Available Organic Carbon Controls Nitrification and Immobilization of Ammonium in an Acid Loam-Textured Soil

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Received March 13 2006; Accepted March 24 2006

Effect of organic-C on immobilization and nitrification patterns in acidic soil was examined during 20 weeks incubation period to verify if organic amendments such as composted material can increase soil retention of N by stimulating microbial immobilization of NH_4^+. Four treatments were laid out: control without fertilizer N and glucose (treatment code: S), ammonium sulfate (SN), ammonium sulfate with single glucose at the commencement (0 week) of incubation (SNG), and ammonium sulfate with double glucose at 0 and 4 weeks of incubation (SNGG). Glucose application (SNG) significantly increased microbial immobilization of NH_4^+ within 1 week of incubation over SN. Immobilization was followed by re-mineralization thereafter; however, second-application of glucose (SNGG) restored NH_4^+ immobilization. At the same time, nitrification was significantly inhibited by glucose application as indicated by consistently low NO_3^- concentration in SNG and SNGG soils, suggesting that microbial assimilation of NH_4^+ is predominant compared to nitrification when available C-source is abundant. These results suggest application of chemical fertilizer-N with organic amendment would have beneficial effect on soil-N retention and environmental conservation by reducing production of NO_3^- which is likely to be lost through leaching or denitrification.

Key words: *Ammonium, microbial immobilization, nitrate, nitrification, organic carbon*

Two dominant fates of ammonium derived from fertilizer N or produced by mineralization of organic N in soils are nitrification (mediated by autotrophs) and microbial immobilization (mediated by heterotrophs).[1,2] Nitrate is susceptible to loss from the soil through leaching and denitrification, while immobilized N is likely to be re-mineralized and thus become available for plant uptake.[2,3] Several studies have reported that immobilization rather than nitrification would be more beneficial in terms of conservation of available soil-N.[1,3-5] As nitrate is one of the most frequently observed contaminants of groundwater and NO_x produced via denitrification are atmospheric pollutants, understanding of the competition between immobilization and nitrification has also environmental implications.[6]

Recently, with the change of agricultural paradigm toward so-called "environmental-friendly" agriculture, blending composted manure (at crop P requirement) enriched in organic C with chemical fertilizer-N (as supplementary N) is considered an environmentally safe way to use the composted material, because such blending reduces the application rate of organic amendment.[3] Under the mixed application conditions, microbial immobilization of fertilizer N blended with compost has been shown to increase due to microbial activity of heterotrophs, stimulated by organic C, which is responsible for immobilization of mineral N.[3,7-10] At the same time, retardation or inhibition of nitrification induced by organic-C-enhanced immobilization of NH_4^+ has often been observed, because immobilization and nitrification compete for the same substrate (NH_4^+).[10-13] However, it is not straightforward to ascribe compost-induced increases in immobilization and decreases in nitrification solely to stimulated activities of heterotrophs by organic C, because compost has phenolic or humified sites available for physico-chemical binding of NH_4^+.[8,12,14,15] Therefore, it is still doubtable if organic-C-induced microbial immobilization rather than physico-chemical fixation reduces nitrification of NH_4^+. In this study, effect of organic-C source (glucose), which does not have phenolic or humified sites, on immobilization-nitrification competition for soil NH_4^+ was examined.

Materials and Methods

Soil. Soil (coarse loamy, Fluventic Haplaquepts) samples were collected from an experimental farm of Chonnam

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National University, and used for aerobic incubation study after passed through a 2-mm sieve. The soil had a 5.8 of pH (1 : 2.5), 0.36 kg kg^{-1} of WHC (water holding capacity), 14.0 g kg^{-1} of total organic-C, 1.38 g kg^{-1} of total N, 15.0 mg kg^{-1} of NH_4^+-N, and 101.0 mg kg^{-1} NO_3^--N.

Incubation Study. Incubation was conducted for 20 weeks after the soil samples were pre-incubated at 25 ± 2°C for 5 days. During the pre-incubation, soil moisture content was adjusted to 40% WHC. To examine the effect of organic C (glucose) on the immobilization and nitrification patterns of fertilizer-N (ammonium sulfate), four treatments were laid out with three replications: control without fertilizer N and glucose (treatment code: S), ammonium sulfate (SN), ammonium sulfate with single glucose at the commencement (0 week) of incubation (SNG), and ammonium sulfate with double glucose at 0 and 4 weeks of incubation (SNGG).

To set up the incubation experiment, 30 g (oven-dry basis) of the pre-incubated soil was placed in a 100-mL polyethylene bottle (3.5 cm in diameter and 5.5 cm depth). For the corresponding treatments, ammonium sulfate solution (100 mg N L^{-1}) and glucose were applied at 173 mg N kg^{-1} and 8650 mg C kg^{-1} (C : N ratio = 50 : 1), respectively, and mixed with the soil. Distilled water was added to bring soil moisture content to 80% WHC. The bottles were capped with perforated caps to ensure gas exchange and incubated at 25 ± 2°C in the dark. Throughout the 20-week incubation period, initial soil moisture content was maintained by daily addition of distilled water as necessary. Three bottles randomly selected from each treatment were destructively sampled 1, 2, 4, 6, 8, 10, 12, 16, and 20 weeks after the commencement of incubation. For SNGG treatment, glucose (8650 mg C kg^{-1}) was added one more time at 4 weeks of incubation.

Chemical Analyses. At each sampling time, the sample in each bottle was homogenized with a spatula. The pH (1 : 2.5) of each soil sample (10 g oven-dry basis) was measured with a pH meter (P 25, EcoMet, Seoul, Korea). To determine NH_4^+ and NO_3^- concentrations, soil sample (15 g oven-dry basis) was placed into a 100-mL centrifuge tube and added with 75 mL of 2 M KCl solution. After shaking for 1 h, the tube was centrifuged, and the supernatant was analyzed for NH_4^+ and NO_3^- concentrations with steam-distillation on a distillation system (Pronitro 1, J.P. Selecta, Barcelona, Spain).[16] For the determination of organic-N concentration, the remaining mineral-N was completely removed from the soil in the tube using deionized water following the procedures of Choi *et al.*[3] The samples were dried at 65°C in an oven, ground to fine powder using a ball mill, and analyzed for N concentration following Kjeldahl method.[17]

Statistical Analyses. Data were tested for homogeneity of variance and normality of distribution. Transformation of data was not needed, because no heterogeneity was detected and distribution was normal. Analysis of variance (ANOVA) was performed on all experimental variables using the general linear models (GLM) procedure of the SPSS 11.5 package (SPSS Inc., Chicago, IL) to assess the effects of organic-C application on immobilization and nitrification. The level of significance for all statistical tests was set at α = 0.05.

Results

Changes in NH_4^+ Concentration. The NH_4^+ concentration of SN soil treated with ammonium sulfate without glucose (SN) showed a gradually decreasing pattern throughout the incubation period (Fig. 1), whereas soil treated with both ammonium sulfate and glucose (SNG) showed a large fluctuation in NH_4^+ concentration. Significantly different from SN soil, the NH_4^+ concentration of the SNG soil sharply decreased from 188.9 mg N kg^{-1} at the commencement of incubation to 33.0 mg N kg^{-1} within 1 week of incubation and increased thereafter to 102.7 mg N kg^{-1} after 4 weeks incubation. In SNGG soil, second application of glucose at 4 weeks of incubation again rapidly decreased the NH_4^+ concentration (102.7 mg N kg^{-1} after 4 weeks to 22.1 mg N kg^{-1} after 6 weeks), while the concentration in SNG (without re-application of glucose) decreased gradually (e.g. 102.7 mg N kg^{-1} after 4 weeks to 87.9 mg N kg^{-1} after 6 weeks). Such different pattern was statistically significant.

Changes in NO_3^- Concentration. In the S treatment (control), NO_3^- concentration gradually increased throughout the incubation period (Fig. 2), whereas the SN treatment resulted in a rapid increase in NO_3^- concentration, and, after 6 weeks of incubation, reached the maximum concentration (203.6 mg N kg^{-1}), which was maintained relatively constant thereafter. Glucose application at the initiation of incubation (SNG) resulted in a short-term depression of NO_3^- concentration, decreasing from 26.6 to 4.2 mg N kg^{-1} within 2

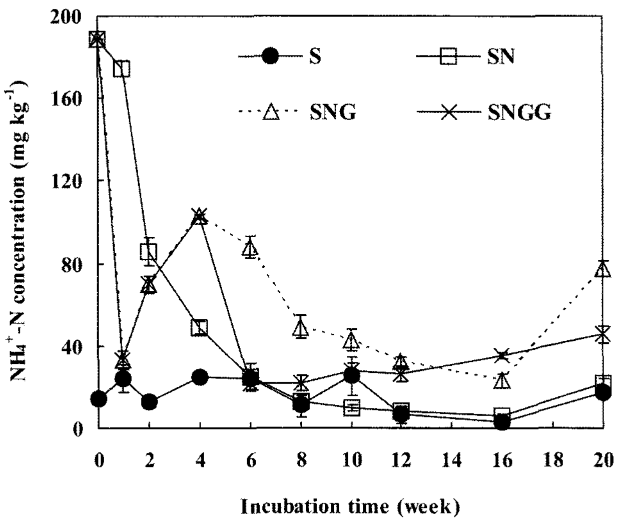

Fig. 1. Changes in NH_4^+-N concentration during the incubation. Treatment codes: S for control, SN for ammonium sulfate, SNG for ammonium sulfate and glucose (applied at 0 week), and SNGG for ammonium sulfate and glucose (applied at 0 and 4 weeks of incubation). Vertical bars indicate standard errors of the means.

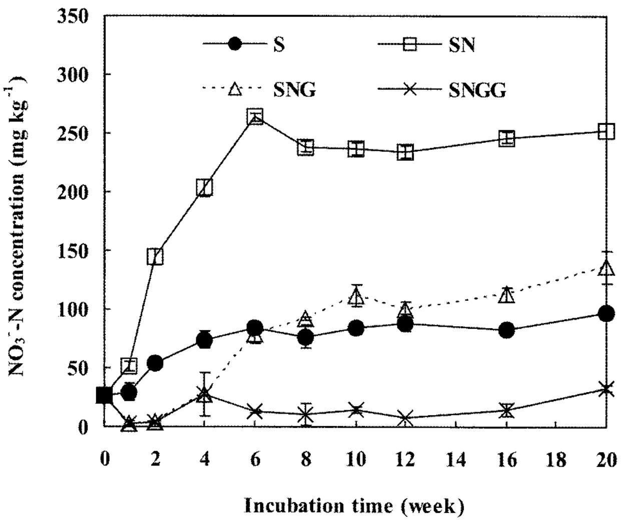

Fig. 2. Changes in NO_3^--N concentration during the incubation. Treatment codes: S for control, SN for ammonium sulfate, SNG for ammonium sulfate and glucose (applied at 0 week), and SNGG for ammonium sulfate and glucose (applied at 0 and 4 weeks of incubation). Vertical bars indicate standard errors of the means.

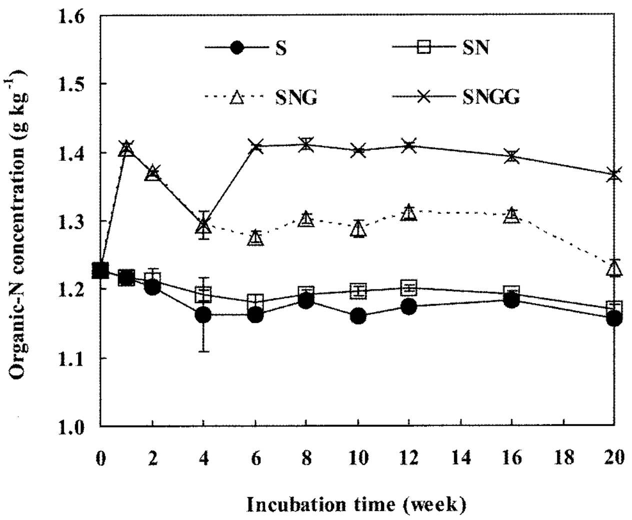

Fig. 3. Changes in organic-N concentration during the incubation. Treatment codes: S for control, SN for ammonium sulfate, SNG for ammonium sulfate and glucose (applied at 0 week), and SNGG for ammonium sulfate and glucose (applied at 0 and 4 weeks of incubation). Vertical bars indicate standard errors of the means.

weeks, thereafter increased, reaching 136.5 mg N kg^{-1} at the end of incubation. On the other hand, re-application of glucose at 4 weeks incubation (SNGG) suppressed NO_3^- production, resulting in a significantly low NO_3^- concentration, ranging 7.8 to 32.9 mg N kg^{-1} between 4 to 20 weeks incubation, compared to the SNG treatment. At the end of incubation, glucose application decreased NO_3^- concentrations by 46% in SNG (136.5 mg N kg^{-1}) and 87% in SNGG (32.9 mg N kg^{-1}) soils as compared to SN soil (252.3 mg N kg^{-1}). The nitrification inhibition efficiencies of glucose-C calculated as the difference between NO_3^- concentration of glucose-untreated (SN) and that of glucose-treated soil per unit glucose-C rate were 13.4 and 12.7 μg N C^{-1} in SNG and SNGG soils, respectively.

Changes in Organic-N Concentration. Application of ammonium sulfate resulted in an increase in organic-N concentration as compared to the control (S), while statistical significance varied with sampling time. Glucose application significantly enhanced immobilization of N; between 1 and 4 weeks the organic-N concentration ranged from 1.19 to 1.22 mg N kg^{-1} in SN soil and from 1.29 to 1.41 mg N kg^{-1} in SNG soil (Fig. 3). During the same period, organic-N concentration in glucose-amended soils showed a decreasing pattern with time; however, this pattern was reversed by re-application of glucose at 4 weeks of incubation (SNGG in Fig. 3). At the end of incubation, the immobilization stimulation efficiencies of glucose-C calculated as the difference between organic-N concentration of glucose-untreated (SN) and that of glucose-treated soil per unit glucose-C rate were 6.9 μg N C^{-1} in SNG and 11.3 μg N C^{-1} in SNGG soils.

Changes in pH. The pH showed a decreasing pattern up to

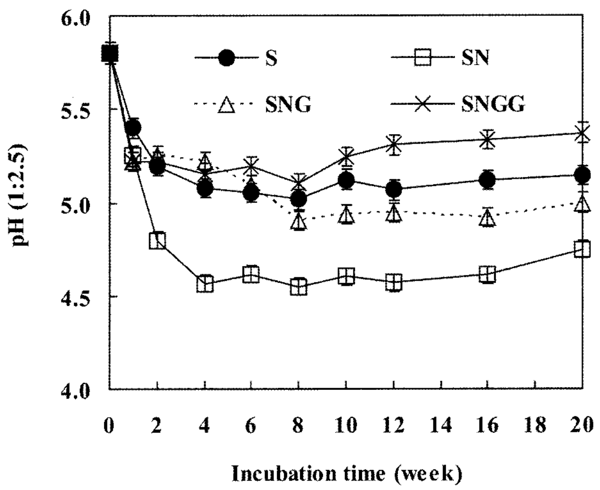

Fig. 4. Changes in pH during the incubation. Treatment codes: S for control, SN for ammonium sulfate, SNG for ammonium sulfate and glucose (applied at 0 week), and SNGG for ammonium sulfate and glucose (applied at 0 and 4 weeks of incubation). Vertical bars indicate standard errors of the means.

4 weeks of incubation; thereafter the value tended not to vary significantly (Fig. 4). Compared to the control (S treatment), ammonium sulfate application significantly lowered the pH throughout the incubation period. This decreasing pattern was retarded by application of glucose; the pH values after 4 weeks of incubation were 4.6 for SN and 5.2 for SNG soils. Between 4 and 8 weeks of incubation, pH of SNG soil decreased further, while that of SNGG soil retreated with glucose did not. Eventually, the pH decreased in the order of SNGG > S > SNG > SN at the end of incubation.

Discussion

Overall, our data clearly show that organic-C application depresses nitrification (Fig. 2) and enhances microbial immobilization of NH_4^+ (Fig. 3), which indicate available organic-C is a critical controller of competition between heterotrophs (responsible for immobilization) and autotrophs (responsible for nitrification). This finding is largely consistent with the immobilization and nitrification patterns observed in soil treated with composted manure as reported by Choi *et al.* and Han *et al.*[3,10] On the other hand, negligible increases in organic-N concentration (Fig. 3), even when NO_3^- concentration was high (Fig. 2), particularly for SN soil, suggest that microbial assimilation of NO_3^- was not an important process in the soil used. Several studies suggested that heterotrophs prefer NH_4^+ to NO_3^- as a N source except in NH_4^+-depleted soils.[5,18-20] Hence, our results suggest that organic-C amendment can increase soil retention of N and thus reduce N loss, which eventually has adverse impacts on water and air qualities.

An evidence of the changed pattern of immobilization and nitrification caused by organic-C amendment was further provided by different pH values among treatments (Fig. 4). It is well understood that nitrification produces proton (H^+), thus lowering soil pH.[21] In our study, the highest pH value was observed in SNGG soil showing the lowest NO_3^- concentration (and the highest organic-N concentration), and the reverse in SN soil (Figs. 2 and 4). These results further suggest that organic-C amendment has a beneficial effect on the retardation of soil acidification.

The organic-N concentration in glucose-amended soils (SNG) showed a decreasing pattern between 1 and 4 weeks of incubation, suggesting immobilized N can be re-mineralized easily in a very short period (Fig. 3), which coincided with an increase in NH_4^+ concentration of the soil, an indicative of N mineralization (Fig. 1). It is well understood that immobilized N is likely to be re-mineralized into NH_4^+, corresponding with the decay of microbes.[1,2] For example, Shindo and Nisho observed that microbial biomass-N increased rapidly within 1 week of incubation, which, thereafter, decreased by 50% within 4 weeks.[2] In our study, because re-application of glucose restored microbial immobilization of N very quickly (within 2 weeks for SNGG; Figs. 1 and 4), repeated imposition of organic-C amendment could be assumed to lead to a higher soil-N retention.

In conclusion, glucose application significantly enhanced microbial immobilization of NH_4^+, which otherwise is likely to be nitrified by autotrophic nitrifiers. However, such effect was diminished in a short-term incubation period due to re-mineralization of immobililzed-N. Re-application of glucose reinforced microbial immobilization of NH_4^+, indicating available organic-C is a critical controller of competition between immobilization by heterotrophs and nitrification by autotrophs. At the end of incubation, nitrification inhibition and immobilization stimulation efficiencies of glucose-C were 13.4 and 6.9 μg N C^{-1} for SNG and 12.7 and 11.3 μg N C^{-1} for SNG, respectively. Considering the susceptibility of NO_3^- to loss via leaching and denitrification, our data suggest that increasing available soil-C is one strategy to retain soil mineral-N, leading to higher uptake of N by plants and less loss of NO_3^- to the environment.

Acknowledgements. This study was supported by "Specific Joint Agricultural Research-Promoting Projects," Rural Development Administration, Republic of Korea.

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