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SHORT COMMUNICATION

Field measurement of net nitrogen mineralization of manured soil cropped to maize

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Abstract We evaluated the in situ net nitrogen (N) mineralization in a soil cropped to maize and fertilized for 11 years with cattle slurry or farmyard manure, both common on livestock farms of the Po River valley in Northern Italy. The net N mineralization of the tilled soil layer was measured in six consecutive incubation periods after manure application, for a total of 12 weeks, using the polyethylene buried bags technique. Results showed that net N mineralization was followed by N immobilization and finally, by mineralization whose rate increase until maize flowering. On average, net N mineralization was 70.4 kg N ha^{-1}, with the majority being released during the last measurement period. The time and extent of net N mineralization and plant N uptakes were not affected by fresh manure application. Instead, the effect of past management increased the maximum net N mineralization rate obtained with farmyard manure. The buried bag technique probably underestimates the total amount of mineralized N available for crop growth because it excludes the presence of the plant.

Keywords Net nitrogen mineralization · Field measurement · Manure fertilization · Buried bags technique

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Introduction

Nitrogen (N) cycle in cropping systems is strongly affected by the use of mineral and organic fertilizers, which have a central role in sustaining crop productions (Jarvis et al. 1996; Tilman et al. 2002). Moreover, in agricultural areas with intensive livestock breeding such as the Po River plain in Northern Italy, animal manures are often applied at rates above optimal levels (Sacco et al. 2003). In order to reduce pollution and to enhance the beneficial effects of manure, it is needed to predict manure N availability for plant nutrition (Gutser et al. 2005). The optimal rate and time of fertilizer application should match nutrient supply with plant demand (Tilman et al. 2002). However, the prediction of short- and long-term fate of manure N presents several conceptual and experimental problems, which makes it a compelling investigational topic in both agricultural and forest soils. Aerobic incubation under controlled temperature and moisture conditions in the laboratory is the most widely used method to evaluate net soil N mineralization (Stanford and Smith 1972; Curtin and Wen 1999; Heumann and Bottcher 2004; Griffin et al. 2008), to evaluate net N mineralization from organic fertilizers (Hadas and Portnoy 1994; Chadwick et al. 2000; Van Kessel and Reeves 2002; Griffin et al. 2005), and to model N mineralization dynamics (Mary et al. 1998; Muller et al. 2003; Probert et al. 2005). However, there are several limitations to this method such as soil perturbations (e.g., storing, mixing, and sieving), which modify physical and microbiological characteristics and demolish the heterogeneity of the profile, and the confinement of soil into an unrealistic micro- or mesocosm can alter the soil N dynamics (e.g., absence of N plant uptake and root exudates, temperature, and soil moisture fluctuations); these limitations make it problematic to transfer the results to the field situation (Lomander et al. 1998; Curtin and McCallum 2004).

Several techniques have been proposed for quantifying N mineralization and immobilization under field conditions (Rees et al. 1994). Net N mineralization has often been estimated by measuring temporal changes in mineral N content of soil samples incubated under actual field conditions. Different techniques are used to enclose soil cores and to prevent N losses during the incubation (i.e., N uptake by plant roots, N leaching, and denitrification losses) including capped tubes (Raison et al. 1987), polyethylene bags (Eno 1960), ion exchange resins to trap leached N (Di Stefano and Gholz 1986), and the addition of acetylene to prevent N losses through denitrification (Hatch et al. 1990). Unfortunately, all incubation techniques present problems such as disturbance of the soil prior to the incubation, physical isolation of the incubated soil, differences in environmental conditions inside and outside the containers, the choice of the duration of incubation (Stenger et al. 1996; Abril et al. 2001; Hanselman et al. 2004), and the need of a high number of replicates because of the great spatial variability in the field. Although the buried bags technique (BBT) is considered less accurate than other in situ techniques, BBT is simple, causes moderate disturbance of the soil, and allows investigation of N dynamics of subsurface soil layers. These characteristics make this method suitable for agronomic investigations.

The results of 11 years of fertilization with farmyard manure and cattle slurry (Grignani et al. 2007) showed that fertilizer type had no significant effect on average N uptake of maize while fertilizers increase total N pool of the tilled layer. Although this increase was higher for farmyard manure than cattle slurry, the amounts of N mineralized by anaerobic incubation (Monaco et al. 2008) were similar for the two fertilizer types.

The main objective of the present work was to evaluate under field condition the contribution of N mineralization by fresh and past additions of cattle slurry and farmyard manure on soil N availability. For this reason, net N mineralization was measured in the tilled soil layer during the early stage of maize growth using in situ incubations in treatments with or without fresh farmyard manure or cattle slurry additions. Another aim was to evaluate the reliability of BBT by comparing the relative results with changes in soil inorganic N content and soil–plant N balance.

Material and methods

Treatments

The experiment was conducted in 2005 from April 8 (day 0) to July 2 (day 81) on two treatments (replicated in three blocks) of a large field experiment established in 1993 (Grignani et al. 2007). The site is located in Northern Italy, western area of the Po valley. The alluvial soil, classified as a typic ustifluvent (Soil Survey Staff 1998), is deep with a first layer characterized by a loam texture and a subalkaline pH (Table 1). The soil was under continuous cultivation and was cropped with irrigated maize (*Zea mays* L.) for silage from 1993 and was subjected to two treatments: (1) MsS, fertilized with 100 Mg ha^{-1} y^{-1} of cattle slurry and (2) MsF, fertilized with 40 Mg ha^{-1} y^{-1} of composted farmyard manure. Both treatments received organic manure before sowing and an additional 100 kg ha^{-1} y^{-1} of urea-N at ridging. As reported by Grignani et al. (2007), the different fertilization significantly affected soil organic C and total N contents of the tilled layer, which were equal to 11.3 and 1.33 g kg^{-1} in MsS and 13.6 and 1.53 g kg^{-1} in MsF, respectively. In April 2005, the main plots of MsS and MsF treatments (10 × 7.5 m^2) were randomly split into two subplots (10 × 3.75 m^2) following the direction of cultivation. One subplot received slurry (MsS+) or farmyard manure (MsF+) depending on the past fertilization treatment while the other received no fertilizer (MsS− and MsF−). Manure was distributed manually in April 5 at a rate of 86 Mg ha^{-1} of slurry and 61 Mg ha^{-1} of farmyard manure, both rates supplying 247 kg ha^{-1} of total N (TN) and 103 kg ha^{-1} of NH_4^+-N (42% on TN) and 21 kg ha^{-1} of NH_4^+-N (9% of TN), respectively. Manures were incorporated into the soil with a digger, followed by the preparation of the seedbed by a rotating harrow. Identical soil tillage was also performed on unfertilized subplots. Maize was then sown in April 8 (day 0 of the experiment) with a density of 7.2 plants per square meter. During the experiment, maize did not receive mineral fertilizers and irrigation in order to reduce the number of experimental factors. Weed control was performed by applying a mixture of isoxaflutole (50 g a.i. ha^{-1}) and flufenacet (240 g a.i. ha^{-1}) in preemergence followed by a treatment in postemergence with foramsulfuron (56 g a.i. ha^{-1}).

Table 1 Soil physical and chemical characteristics

Soil horizon Depth (m)	m	Ap 0–0.4	CA 0.4–0.9	C 0.9–1.2	Cg 1.2–1.4
Sand (0.05–2 mm)	g kg^{-1}	484	269	444	294
Silt (0.002–0.05 mm)	g kg^{-1}	431	661	489	633
Clay (<0.002 mm)	g kg^{-1}	85	70	67	73
pH (H_2O)		7.9	8.1	8	8.2
$CaCO_3$	g kg^{-1}	0	5	15	20
Organic C	g kg^{-1}	-[a]	5.5	3.9	5.0
C.E.C.	$cmol_{(+)}kg^{-1}$	10	8	5	7

[a] Organic C and total N contents of the tilled layer for the different treatments are reported on the text (see "Material and methods" section)

Measurements

In field net N mineralization of the tilled soil layers was measured using BBT as described by Hart et al. (1994) in six sequential incubations from sowing (day 0) to flowering (day 81) of maize. At each interval, eight intact soil cores were collected from each subplot at 0–15 and 15–30 cm depth. Four cores were mixed and extracted immediately for determining the amount of mineral N before incubation. The other four cores were enclosed in polyethylene bags, placed in their original holes, and incubated for about 2 weeks. At the end of the incubation period, soil cores from the bags were mixed to produce one sample for each replicate. The extractions of pre- and postincubated soil samples were performed using 2 M KCl (soil, KCl solution ratio of 1:2) and shaking the suspension for 1 h. Ammonium and nitrate contents of soil extracts were analyzed by the Berthelot reaction and the Griess–Illosvay method as reported by Mulvaney (1996) with a continuous flow analyser (Alliance Instruments Evolution II, France). The difference between the amount of inorganic N in post- and preincubated soil was used to calculate net N mineralization rates (NNM) during the period of incubation. The sum of net N mineralized during each incubation provided the estimate of the total mineralized N in the tilled layer for the experiment.

During each incubation period, soil temperature was measured every hour at 7.5 and 22.5 cm depth. Gravimetric moisture of the postincubated soil sample was determined by drying at 105 °C for 24 h. The condition of soil moisture during the incubation was expressed as water-filled pore space (WFPS), calculated assuming a particle density of 2.65 g cm^{-3}, and a measured bulk density of 1.38 g cm^{-3}. Aerobic condition during the incubation was always verified using Microbiology Anaerotest strips (Merck).

The preincubation measurements also provided the dynamic of actual soil inorganic N (IN) contents in the tilled layer (0–30 cm). In the deeper soil layers (30–50, 50–70, and 70–100 cm), IN was measured at day 0 and day 81 following the same procedure of N determination of the preincubated samples. At each sampling date from the emergence to flowering of maize, dry matter, N concentration, and total N uptake of maize (NU) were also determined. A representative sample of the aboveground biomass was collected from each plot, dried at 65 °C for 48 h, weighed, ground to pass through a 0.5-mm screen, and analyzed for N concentration using an elemental analyzer.

The soil–plant mineral N balance (Silgram and Shepherd 1999) also provides an estimate of the net mineralized N for the entire period ($N_{mineraliz}$), according to the general equation:

$$N_{mineraliz} = N_{Upt} - (IN_0 - IN_{end}) - N_{fert} + N_{losses}, \qquad (1)$$

where N_{Upt} is the aboveground crop N uptake for the entire period, IN_0 and IN_{end} are the initial and the final amounts of mineral N contents for the entire profile, and N_{fert} is the amount of N from the fertilization of the year. If we assume no N losses from the profile (N_{losses}) and no mineralization below the tilled layer, and if we exclude the fertilized treatments, the result of Eq. (1) can be compared with the total mineralized N estimated with BBT.

Statistical analysis and results presentation

All data were analyzed using SPSS 12.1.1 software (2003). The results of NNM and IN for the tilled layer (0–30 cm), IN for the entire profile (0–100 cm), and NU were analyzed using ANOVA procedures. The experimental design was a split-plot with three replicates, with past fertilization (MsS and MsF) as the main plots and fresh fertilization (+ and −) as the subplots. Also, the first order interaction (past * fresh) and block were considered. Analysis was performed separately for each date of sampling. Net N mineralization and the dynamic of IN were also tested to evaluate if measured fluxes were different from zero using one-sided confidence interval. The errors used in the tests were the residuals from the analyses of variances.

Results

Soil temperature of the tilled 0–30 cm layer increased throughout the experiment from 10.7 °C (average during first incubation period) to 22.0 °C, while soil water content ranged between 45.1 and 85.1% of WFPS (Fig. 1a).

Net N mineralization rates (Fig. 1b) were zero during the first and fourth incubation periods and were significantly positive during the second, fifth, and final incubation periods. Net N immobilization occurred only during the third period. During the experiment, the observed NNM pattern progressed from an initial release of N, followed by a net N immobilization, and then a last period with an increasing net N mineralization rate.

Excluding the first incubation period during which variances were not homogeneous, fresh fertilizer applications did not cause statistically significant differences in the amount of N mineralized throughout the experiment, while past fertilization was statistically significant during the last incubation period (p=0.039) when high mineralization rates were measured. During this period, NNM rate was higher in MsF than in MsS (1,109 versus 650 µg N kg^{-1} day^{-1} that is 59.7 versus 35.0 kg ha^{-1} of N mineralized) treatments.

During the overall experiment, the average NNM rate was positive, not affected by any factor, and equal to 210 µg N kg^{-1} day^{-1} (that is 70.4 kg ha^{-1} of total N mineralized).

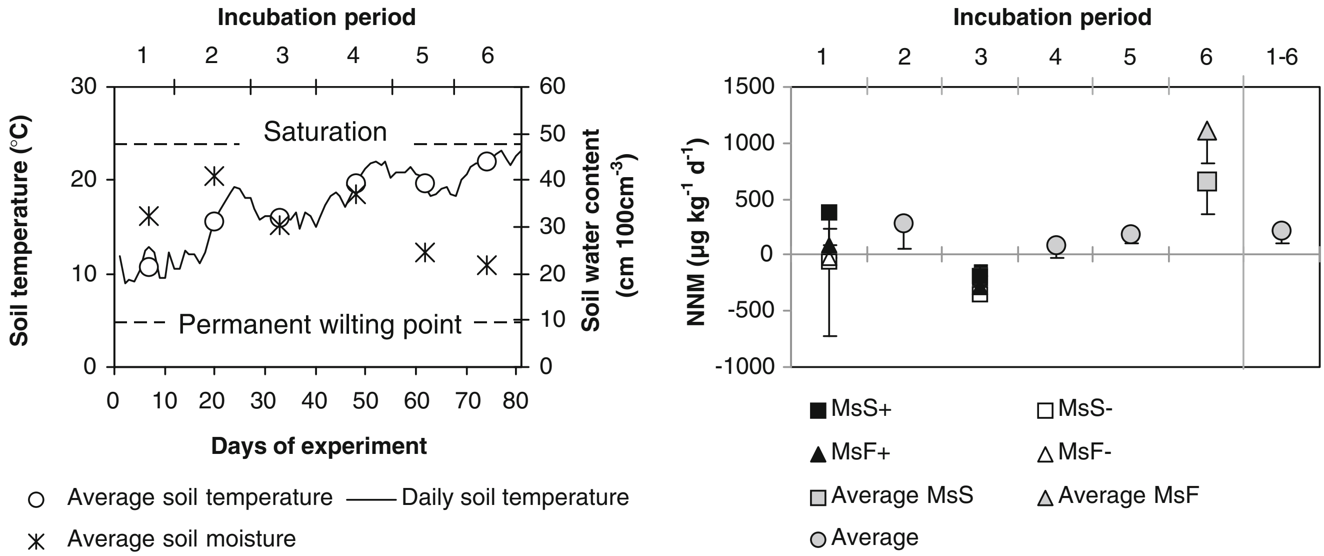

Fig. 1 Average soil temperature, daily soil temperature, and average soil moisture content of each incubation period (**a**) and soil net N mineralization rate measured during each incubation period (**b**) in the 0–30 cm layer. Treatments in (**b**) are: cattle slurry (MsS), farmyard manure (MsF), with (+), and without (−) fertilizer application in the year of the experiment. *Error bars* in (**b**) represent a one-tailed confidence interval (95%)

The organic N mineralized throughout the experiment was almost totally nitrified; in fact, NH_4^+-N represented always a very small amount (4.3% on average) of the total mineral N detected in the polyethylene bags at the end of each incubation period.

At the first measurement, that is 3 days after the application of manures, IN was higher in fertilized than unfertilized treatments (p=0.012) in the tilled layer: 21.7 and 12.7 mg N kg^{-1} of N, respectively. If compared to unfertilized treatments, the application of slurry and farmyard manure increased IN to 51.6 and 22.8 kg N ha^{-1} of N, respectively, which correspond to 50.0% and 108.6% of the mineral N added with the fresh fertilizers. The effect of fresh fertilizer applications on IN was also significant at the beginning of the second incubation period (p=0.031); but after day 13, measured IN was not affected by any factor.

The difference in mineral N of the entire soil profile (0-100 cm layer), calculated between the last and first measurements, showed a significant reduction of −6.33 mg N kg^{-1} (that is −86.1 kg N ha^{-1}) without any significant difference between treatments.

Crop N uptake started during the second period of incubation (0.62 kg N ha^{-1} of N on average). It was significantly higher in MsF than in MsS treatments at the end of the third (7.34 versus 5.06 kg N ha^{-1} of N; p=0.008) and fourth (61.9 versus 46.6 kg N ha^{-1}; p=0.028) periods of incubation. Nitrogen uptakes detected at the end of the fifth incubation period and at the end of the experiment were not affected by any factor and were equal to 127.1 and 189.8 kg N ha^{-1}, respectively.

Discussion

While fresh organic fertilization never affected NNM rates in any incubation period, past fertilization management caused significant differences in the last incubation period when the highest amounts of mineralized N were measured. Our data seem to confirm the hypothesis that mineralization of previous additions of animal manure N masks the effect of fresh amendments (Luxhoi et al. 2004). We also showed a higher NNM for farmyard manure when compared with slurry. The observed pattern of short-term net N release, characterized by an initial net N mineralization, followed by immobilization, and then, by a period of increasing net N mineralization rates, was also observed during aerobic incubations of fertilized soils under laboratory conditions (Calderon et al. 2004; Probert et al. 2005).

The variations in the environmental conditions during the experiment probably contributed to the pattern of net N mineralization. In particular, soil temperatures could explain the null mineralization rates detected in all unfertilized treatments during the first incubation (10.8 °C; −41.1 µg N kg^{-1} day^{-1}) and the high values of NNM measured during the last incubation (22.0 °C; 880 µg N kg^{-1} day^{-1}). Under field conditions, decomposition rates after liquid and solid manure applications measured as CO_2 evolution were low due to a soil temperature of about 10 C (Rochette et al. 2006), similar to that detected during the first incubation period of our experiment. In the case of soil moisture, the lowest level of soil water content detected (45.1% of WFPS during the last incubation) was not limiting microbial activity. In fact, Paul et al. (2003), reviewing data from 12 laboratory incubations, found that net N mineralization was only slightly limited at 45% of WFPS. On the other hand, the high water content measured during the second and fourth incubations (85.1% and 77.1% of WFPS) may have caused a lack of oxygen in the soil, limiting aerobic microbial activity and also supporting denitrification processes.

During the first period, characterized by low soil temperatures, high precipitations, and absent crop N uptake, fertilization with both manures clearly increased soil mineral N contents. The increase in soil mineral N caused by application of the farmyard manure was consistent with the amount of mineral N added while it represented only half of that added with slurry, probably due to NH_3 volatilization because the liquid manure was not directly injected into soil. Under these conditions, NH_3 losses from applied slurry can reach 40% of supplied NH_4^+-N (Mattila and Joki-Tokola 2003).

The measured amounts of NNM (x, kg N ha^{-1}) during the incubation periods were significantly correlated with the changes in soil IN content (y, kg N ha^{-1}) in the 0–30 cm layer (n=72; p=0.000; R^2=0.48; and y=0.69x−10.2). By excluding the first measurement period of MsS+ (n=69) from the analysis, since N losses probably occurred in this treatment, the coefficient of correlation (R^2) increased to 0.63 (p=0.000; y=0.80x−10.3). This result shows that BBT provides a consistent assessment of the dynamic of net N mineralization. Comparing different techniques for enclosing soil during in field measurements, Hanselman et al. (2004) showed that buried bags give reasonable estimates of short-term (<45 days) net N mineralization of soil treated with different manures.

A positive correlation between soil–plant N balance (y, kg N ha^{-1}) and cumulated BBT (x, kg N ha^{-1}) was found (n=6; p=0.012; R^2=0.83; and y=1.45x+40.8) in the unfertilized treatments, with an underestimation by BBT (45.6% of N mineralization calculated by plant–soil N balance). Abril et al. (2001) argue that BBT underestimates the N mineralization rate in soils because the oxygen depletion due to microbial activity can reduce rates of aerobic processes. Moreover, living plants can release organic N through root exudation, and this can promote microbial activity; in addition, root uptake can reduce N immobilization due to the competition between plants and microorganisms for N (Kuzyakov 2002; Parkin et al. 2002; Sauer et al. 2006; Blagodatskaya and Kuzyakov 2008). Thus, enclosing the soil in the buried bags for 2 weeks, as done in our experiment, excludes living roots and prevents plant N uptake during the incubation, leading to an underestimation of total mineralized N.

Conclusions

Fresh fertilizations of farmyard manure or cattle slurry caused no significant differences in the time or extent of net N mineralization when compared to the residual effect of past fertilizations. This result highlights the importance of fertilization management history on soil N availability when animal manures are supplied.

Although the BBT can be considered a good indicator of soil net N mineralization dynamics, when utilized for comparing different agronomic treatments in relative terms, it can underestimate the amount of plant available in mineralized N.

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