Short communication

Microbial control of nitrate concentrations in an agricultural soil treated with dairy waste compost or ammonium fertilizer

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Abstract

We conducted a 112-day laboratory incubation of an agricultural soil treated with dairy-waste compost or ammonium sulfate ((NH4)2SO4) to examine the role of microbial production and consumption of NO3– in controlling soil NO3– concentrations. Inorganic N, net N process rates, nitrification potentials and gross N process rates were measured at various time periods in the treated soils. Microbial consumption of NO3– was not an important process in controlling soil NO3– concentrations in these soil systems. Transient growth in the nitrifier population was observed with ammonium sulfate but not compost addition. Nitrification rates were significantly correlated with and comprised about 50% of the gross N mineralization rates, suggesting that nitrifying bacteria were not weaker competitors for soil NH4+ than heterotrophs in these systems. © 2000 Elsevier Science Ltd. All rights reserved.

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Control of soil NO3– concentrations has both agricultural and environmental importance. Appropriate agricultural N management should control NO3– concentrations at levels meeting crop N requirements without excessive NO3– accumulation in soil, because excess NO3– is susceptible to loss by leaching or denitrification, and NO3– loss decreases N-fertilizer use efficiency. Microbial production and consumption of NO3– occur simultaneously and their relationship controls soil NO3– concentrations.

Microbial assimilation of NO3– has not been considered an important process in controlling soil NO3– concentrations in most agricultural soils, because microorganisms generally prefer NH4+ for their growth (Jansson et al., 1955; Jansson, 1958; Jones and Richards, 1977), and NH4+ even at relatively low concentrations (i.e., < 1 µg N g–1 soil) may decrease microbial utilization of NO3– (Rice and Tiedje, 1989). Nitrate accumulation is often observed in many agricultural soils. One explanation is that microbial assimilation of NO3– is negligible in those soil systems, and that nitrification is the dominant process controlling soil NO3– concentrations. In contrast, it may be that significant microbial assimilation of NO3– does occur, but that production of NO3– greatly exceeds NO3– consumption by both microorganisms and plants. The determination of the gross rates of simultaneous production and consumption requires the use of isotopic methods.

The functional groups of soil microorganisms act variously as producers, consumers, and competitors of the different forms of soil N. For example, heterotrophs not only decompose soil organic matter, supplying NH4+ to soil nitrifiers, but may also compete with nitrifiers for soil NH4+ (Verhagen and Laanbroek, 1991). Though soil organic amendments such as compost significantly increase the activities of soil heterotrophs (Schnurer et al., 1985; Fauci and Dick, 1994),
the effects of this enhanced microbial activity on ammonification, nitrification, microbial N assimilation, and the interactions of these processes have not been well characterized. Our objectives were to examine if microbial consumption of NO$_3^-$ was an important process controlling soil NO$_3^-$ concentrations, and whether soil nitrifiers can strongly compete for NH$_4^+$ with soil heterotrophs in N amended soils.

Timpanogos silt loam soil (fine-loamy, mixed, superactive, mesic Calcic Argixeroll) was collected from the 0–15 cm surface layer in bulk from the Blue Creek Farm of Utah State University. Ammonium sulfate was used as the inorganic N source, while mature dairy-waste compost (M.G. Pace, unpub. MS thesis, Utah State University, 1996) was used as the organic N source. Soil and dairy-waste compost were sieved (2 mm) and stored at 4°C until use. Selected properties of the soil and the compost are given in Table 1. Three soil treatments in this laboratory incubation experiment were (1) control, soil without additions; (2) NH$_4^+$, soil with the addition of (NH$_4$)$_2$SO$_4$ at 50 mg N kg$^{-1}$ soil (equivalent to 100 kg N ha$^{-1}$); and (3) compost, soil with the addition of dairy-waste compost at 2.0 g (dry wt.) per 100 g soil (equivalent to 40 Mg dry wt. ha$^{-1}$). Treated soil samples (20 g dry wt. equivalent) were weighed into 120-ml specimen containers and placed in an incubator at 20°C. The gravimetric water content of soil samples was adjusted to 19% (60% of field capacity) every 4–6 days. Three samples of each treatment were randomly withdrawn at 0, 7, 25, 40, 70, and 112 days for measuring inorganic N and nitrification potential (Hart et al., 1994). The nitrification potential soil slurries were continuously shaken for 24 h at 200 rpm (Stark, 1996), and the pH of the soil slurries was monitored and adjusted four times to maintain the pH near 7.5.

Gross N process rates were measured by $^{15}$N pool dilution techniques (Hart et al., 1994) for the three soil treatments, at the four labeling dates (incubation-day 7, 40, 70, and 112). For measuring gross nitrification and microbial assimilation of NO$_3^-$ at each labeling date, three pairs of soil samples per treatment (as three replications) were withdrawn, and each soil sample received 1 mg N kg$^{-1}$ soil as K$^{15}$NO$_3$ solution (99% enrichment, 20 mg NO$_3^-$-N L$^{-1}$). For measuring gross N mineralization and microbial assimilation of NH$_4^+$, we used the same procedure as described above, except that soil samples were labeled with $^{15}$NH$_4$Cl. Depending on the labeling date, the amount of $^{15}$NH$_4$Cl injected varied from 1 to 5 N mg kg$^{-1}$ soil, along with the enrichment decreasing from 99% to 50%. For each pair of labeled soil samples, one was extracted 15 min after and the other 24.25 h after the injection. A diffusion procedure (Stark and Hart, 1996) was used to prepare samples for $^{15}$N analysis. The $^{15}$N enrichments in the NH$_4^+$ or NO$_3^-$ pools were analyzed by continuous-flow direct combustion and mass spectrometry with ANCA 2020 system (Europa Scientific, Cincinnati, OH).

The $^{15}$N excesses and recoveries measured shortly and one day after $^{15}$N injection were compared by two-way ANOVA with the labeling dates and treatments as factors. If the $^{15}$N excesses measured one day after $^{15}$N injection were significantly lower than those measured initially, the gross N process rates were calculated by the equations of Kirkham and Bartholomew (1954). Effects of treatments and incubation days on soil process rates of N mineralization, nitrification, microbial N assimilation, and the ratios of these rates were analyzed using two-way ANOVA with the treatments and sampling dates as factors. All statistical analyses were performed using SuperANOVA software (Abacus Concepts, 1995, Berkeley, CA).

Throughout the 112-day incubation, NH$_4^+$-N concentrations in the control soil and soil added with compost were very low (<1 mg kg$^{-1}$ soil), whereas NO$_3^-$-N concentrations were ten to hundred times NH$_4^+$-N concentrations and increased almost linearly with the incubation (Fig. 1). In the soil with added (NH$_4$)$_2$SO$_4$, NH$_4^+$-N concentrations rapidly decreased and reached levels similar to the control soil or the soil with compost at 40 days, while the NO$_3^-$-N concentrations increased rapidly and non-linearly (Fig. 1).

We measured the recoveries of $^{15}$NO$_3^-$ one day after the $^{15}$N injections and found that they did not differ from those measured shortly after the $^{15}$N injections ($p = 0.26$). The 100% recovery of $^{15}$NO$_3^-$ combined with the accumulation of soil NO$_3^-$ suggests that microbial assimilation of NO$_3^-$ and denitrification were both very low, and that they can be ignored as important processes controlling NO$_3^-$ concentrations under the experimental conditions. Additionally, the $^{15}$N excess measured one day after the $^{15}$N injection did not significantly differ from those measured shortly after injection ($p = 0.10$), which indicates that gross nitrification rates could not be measured by the $^{15}$N pool di-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The selected properties of Timpanogos soil and dairy-waste compost</th>
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<tr>
<td></td>
<td>Organic C (g kg$^{-1}$)</td>
</tr>
<tr>
<td>Soil</td>
<td>14</td>
</tr>
<tr>
<td>Compost</td>
<td>237</td>
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</table>
olution method and that the observed net rates represent nitrate production. Rice and Tiedje (1989) documented that NH$_4^+$ could decrease microbial assimilation of NO$_3^-$ even at relatively low concentrations (<1 µg N g$^{-1}$ soil). They suggested that microbial assimilation of NO$_3^-$ would not be an important process in most agricultural soils. Wichramasinghe et al. (1985) showed that there was no microbial assimilation of NO$_3^-$ in agricultural soils with 4% organic C and C:N ratios of 13. Recous and Mary (1990) also reported that microbial assimilation of NO$_3^-$ in cultivated soil was negligible when KNO$_3$ was added at 50 µg N g$^{-1}$ soil without the addition of glucose C. In contrast, when glucose at 500 µg C g$^{-1}$ soil was added along with the same amount of KNO$_3$, microbial assimilation of NO$_3^-$ occurred. The absence of microbial assimilation of NO$_3^-$ in our experiment suggests a C limitation of the heterotrophic microorganisms even in the compost treated soil. While several studies have documented the importance of microbial NO$_3^-$ consumption in forest and grassland soils (Stark and Hart, 1997; Jackson et al., 1989; Schimel et al., 1990), our data supports the paradigm of a C-limited but N sufficient microbial biomass in agricultural soils. Additional in situ N cycle experiments in similarly treated field soils also support this interpretation (data not shown).

Soil nitrifiers get their energy solely from the oxidation of NH$_4^+$ to NO$_3^-$: increased nitrification rates with increasing additions of mineral NH$_4^+$ have been reported in studies of nitrification kinetics (Darrah et al., 1985; Nishio and Fujimoto, 1990). Nishio and Fujimoto (1990) observed that an increase in nitrification rate was attributed to the growth of nitrifiers when NH$_4^+$ was added at levels >50 mg N kg$^{-1}$ soil. The increased nitrification rates and potentials along with the addition of (NH$_4$)$_2$SO$_4$ in this soil system (Fig. 2) support the observation that available NH$_4^+$ limited both the nitrification rate and nitrifier population size. Initially, the existing nitrifier popu-
The metabolic energy produced exceeded the maintenance requirement of the population and allowed for population growth (Fig. 2). An apparent specific growth rate for nitrifiers in the (NH₄)₂SO₄ treated soil was calculated based on the assumption that nitrifier population growth was represented by an exponential increase in the nitrification potential between days 7 and 25 (Fig. 2). The apparent specific growth rate calculated was 0.01 day⁻¹, equivalent to a doubling time of 69 days. The observed specific growth rate is far below maximum specific growth rates reported for soil nitrifier populations under non-limiting NH₄⁺ supply (Belser, 1979; Darrah et al., 1985) suggesting that growth remained NH₄⁺ limited under these experimental conditions. As the added NH₄⁺ was depleted, nitrification rates and the ratio of nitrification rate to nitrification potential began to decrease (Fig. 2, Table 2). The supply of mineralized NH₄⁺ could not maintain the larger nitrifier population. Nitrification potentials began decreasing at day 40 until they were equivalent to those of the control soil by the end of the incubation (Fig. 2).

In the control soil or the soil receiving compost, N mineralization was the primary source of NH₄⁺ available to soil nitrifiers and was the rate limiting process for subsequent nitrification. N mineralization supplied substrate approximately equivalent to or less than the maintenance requirement of the existing nitrifier population.

Gross N mineralization rates were significantly different at the different incubation times (p < 0.01) and among the three soil treatments (p < 0.01) (Table 3). Generally, gross N mineralization rates decreased with incubation, and the soil receiving compost had higher gross N mineralization rates than the control soil. Because of low NH₄⁺ concentrations in the control soil or the soil added with compost, the addition of NH₄⁺ from ¹⁵N injections even at 1 mg N kg⁻¹ soil could enhance the rates of those N processes that utilize NH₄⁺. Our data (Table 3) showed that NH₄⁺ consumption rates were much higher than the gross N mineralization rates, implying that the NH₄⁺ addition enhanced the combined consumption by nitrifiers and immobilizing heterotrophs. Since the enhanced nitrification rates were almost equal to the enhanced NH₄⁺ consumption rates (data not shown), we conclude that nitrifiers were the main consumers of the added NH₄⁺ in these systems.

The ratios of nitrification rates to gross N mineralization rates were not significantly different among the

<table>
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<th>Incubation days</th>
<th>Nitrification rate/gross N mineralization rate</th>
<th>Nitrification rate/nitrification potential</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Compost</td>
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<tr>
<td>7</td>
<td>0.52 (0.03)°</td>
<td>0.51 (0.03)</td>
</tr>
<tr>
<td>40</td>
<td>0.45 (0.11)</td>
<td>0.26 (0.02)</td>
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<tr>
<td>70</td>
<td>0.68 (0.03)</td>
<td>0.63 (0.15)</td>
</tr>
<tr>
<td>112</td>
<td>3.53 (1.50)</td>
<td>0.50 (0.09)</td>
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° Values are means (standard error) for n = 3.
three soil treatments \((p = 0.10)\) (Table 2). Nitrification rates were about 50% of the gross N mineralization rates or higher. High ratios of nitrification rates to gross N mineralization rates in all the three soil treatments throughout the 112-day incubation (Table 2) further indicate that nitrifiers are strong competitors for NH\(_4^+\) in these systems.

### Acknowledgements

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### References


