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Abstract

We compared gross N fluxes by ^{15}N pool dilution in a coarse-textured agricultural soil when ^{15}N was applied to the soil NH_4^+ pool by either: (i) mixing a $^{15}NH_4NO_3$ solution into disturbed soil or (ii) injection of $^{15}NH_3$ gas into intact soil cores. The two techniques produced similar results for gross N mineralization rates indicating that NH_4^+ production in soil was not altered by soil disturbance, method of application (gas vs. solution), or amount of N applied. This was not the case for immobilization rates, which were twofold higher when ^{15}N label was applied to the soil NH_4^+ pool with the mixing technique compared to the injection technique. This was attributed to the fact that more NH_4^+ was applied with the mixing technique. Estimates of gross nitrification were accompanied by large error terms meaning differences between ^{15}N labeling methods could not be accurately assessed for this process rate.
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One assumption in the measurement of gross N flux rates (mineralization, immobilization and nitrification) in soil, using ^{15}N pool dilution, is uniformity in labeling of the soil NH_4^+ pool with ^{15}N (see review Murphy et al., 2003). In reality this is nearly impossible to achieve due to the spatial heterogeneity in both the applied ^{15}N but also the indigenous soil NH_4^+ pool, microbial community and location of soil organic matter (Luxhøi et al., 2004b). To achieve relatively uniform distribution of ^{15}N in soil, $^{15}NH_4^+$ solutions can be sprayed by use of an atomizer onto the soil followed by mixing (Recous et al., 1995). However, this technique introduces both soil disturbance and water addition as factors in the experimental design. Use of a multiple-needle injection apparatus to label the soil NH_4^+ pool via delivery of $^{15}NH_3$ gas (Murphy et al., 1997),

has been less widely used but has the advantage of not adding water to the soil and also minimizing soil disturbance. The aim of this study was to compare gross N flux rates (mineralization, immobilization, nitrification) from a coarse-textured agricultural soil where the soil NH_4^+ pool was enriched via either (i) use of an atomizer to spray $^{15}NH_4NO_3$ solution and then mixing the soil or (ii) the $^{15}NH_3$ gas injection system.

Soil was collected from an agricultural trial located 14 km west of the town of Moora, Western Australia. The top 10 cm of soil (typic Xeric Psamment) was sandy in texture (94% sand, 4.5% clay, 1.5% silt), contained 0.06% N, and 0.66% C, and had a pH (0.01 M $CaCl_2$) of 5.5. Intact soil cores ($d = 5.5$ cm) were collected soon after harvest (December 1999) of wheat, canola or lupine by pressing 12 cm long sharpened galvanized pipe 10 cm into soil. In the laboratory, soil was adjusted to soil water potentials of -0.05 MPa (10% w/w soil water content) and pre-incubated for 24 h at 30 °C. In the case of

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the spraying technique, four replicate soil cores from each field plot were segmented into layers (0–2.5, 2.5–5.0, 5.0–10.0 cm). Soil from each depth section of each replicate was mixed, and a sub-sample of soil (70 g on dry weight basis) was spread thinly in a tray placed on an electronic balance and then sprayed with a $^{15}NH_4NO_3$ solution (until the correct weight of addition was achieved 15 mg NH_4^+-N kg^{-1} soil enriched with 5 atom% ^{15}N). Soil was then thoroughly remixed before it was divided equally between two glass vessels that were subsequently incubated at 30 °C. After 2 h (t_0), or a further 24 h (t_1) one vessel from each depth section of each replicate was removed from the incubator, and 20 g of moist soil was sub-sampled from the vessel. In the case of the $^{15}NH_3$ injection technique (see Murphy et al., 1997 for details) eight replicate soil cores from each plot were individually injected with a mixture of $^{15}NH_3$ gas and air so as to apply 4 mg NH_4^+-N kg^{-1} soil at 5 atom % ^{15}N. Four of the injected soil cores were segmented into layers (0–2.5, 2.5–5.0, 5.0–10.0 cm) at t_0, while the remaining four soil cores were segmented into the same layers at t_1. After sub-sampled soil was shaken with 80 ml 1 M KCl for 1 h, the soil plus extract was filtered under suction using Macherey-Nagel 615 round-filters placed into Buchner flasks. As suggested by Recous et al. (1999), any inorganic N remaining in the soil on the filter was removed by washing an additional two times with 1 M KCl and then a further two times with deionised water. A sub-sample of the extracted soil was retained for ^{15}N analysis. The remainder of extracted soil was used to measure soil water content (application of N-solution increased soil water content from 10.0 to 11.5% (w/w), corresponding to a change in water potential from -0.05 to -0.01 MPa). The ^{15}N enrichment of the NH_4^+-N and NO_3^--N pools in KCl extracts were determined after diffusion (Brooks et al., 1989) with modification (Sparling et al., 1996), and subsequent analysis using a VG-Micromass Sira-10 mass-spectrometer connected to the output of an Europa Roboprep C–N analyzer. Remaining organic-^{15}N and total organic-N in the residual soil after KCl extraction was measured on the mass-spectrometer.

Gross mineralization, gross immobilization and gross nitrification rates were calculated using the numerical model FLUAZ (Mary et al., 1998). With this model, numerical solutions of partial differential equations describing the N and ^{15}N fluxes between NH_4^+-N, NO_3^--N, organic-N and biomass-N are combined with a non-linear fitting method that searches for rate parameters giving the best approximation of modeled pools to measured values. Normalized rates of gross N immobilization were expressed in terms of apparent NH_4^+ availability, including the initial NH_4^+ content, added NH_4^+ and NH_4^+ produced during gross N mineralization (Eq. (1)). All results were calculated on the basis of oven dry (105 °C) soil. The data was subjected to a three-way weighted analysis of variance to test for difference between techniques, soil depth and crop residues

(Luxhøi and Brockhoff, 2004).

Normalised immobilization

$$= \frac{\text{immobilization}}{NH_4^+ \text{ content} + NH_4^+ \text{ applied} + \text{mineralization}} \quad (1)$$

Error terms for the ^{15}N enrichment at t_0 of the NH_4^+ and NO_3^- pools were on average 50% higher for the injected soil than for the sprayed and mixed soil (data not presented). The average ^{15}N recovery was 96% for the mixing technique and 78% for the injection technique. The unaccounted for ^{15}N, particularly in the case of the injection technique (22% of injected ^{15}N) is assumed to have been lost through ammonia volatilization. The coefficient of variance for gross N transformation measurements was reduced, on average, from 30 to 10% with the use of the mixing technique compared to the injection technique (data not shown). This outcome is attributed to the sub-sampling of soil for t_0 and t_1 samples from a common soil core in the case of the mixing technique, but from different soil cores in the case of injection technique.

Although the two ^{15}N labeling techniques differed in five main points, namely dry (gas) vs. wet (solution) application, the form of N applied, the amount of NH_4^+ applied, the homogeneity of distribution, and the degree of soil disturbance, it is notable that the two labeling techniques resulted in similar estimates of gross N mineralization rates (Fig. 1a). In contrast, estimates of gross N immobilization were significantly affected by the technique used, with an at least twofold higher rate of N immobilization, estimated using the mixing technique compared to the injection technique (Fig. 1b). The high rates of gross N immobilization, obtained using the mixing technique compared to the injection technique, gave estimates of net N immobilization (gross immobilization ≥ gross mineralization) that are likely artificial, since unpublished in situ measurements from the experimental site show net mineralization.

It is unlikely that the dry (gas) vs. wet (solution) application, the form of N applied, the homogeneity of distribution, and the degree of soil disturbance would account for the twofold higher immobilization rates, particularly since these factors did not significantly affect gross N mineralization measured by either labeling method (Fig. 1). However, since NH_4^+ is substrate for immobilization, the higher rate of NH_4^+-application used in the mixing technique could potentially have stimulated immobilization rate compared to the injection technique (Recous et al., 1995; Luxhøi et al., 2004a). To evaluate the effect of substrate concentration on gross N immobilization, normalized immobilization rates were expressed in terms of apparent NH_4^+ availability (Eq. (1)). The normalized rates were largely aligned along the 1:1 line (Fig. 1d), but with a poor regression coefficient ($r^2 = 0.2$), indicating that the variability of the factors on the right hand side in Eq. (1), was transferred on to the normalized immobilization.
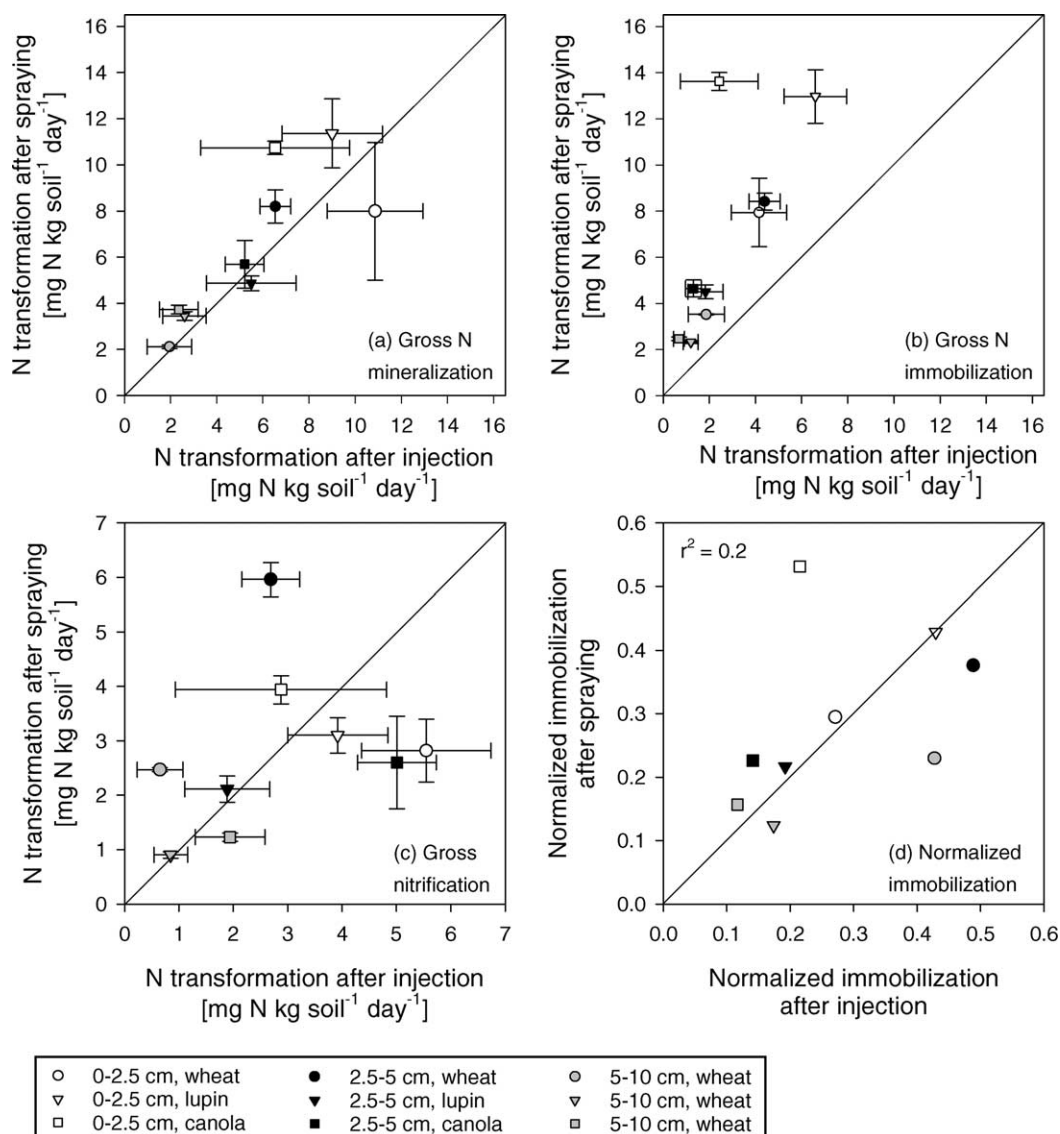
Fig. 1. (a) Gross N mineralisation rates, (b) gross N immobilisation rates and (c) gross nitrification rates, measured by $^{15}NH_3$ gas-injection vs. $^{15}NH_4^+$ mixing technique and (d) normalised gross N immobilisation, measured by $^{15}NH_3$ gas-injection vs. $^{15}NH_4^+$ spraying technique. Normalised gross N immobilisation is calculated as immobilisation rate per unit of available ammonium, by Eq. (1). r^2-value gives the coefficient for a linear regression. Error bars are standard errors.

The larger amount of NH_4^+ added to soil with the mixing technique, compared with the injection technique, was expected to produce higher estimates of gross nitrification with the mixing method because NH_4^+ is substrate for nitrification. However, in this study gross nitrification estimates were unaffected by the technique used. It is notable that the effect of labeling technique on measured nitrification rate was inconsistent across and within soil depths for samples with different crop history (Fig. 1c). Estimates of gross nitrification for each soil depth treatment also had large error terms that possibly obscured differences in nitrification expected between the two techniques.

In conclusion, the mixing technique and the injection technique used to add $^{15}NH_4^+$ to soil in ^{15}N dilution studies of gross N transformation gave comparable estimates of gross N mineralization but twofold differences in estimates of gross N immobilization, probably due to higher NH_4^+ application rate with the injection technique. No conclusion can be drawn on the relative merit of the mixing technique compared to the injection technique to measure gross nitrification.

Acknowledgements

References

Brooks, P.D., Stark, J.M., McInteer, B.B., Preston, T., 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Science Society of America Journal 53, 1707–1711.

Luxhøi, J., Brockhoff, P.B., 2004. Analysis of variance on gross nitrogen mineralization data. Soil Biology & Biochemistry 36, 735–736.

Luxhøi, J., Debosz, K., Elsgaard, L., Jensen, L.S., 2004a. Mineralization of nitrogen in Danish soils, as affected by short-, medium- and long-term annual inputs of animal slurries. Biology and Fertility of Soils 39, 352–359.

Luxhøi, J., Jensen, L.S., Nielsen, N.E., 2004b. Effect of soil heterogeneity on gross nitrogen mineralization measured by ^{15}N-pool dilution techniques Plant and Soil. 262; 263–275.

Mary, B., Recous, S., Robin, D., 1998. A model for calculating nitrogen fluxes in soil using ^{15}N tracing. Soil Biology & Biochemistry 30, 1963–1979.

Murphy, D.V., Fillery, I.R.P., Sparling, G.P., 1997. Method to label soil cores with ^{15}NH$_3$ gas as a prerequisite for ^{15}N isotopic dilution and measurement of gross N mineralization. Soil Biology & Biochemistry 29, 1731–1741.

Murphy, D.V., Recous, S., Stockdale, E.A., Fillery, I.R.P., Jensen, L.S., Hatch, D.J., Goulding, K.W.T., 2003. Gross nitrogen fluxes in soil: theory, measurement and application of ^{15}N pool dilution techniques. Advances in Agronomy 79, 69–118.

Recous, S., Robin, D., Darwis, D., Mary, B., 1995. Soil inorganic N availability: effect on maize residue decomposition. Soil Biology & Biochemistry 27, 1529–1538.

Recous, S., Aita, C., Mary, B., 1999. In situ changes in gross N transformations in bare soil after addition of straw. Soil Biology & Biochemistry 31, 119–133.

Sparling, G.P., Zhu, C., Fillery, I.R.P., 1996. Microbial immobilization of 15N from legume residues in soils of differing textures: Measurement by persulphate oxidation and ammonia diffusion methods. Soil Biology & Biochemistry 28, 1707–1715.