by soils and crops, thus leading to nitrate leaching (Andrew et al., 2002). Temporary N immobilization in soil microbial biomass and organic matter can be achieved by adding amendments with a large C/N ratio which can reduce nitrate

leaching (Vinten et al., 1998). Aulakh et al. (2001) demonstrated that rice and wheat residues can be incorporated shortly after crop harvest without affecting yields of the following crop. Others have expressed concerns about the unavailability of N for the next crop following cereal residue application to soil (Foth and Ellis, 1997). Corn residues are generally considered as low-N, low quality plant materials. Incorporation of these residues into soils would presumably result in temporary N immobilization and consequently reduce potential nitrate leaching (Recous et al., 1995). The interactions of N-rich organic amendment such as manure and N-poor corn residues have not yet been clearly demonstrated.

Soil enzymes influence the release of nutrients for plant and microbial growth (Tate, 2000). This article considers urease, L-glutaminase and L-asparaginase as those soil enzymes participating in the mineralization of organic N. The enzymes are central regulators of the N cycle in microbial cells and produce mineral N during the decomposition of aliphatic and aromatic N compounds in soil organic matter (Monreal and Bergstrom, 2000). The enzyme β -glucosidase, by hydrolysing cellobiose, provides essential carbon skeletons and energy sources for the growth of soil heterotrophs and initiates processes leading to the mineralization and/or stabilization of plant residue carbon (Monreal and Bergstrom, 2000; Tate, 2000). In general, the activities of soil enzymes are increased

*Corresponding author: farshid@cc.iut.ac.ir

by farming practices conserving plant residues in a variety of soils and climates (Deng and Tabatabai, 1996; Bandick and Dick, 1999; Kandeler et al., 1999). A system capable of monitoring changes in soil biochemical health needs to identify biochemical pools with ecological functions and expressing environmental stress (Monreal and Bergstrom, 2000). Soil enzyme activities are generally considered as practical tools to evaluate ecological functions within a soil ecosystem (Nannipieri et al., 2002).

We hypothesized that the dynamics of inorganic N concentration in manure-applied soils will be affected by corn residue application and thus may help reduce the potential of N leaching. We also hypothesized that soil enzymes are different in terms of their response to the treatments. Therefore, a laboratory experiment was conducted to investigate the effects of corn residue amendment on inorganic N dynamics and soil urease, L-asparaginase, L-glutaminase and β -glucosidase activities in soils that had previously received different rates of cow manure.

2. MATERIALS AND METHODS

A field experiment was started in 1999 on a Typic Haplargid, silty clay loam soil, in Lavark research station, 32° 30' N, 51° 20' E, Isfahan, Iran. The field is located on a nearly flat area with a very mild slope of less than 0.5%. The soil is calcareous, containing 390 g kg⁻¹ Ca-carbonate equivalent, 5.0 g kg⁻¹ organic C and 0.77 g kg⁻¹ total N, with pH 8.3. The soil is non-saline and non-sodic, and the electrical conductivity and the sodium adsorption ratio of the soil saturated extract are 1.6 dS m⁻¹ and 1.4 mmol_c^{0.5}, respectively. The mean annual temperature and precipitation are 14.5 °C and 140 mm, respectively. Fall and winter precipitation comprise about 75% of annual precipitation. Repeated annual applications of cow manure at three levels of application had been arranged in a complete block design with three replicates. The levels of manure application were 0, 50 and 100 Mg ha⁻¹ and the average of C and N concentrations in the manure were 24.9 and 1.3%, respectively. The field had been cropped to corn (*Zea mays* L.) – wheat (*Triticum aestivum* L.) rotation, under conventional tillage and irrigated conditions. Before 1999, similar tillage and rotation had been applied without manure application. Instead, about 250 kg N ha⁻¹ y⁻¹ as urea and 80 kg P ha⁻¹ y⁻¹ as superphosphate had been used. No pesticide was used either before or during the experiment.

Composite soil samples were collected from the 0–15 cm depth by a core sampler with 11.2 cm inside diameter and 15 cm length. Ten soil cores were taken from each 5 by 15 m field plot and mixed thoroughly. The sampling was performed in 2004 at the end of the 5th annual consecutive manure application. The soils were air-dried and passed through a 2-mm sieve. Corn shoots and roots were sampled from the same field, washed with running tap water, rinsed three times with distilled water, dried at 65 °C for 24 h, milled and passed through a 1-mm sieve. The hemicellulose, cellulose and lignin contents of the plant materials were measured sequentially in duplicate samples (Van Soest et al., 1991). Kjeldahl digestion and dis-

tillation were used to analyze the residue for total N (Bremner and Mulvaney, 1982). Organic C was determined using wet digestion procedures (Nelson and Sommers, 1982). Corn shoots contained 42.3% C, 1.0% N, 25.6% cellulose, 25.4% hemicellulose and 3.3% lignin. Corn roots contained 46.5% C, 0.5% N, 40.8% cellulose, 26.3% hemicellulose and 4.3% lignin.

To study the N mineralization/immobilization, 100-g soil samples were mixed thoroughly with plant residues at the rate of 10 g residue C kg⁻¹ soil. The laboratory experiment was a factorial combination of three fertilization histories including M0: no manure added; M1: 50 Mg manure ha⁻¹ y⁻¹ and M2: 100 Mg manure ha⁻¹ y⁻¹ and three residue applications including P0: no residue added; P1: corn shoots added and P2: corn roots added. The experiment was run with three replications. Soils were incubated at 25 °C and 50% water-holding capacity for 20 weeks. During the 20 weeks of incubation inorganic N was monitored by destructive soil sampling on 10 occasions 0, 1, 2, 4, 6, 8, 10, 13, 16 and 20 weeks after incubation. Inorganic N was extracted with 2M KCl and measured by the steam distillation procedure (Keeney and Nelson, 1982).

The hydrolases of L-glutaminase (LG), L-asparaginase (LA), urease (UR) and β -glucosidase (β G) activities were determined both immediately after residue incorporation (week 0) and after 20 weeks of incubation according to standard procedures described by Tabatabai (Tabatabai, 1994), using L-glutamine, L-asparagine, urea and *p*-nitrophenyl- β -D-glucoside as substrates, respectively. All measurements were expressed on a moisture-free basis. Analysis of variance and the least significant difference (LSD) test were performed for mean separation.

3. RESULTS AND DISCUSSION

3.1. N mineralization and immobilization

The treatments with no application of corn residues, including M0P0, M1P0 and M2P0, showed flushes of mineralized N during the first week of incubation followed by a slower release phase. Regardless of the manure application history, similar patterns were observed in inorganic N accumulation with time (Fig. 1A). However, on all sampling occasions, inorganic N concentrations were significantly different in soils with different manure application histories (M2P0 > M1P0 > M0P0).

A different pattern was observed for those treatments that received corn shoots (P1) and/or roots (P2). The inorganic N declined in the first 2 weeks of incubation, followed by gradual accumulation of inorganic N during the rest of the incubation period (Figs. 1B, C). Moreover, we observed that during the first 13 weeks of incubation, the concentrations of inorganic N in corn residue-applied soils (Figs. 1B, C) were not significantly affected by manure application history. However, at week 20, there was an increase in inorganic N concentrations as the rate of manure application increased. The trend was similarly observed both in corn shoot-treated: M2P1 > M1P1 > M0P1 and corn root-treated: M2P2 > M1P2 > M0P2 soils.

Figure 1. The pattern of cumulative inorganic nitrogen concentration (Ni) in no residue-amended (A), corn shoot-amended (B) and corn root-amended soils (C) as affected by manure application history (M0, M1 and M2 correspond to 0, 50 and 100 Mg ha⁻¹ y⁻¹, and P0, P1 and P2 correspond to no residue, shoot and root-amended soils, respectively).

In those treatments that were not amended by corn residues, a rapid inorganic N accumulation was observed. The rapid accumulation of inorganic N can be attributed to the release of inorganic N from more readily mineralizable fractions of soil organic N. Higher manure application rates increased the rate of N mineralization due to the high N content of manure. The stimulating effect of air-drying on the decomposability of N-containing organic molecules, which is called the Birch effect (Tate, 2000), can enhance the release of inorganic N during the first week.

Whalen et al. (2001) reported that long-term manure application increased the potentially mineralizable N in soil. They also showed that N mineralization potential increased linearly as manure application rates increased. Our findings corroborate previous results (Whalen et al., 2001; Haney et al., 2001)

indicating that the manure application history influences N mineralization.

The early decline in the inorganic N pool in the first 2 weeks of incubation is due to the fact that growing microbial populations deplete the available pools of N to compensate for the decomposition of N-poor corn residues. Following the first 2 weeks of incubation, the easily biodegradable fractions of corn residues were consumed (Nourbakhsh, 2006), so that the immobilized N in the microbial biomass would be subjected to gradual release (Figs. 1B, C). The results imply that there are two distinctive phases in inorganic N dynamics following corn residue application: first, the early 2 weeks in which net N immobilization occurs and second, the rest of the incubation period (weeks 2–20) in which net N mineralization happens and hence, inorganic N content increases. A maximum microbial biomass buildup has been reported, 15 days after wheat

Table I. Enzyme activities (mg product kg⁻¹ soil h⁻¹) as affected by manure application histories, M0: control; M1 and M2: 50 and 100 Mg manure ha⁻¹ y⁻¹, (means within a row and an incubation time followed by different letters are significantly different: LSD, $P < 0.05$).

	Week 0			Week 20		
	M0	M1	M2	M0	M1	M2
Urease	91.5c	129.7b	172.0a	151.3c	225.4b	276.9a
L-asparaginase	11.9c	27.9b	39.3a	29.5c	51.2b	73.7a
L-glutaminase	82.1c	146.8b	204.3a	144.6c	229.5b	257.3a
β-glucosidase	86.3c	242.1b	397.1a	136.9c	262.1b	441.1a

Table II. Soil enzyme activities (mg product kg⁻¹ soil h⁻¹) as affected by corn residue application; P0: no residue-amended, P1: corn shoot-amended, P2: corn root-amended (means within a row and an incubation time followed by different letters are significantly different: LSD, $P < 0.05$).

	Week 0			Week 20		
	P0	P1	P2	P0	P1	P2
Urease	129.7a	132.2a	131.2a	209.3c	230.0a	214.3b
L-asparaginase	25.8a	26.9a	26.5a	46.4c	58.4a	49.5b
L-glutaminase	143.8a	144.7a	144.7a	203.5c	220.3a	207.7b
β-glucosidase	233.0c	253.9a	243.6b	273.7c	292.6a	283.9b

residue incorporation (Tarafdar et al., 2001), indicating that rapid growth of soil microorganisms is responsible for inorganic N decline after low quality residue incorporation. It has also been suggested that microbial biomass and accumulation of microbial recalcitrant metabolites are the main sinks for the N immobilized (Andrew et al., 2002).

The remineralization kinetics of immobilized N are thought to be influenced by the fungal/bacterial ratio, intensity of grazing pressure on primary decomposers, the particle size of decomposing material, the extent of organic matter coverage of soil colloids and clay content of soils (Andrew et al., 2002).

3.2. Soil enzyme activities

Manure application significantly increased the activity of the enzymes studied, both at week 0 and at week 20 (Tab. I). Before incubation, the averages of urease, L-asparaginase, L-glutaminase and β-glucosidase activities in soils that received 100 Mg ha⁻¹ y⁻¹ (M2 treatments) were increased by 47, 70, 60 and 78%, respectively, compared with those of control soils. Significant increases were also observed after 20 weeks of incubation. At week 20, urease, L-asparaginase, L-glutaminase and β-glucosidase activities were increased by 45, 60, 44 and 69%, respectively. Both at week 0 and 20, the maximum response was observed in β-glucosidase. The enzyme activities were also significantly increased in the soils receiving 50 Mg ha⁻¹ y⁻¹, M1 treatments, compared with the control soils. Interestingly, we observed dissimilar patterns among soil enzymes in response to corn residue application (Tab. II). While β-glucosidase activity increased significantly at week 0, no significant difference was observed in urease, L-asparaginase or L-glutaminase before incubation. After 20 weeks of incubation, the four enzyme activities were significantly increased compared with the soils which

were not amended by corn residues. Corn shoot-applied soils showed higher enzyme activities than those of root-applied soils (Tab. II).

The results demonstrate that manure application has a promoting effect on catalytic activity of soils (Tab. I). The higher enzyme activities of the manured soils can be attributed to the higher enzyme content of the soils. The higher humic material in manured soils can increase the capacity of the soils to protect extracellular pools of the soil enzymes against proteolytic activities and hence, provide more functional enzyme molecules in soil (Nannipieri et al., 1996). Moreover, application of organic amendments may provide more C and energy sources for proliferating soil microorganisms which are partially responsible for enzyme production in soil (Tate, 2000). Therefore, repeated application of manure can enhance both extracellular and intracellular pools of enzymes. Kanchikerimath and Singh (2001) reported that urease, alkaline phosphatase and dehydrogenase activities, and soil microbial biomass C increased in manure-treated soils. They suggested that soil enzyme activities are closely related to the organic matter build-up and therefore, manure application improves the organic matter status, which is in turn reflected in higher enzymatic activity.

The effects of plant residue amendment on soil enzymatic activities in calcareous soils have rarely been studied. The higher enzyme activities of the shoot-applied soils can be attributed to the higher decomposability of the corn shoots (Nourbakhsh, 2006) which provide more energy and readily decomposable C and N for living microorganisms. Bandick and Dick (1999) showed that deaminase activity was not sensitive to management practices, while β-glucosidase activity was suggested as an assay that reflects soil management effects. Acosta-Martinez and Tabatabai (2001) reported that arylamidase activity is greatly affected by residue management. Positive responses in amidohydrolases have been reported by

Deng and Tabatabai (1996) to those residue management practices associated with higher crop residue amendment. Monreal and Bergstrom (2000) demonstrated that increased activities of β -glucosidase, L-glutaminase and urease are associated with those management practices that incorporate more plant residues into the soil. Similar observations have been reported by Bandick and Dick (1999) when comparing different management practices. In agreement with the above literature, we observed that urease, L-asparaginase, L-glutaminase and β -glucosidase responded to both manure application history and corn residue application; however, β -glucosidase was the most responsive enzyme to both manure and corn residue application.

4. CONCLUSION

Our findings clearly demonstrate that the inorganic N dynamics in manure-applied soils is affected by manure application history. Following corn residue application, N flush did not happen. This finding implies that soil microorganisms tend to immobilize inorganic N and thus supports the hypothesis that corn residue application may help to reduce N leaching in heavily manure-applied soils. The rate of N remineralization was affected by the amount of manure that had been previously applied. The enzyme activities responded to the treatments, to indicate their potential ecological significance in soil C and N cycles: moreover, β -glucosidase was the most responsive.

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