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Short communication

Heterotrophic and autotrophic nitrification in two acid pasture soils

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Abstract

Laboratory incubation experiments, using ^{15}N-labeling techniques and simple analytical models, were conducted to measure heterotrophic and autotrophic nitrification rates in two acid soils (pH 4.8–5.3; 1/5 in H_2O) with high organic carbon contents (6.2–6.8% in top 5 cm soil). The soils were from pastures located near Maindample and Ruffy in the Northeast Victoria, Australia. Gross rates of N mineralization, nitrification and immobilization were measured. The gross rates of autotrophic nitrification were 0.157 and 0.119 $\mu g\,N\,g^{-1}\,h^{-1}$ and heterotrophic nitrification rates were 0.036 and 0.009 $\mu g\,N\,g^{-1}\,h^{-1}$ for the Maindample and Ruffy soils, respectively. Heterotrophic nitrification accounted for 19% and 7% of the total nitrification in the Maindample and Ruffy soils, respectively. The heterotrophic nitrifiers used organic N compounds and no NH_4^+-N as the substrate for nitrification.

Keywords: N cycling; ^{15}N-labeling; Heterotrophic nitrification

In laboratory studies on pasture soils from northeast Victoria, Australia, it was found that active nitrification existed in acid soils that have a field pH of 4.8–5.3 (1/5 in H_2O) (Islam et al., 2006). Active nitrification under acid conditions has been suggested as evidence of heterotrophic nitrification (Focht and Verstraete, 1977; Papen and von Berg, 1998). However, it has been suggested that acid-tolerant autotrophs could also be responsible for nitrification in such soils (De Boer and Kowalchuk, 2001). Few studies have provided quantitative data on the relative importance of heterotrophic and autotrophic nitrification in acid pasture soils (Pennington and Ellis, 1993). Using the nitrification inhibitor N-serve and ^{15}N-dilution technique, Barraclough and Puri (1995) reported that up to 8% of the observed nitrification in an acidic woodland soil could be the result of heterotrophic nitrifiers oxidizing organic-N to nitrite and nitrate, without passing through the exchangeable ammonium (NH_4^+) pool. However, Honda et al. (1998) and De Boer and Kowalchuk (2001) found that heterotrophic nitrifiers can use both inorganic and organic substrates for nitrification. In addition, because some heterotrophic nitrifiers denitrify simultaneously (Matheson et al., 2003), and therefore, accumulate little or no nitrite, actual heterotrophic nitrification rates may have been underestimated (Robertson et al., 1988).

The main objective of this study was to determine the significance of heterotrophic nitrification in two acid pasture soils in southeastern Australia, and to investigate whether NH_4^+ was also a substrate for heterotrophic nitrifiers. With this information, the importance and relative contribution of heterotrophic and autotrophic nitrifying populations in the N dynamics of these soils could be assessed.

Three composite samples (0–5 cm) from two acid soils were collected in June 2000 from sheep-grazed pastures in northeast Victoria: a Brown Sodosol (Isbell, 1996) on Palaeozoic sedimentary rocks at Maindample (pH 4.8; 1/5 in H_2O, 6.2% organic C, 0.62% N), and a bleached-mottled magnesic Yellow Kurosol on Palaeozoic granite at Ruffy (pH 5.0, 6.8% organic C, 0.63% N). The soils were brought immediately to the laboratory, sieved (<2 mm) and stored at 4 °C in sealed plastic bags prior to analyses. The texture of the soils at Maindample and Ruffy was sandy loam and coarse sandy loam, respectively.

Soil subsamples of 400 g (oven-dry equivalent) were spread over a plastic sheet to form a thin layer, and either $(^{15}NH_4)_2SO_4$ (9.79 at% ^{15}N) plus KNO_3 or $(NH_4)_2SO_4$

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plus $K^{15}NO_3$ (9.50 at% ^{15}N), with or without nitrification inhibitor of N-serve at 80 μg g^{-1}, was applied in a fine spray by using a syringe fitted with a 23-gauge needle. Each treatment received 100 μg NH_4^+-N and 20 μg NO_3^--N g^{-1}. The source of N-serve was N-serve 24E, which is an emulsified nitrapyrin (Dow Chemical Company, USA). An equivalent volume of deionized water instead of N-serve was used in the control treatment and the soil moisture content adjusted to 70% of the field capacity (45% water-filled pore space). Immediately after mixing, 20 g soil (oven-dry equivalent) was placed in a 250 ml plastic vial, covered with parafilm in which four pin holes were made to facilitate air exchange, and incubated at 22 °C. Water was added every day as needed to replenish moisture loss. Three replicate subsamples of each treatment were taken after 0, 5 and 14 d of incubation and extracted with 1 M KCl (1:5 soil:solution ratio) containing 10 μg ml^{-1} phenylmercuric acetate and filtered through glass-fiber filter paper (Whatman GF/C). After filtration, the soil was washed twice with 0.5 M KCl to ensure that all the nitrite and nitrate was removed, dried at 60 °C, ground to <0.15 mm and analyzed for total C and N using a CNS elemental analyzer (Carlo Erba NA 1500, Italy). Ammonium and nitrate in the extracts were determined by steam distillation (Keeney and Nelson, 1982). Labeled distillates were dried and isotope ratios were determined on N_2 generated by hypobromite oxidation (Chen et al., 1990). Isotope ratios were measured on a mass spectrometer (Sira 10, VG Isogas, UK). Gross rates of nitrification, mineralization and immobilization were estimated by ^{15}N dilution technique using the equations given by Chen et al. (1995).

The $^{15}NH_4^+$ applied to the Maindample and Ruffy soils was not quantitatively recovered in the exchangeable ammonium pool at zero time (Fig. 1). The part of labeled ^{15}N was recovered in the $^{15}NO_3^-$ pool and part in the soil organic-N pool (Figs. 2 and 3), indicating that part of the $^{15}NH_4^+$ was oxidized during sample processing, which took approximately 5 h, and part was immobilized into the soil

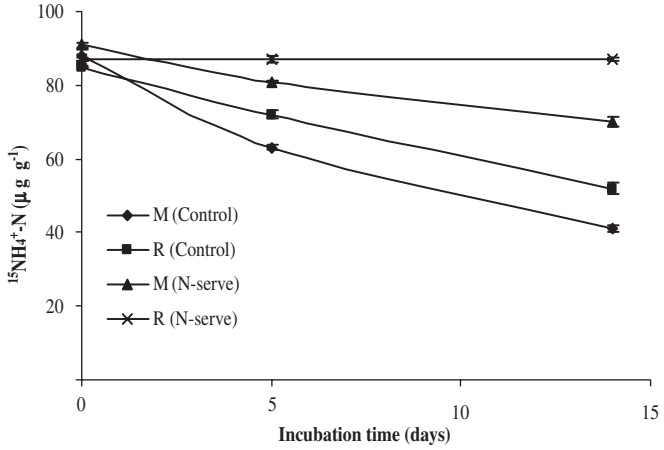

Fig. 1. Change in labeled ^{15}N-ammonium in Maindample (M) and Ruffy (R) soil with time. Error bars are the standard errors of three replicates.

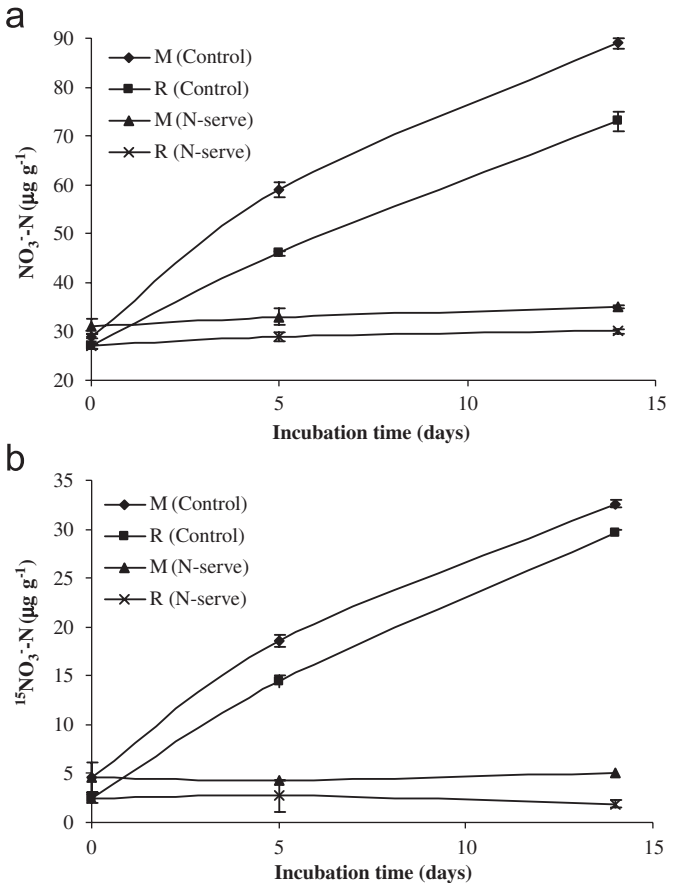

Fig. 2. Accumulation of total (a) and labeled (b) NO_3^- with time in samples of Maindample (M) and Ruffy (R) soil supplemented with NH_4^+. Error bars are the standard errors of three replicates.

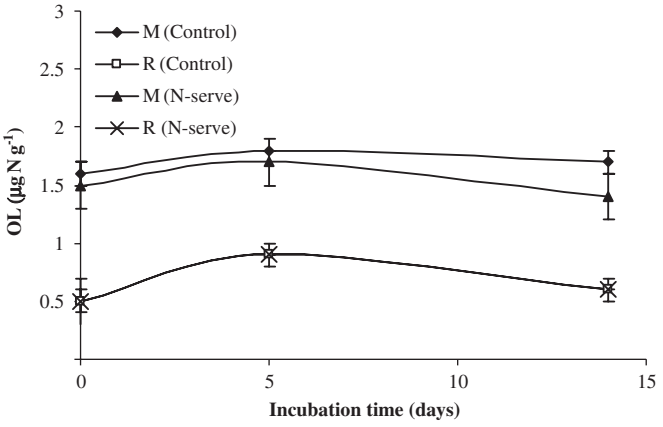

Fig. 3. Labeled organic N (OL) in the Maindample (M) and Ruffy (R) soil over time. Error bars are the standard errors of three replicates.

organic N-pool. The non-recovered ^{15}N may be attributed to clay fixation of NH_4^+, emphasing the necessity of conducting the time zero measurement.

Of the labeled nitrate applied, quantitative recovery was achieved at the time zero sampling. However, considerable ^{15}N labeled nitrate was recovered in the soil organic-N pool