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Christie, P., & Wasson, E. A. (2001). Short-term immobilization of ammonium and nitrate added to a grassland soil. *Soil Biology and Biochemistry*, 33(9), 1277-1278.

Published in:
Soil Biology and Biochemistry

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Short communication

Short-term immobilization of ammonium and nitrate added
to a grassland soil

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Received 6 March 2000; received in revised form 15 August 2000; accepted 6 September 2000

Abstract

Short-term immobilization of added $^{15}\text{NH}_4^+$ -N and $^{15}\text{NO}_3^-$ -N (with or without added glucose) was determined at time intervals in a pre-conditioned grassland soil over laboratory incubation periods of up to 15 days at 25 °C. Exchangeable N was removed from soil sub-samples by repeated 2 M KCl extraction and centrifugation. The solid residues were immediately blast frozen, freeze-dried and analysed for total N and N isotope ratio. Sub-samples without glucose amendment showed very little immobilization of added N (5 and <2% of added NH_4^+ -N and NO_3^- -N respectively after 15 days). In contrast, when glucose was added, 16% of added NH_4^+ -N and 8% of added NO_3^- -N appeared to be immobilized, and most immobilization occurred within about 3 days of N application. This preliminary experiment indicates that this simple method could be used routinely to compare the N immobilization potentials of different soils. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Ammonium; Nitrate; Short-term immobilization; Grassland soil

Net rates of immobilization of NH_4^+ and NO_3^- by the soil microbial biomass have been measured under field conditions over long periods using ^{15}N techniques. For example, Puri and Ashman (1999) found that NH_4^+ showed twice the immobilization rate of NO_3^- on average in an acid woodland soil over 12 months under field conditions. The preferential microbial uptake of NH_4^+ rather than NO_3^- has also been demonstrated in several studies including Jackson et al. (1989); Recous and Mary (1990); Recous et al. (1992); Rice and Tiedje (1989); Wickramasinge et al. (1985). Puri and Ashman (1999) found a relatively low ratio of NH_4^+ to NO_3^- immobilization in their woodland soil and pointed out that this ratio is likely to vary among soil types. This paper describes a very simple laboratory incubation technique that could be used as a routine method for comparing the N immobilization potential of different soils and presents some results from a preliminary experiment on a temperate grassland soil.

A clay loam grassland soil (Typic Dystrachrept) with a pH (in H_2O) of 6.0, 0.34% N and 3.7% C overlying Silurian shale and greywacke was used. Soil was collected from the top 7.5 cm of the profile at the Agricultural Research Insti-

tute of Northern Ireland at Hillsborough, County Down (O.S. Grid Reference J 244577). Sixty sub-samples (50 g) of air dried sieved (<2 mm) soil were adjusted to 75% of water holding capacity (WHC) in 500 cm^3 polyethylene bottles and pre-incubated at 25 °C for 2 weeks with loose screw caps to allow gas exchange. Two aqueous solutions of inorganic N were prepared: 12.26 g l^{-1} 99.7 atom % ($^{15}\text{NH}_4$) $_2\text{SO}_4$ and 18.25 g l^{-1} 99.4 atom % K^{15}NO_3 . The solutions were also prepared containing 31.64 g l^{-1} D-glucose, giving a total of four solutions. A 5-ml aliquot of each solution was applied evenly by pipette to the surface of 15 replicate sub-samples of pre-conditioned soil. Thus NH_4^+ -N and NO_3^- -N were applied at 27 mg per 100 g of soil with or without 130 mg C (as glucose) per 100 g. This addition of N was selected to be approximately equivalent to an application rate of 200 kg N ha^{-1} , the rate equivalent on average to herbage offtake in cut grass at the sampling site.

The N and N + C solutions were applied evenly to the surfaces of the soil samples by pipette and the soils were then adjusted to WHC by surface application of deionized water to transport the added C and N into the soil. The soils were incubated at 25 °C for 0, 1, 3, 7 or 15 days when triplicate sub-samples were removed and re-adjusted to WHC. Exchangeable N was extracted by adding 2 M KCl (125 ml) to each sample and shaking for 30 min. The suspensions were centrifuged for 30 min at 1000 rpm at

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Fig. 1. Percent recovery of added N in soil residue following KCl extraction at different times from N addition during incubation at 25°C. Treatments: ■, NH₄⁺; □, NH₄⁺ + C; ●, NO₃⁻; ○, NO₃⁻ + C. Bars: standard error of the mean.

0–5°C. Extraction and centrifugation was repeated twice to give three KCl extractions in total to ensure complete removal of inorganic N (Widmer et al., 1989). The residues from the centrifugation stage were immediately blast frozen and freeze dried to prevent further biological or chemical changes. The dried residues were ball-milled to pass a 150 µm sieve and total N was determined by dry combustion using a Carlo Erba Model 1500 Nitrogen Analyzer and N isotope ratio was determined using a VG Micromass Model 622 mass spectrometer. No attempt was made to determine gaseous losses of applied N from the soil. The study was restricted to immobilization of applied N.

The percent recovery of added N in the soil residues is shown in Fig. 1. As expected, the pre-conditioning incubation of the soil had allowed microbial activity to utilize substrates released as a result of disturbance during the setting up of the sub-samples, and thus the aliquots not amended with glucose showed very little immobilization of added N (5% and under 2% of added NH₄⁺-N and NO₃⁻-N respectively after 15 days). In contrast, when glucose was added as C substrate, 16% of added NH₄⁺-N and 8% of added NO₃⁻-N were immobilized, and most immobilization occurred within about 3 days of N application. Thus NH₄⁺ showed twice the immobilization rate of NO₃⁻ when C substrate was available for microbial activity, a ratio in accord with those of Puri and Ashman (1999) in an acid temperate woodland soil (pH 3.9) but lower than those reported in other studies (Recous and Mary, 1990; Recous et al., 1992).

The small recoveries of added NH₄⁺ and NO₃⁻ immobilized in the absence of added glucose (5 and <2% respectively) may represent non-microbial fixation to give

nonexchangeable NH₄⁺ (Mulvaney, 1996) and abiotically immobilized NO₃⁻ (Azhar et al., 1986). These could perhaps be regarded as control values that could be subtracted from the results with glucose amendment to give a more accurate estimate of microbial immobilization. With some further development this relatively simple method could be used to compare the immobilization ratios of NH₄⁺ to NO₃⁻ in a wide range of soils under standardized conditions without the complications and assumptions required for ¹⁵N pool dilution techniques. Such studies on a wide range of soils could be combined with more detailed studies on a more restricted range of soils under field conditions. It is important to determine this ratio because N fertilizer use efficiencies will be influenced by the preferential uptake and storage of NH₄⁺ by the soil microbial biomass. Portions of the residue after KCl extraction could be subjected to acid hydrolysis and partitioned into various fractions of organic N (Stevenson, 1996) to give additional information on the forms of organic N into which microbially immobilized N has been incorporated.

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