

Short communication

## Nitrogen mineralization and assimilation at millimeter scales

John B. Cliff<sup>a,c</sup>, Peter J. Bottomley<sup>a,b</sup>, Daniel J. Gaspar<sup>d</sup>, David D. Myrold<sup>a,\*</sup>

<sup>a</sup>Department of Crop and Soil Science, Oregon State University, Agriculture Life Science Building 3017, Corvallis, OR 97331, USA

<sup>b</sup>Department of Microbiology, Oregon State University, 220 Nash Hall, Corvallis, OR 97331, USA

<sup>c</sup>Advanced Radioanalytical Chemistry, Pacific Northwest National Laboratory, Richland, WA 99352, USA

<sup>d</sup>William R. Wiley Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352, USA

Received 25 February 2006; received in revised form 7 October 2006; accepted 10 October 2006

Available online 15 November 2006

### Abstract

This study used inoculated, artificial soil microcosms containing sand, clay, cellulose, and localized “hotspots” of highly labile, organic N-containing dead bacteria to study N mineralization and assimilation at millimeter scales. Labeling with  $^{15}\text{NH}_4^+$  along with measurement of its assimilation into microbial biomass at the bulk scale allowed estimation of gross rates of ammonification and N assimilation using isotope dilution. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) analyses of transects of organic- $^{15}\text{N}$  across Si wafers in contact with the microcosms indicated strong gradients of  $^{15}\text{NH}_4^+$  assimilation as a function of proximity to the hotspots that were not apparent using bulk analyses. This combination of bulk and ToF-SIMS analyses represents a powerful approach to explore the physical and biochemical factors that affect N process heterogeneities in soils.

© 2006 Elsevier Ltd. All rights reserved.

**Keywords:** Ammonification; Immobilization; Isotope dilution; Time-of-flight secondary ion mass spectrometry; SIMS; Nitrogen

Increased accessibility and affordability of  $^{15}\text{N}$  methods has led to insightful discoveries about the nature of N-cycling dynamics in soils. For instance, isotope dilution studies have shown that high rates of both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  assimilation may exist in the same soil volume (Schimel and Firestone, 1989; Davidson et al., 1990; Chen and Stark, 2000) despite the fact that  $\text{NO}_3^-$  assimilation is generally repressed in the presence of  $\text{NH}_4^+$  (Van't Riet et al., 1968; Betlach et al., 1981; Rice and Tiedje, 1989). This phenomenon has been hypothesized to be due to the presence of “microsite” heterogeneities in soils (Schimel and Firestone, 1989; Davidson et al., 1990; Chen and Stark, 2000). Because continuous flow combustion isotope ratio mass spectrometry (CF-IRMS) requires relatively large sample sizes, direct study of this phenomenon has been limited. We have used time-of-flight secondary ion mass spectrometry (ToF-SIMS; Vickerman and Briggs, 2001) in conjunction with  $^{15}\text{N}$  labeling to directly explore spatial heterogeneity of inorganic-N assimilation on Si wafer contact slides in direct contact with soil and model

soil systems. These studies showed that the factors controlling inorganic-N assimilation in soils have the potential to act at the scale of tens to hundreds of microns (Cliff et al., 2002). The objective of the present study was to explore the spatial relationship of  $^{15}\text{NH}_4^+$  assimilation and mineralization in microcosms constructed of an organo-mineral matrix and containing a region of highly labile organic N. We measured these processes at two different spatial scales: at the centimeter scale using bulk analyses and at the millimeter scale using ToF-SIMS.

Fig. 1 represents the basic experimental unit used in this study. Microcosms were constructed using rectangular Plexiglas<sup>®</sup> containers open on the top, and capped with 0.65  $\mu\text{m}$  Acetate Plus filters (Osmionics, Minnetonka, MN) on the bottom. The microcosms consisted of 0.25 g of dead *Pseudomonas fluorescens* cells (grown in nutrient broth, dried at 105 °C, and ground in a roller mill overnight) with a C:N ratio of 4.1 sandwiched between two layers (5.5 g) of an organomineral matrix containing 20% kaolin clay, 79% sand, and 1% C as cellulose (Fig. 1). A microbial inoculum was prepared by suspending 100 g of soil in 250 ml of cold Hoagland's pH 7.0 N-free medium (Hoagland and Arnon, 1950), centrifuging at 1000  $\times g$  for 10 min, and diluting the

\*Corresponding author. Tel.: 541 737 5737; fax: 541 737 5725.

E-mail address: David.Myrold@oregonstate.edu (D.D. Myrold).

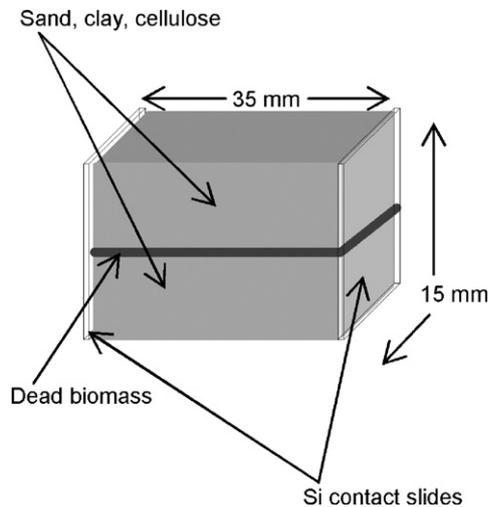


Fig. 1. Schematic of experimental microcosm showing the model soil including organic, N-rich layer in contact with Si wafers. The wafers preserved spatial heterogeneity of organic  $^{15}\text{N}$  signatures for ToF-SIMS analyses.

supernatant to an  $\text{OD}_{600}$  of 0.1 with more Hoagland's medium. Aliquots of the microbial suspension (4 ml), supplemented with  $\text{NO}_3^-$  and 99 atom%  $^{15}\text{NH}_4^+$  to a final concentration of 1 mM N from each inorganic-N source (about  $11 \mu\text{g NH}_4^+ \text{-N g}^{-1}$  dry soil), were added to the microcosms. The microcosms were equilibrated to  $-0.1$  MPa on a ceramic pressure plate in a  $4^\circ\text{C}$  cold room overnight. This resulted in a mean water content of  $11.99 \pm 0.35\%$  (mean  $\pm$  one standard error,  $n = 15$ ).

Triplicate microcosms were sampled before incubation at room temperature and the 12 remaining microcosms were placed in 0.25 l canning jars, over but not in contact with, a 0.02 M NaCl solution that possessed a vapor pressure compatible with keeping the soil microcosms at a water potential of  $-0.1$  MPa (Harris, 1981). Lids were placed on the jars and the samples were incubated at room temperature. Triplicate microcosms were sacrificed daily and the remaining microcosms were opened to flush the headspace. Samples were cross-sectioned so that each subsample contained upper and lower mineral portions as well as the labile N source. Approximately, 5 g portions were extracted in 25 ml of 0.5 M  $\text{K}_2\text{SO}_4$ , and 1 g saved for water content determination. Bulk  $\text{NH}_4^+$  was analyzed colorimetrically with an autoanalyzer (Astoria-Pacific, Inc., Clackamas, OR) using the salicylate–hypochlorite method described by the manufacturer. Isotope abundance of the bulk soil  $^{15}\text{NH}_4^+$ , was measured using the diffusion technique (Brooks et al., 1989) followed by analysis using a Europa 20-20 CF-IRMS (PDZ Europa, Cheshire, UK). The equations of Kirkham and Bartholomew (1954) were used to estimate N-process rates, with standard errors calculated by propagation of error (Luxhøi and Brockhoff, 2004). A further subsample of each microcosm was used for estimating organic- $^{15}\text{N}$  content. Samples of microcosm contents (1 g) were washed in 25 ml of 2 M KCl and

centrifuged at  $10\,000 \times g$  for 10 min three times followed by three washes in distilled deionized  $\text{H}_2\text{O}$ . The samples were ground in a roller grinder overnight and analyzed for organic- $^{15}\text{N}$  content by CF-IRMS. One-way ANOVA and LSD tests ( $\alpha = 0.05$ ) were used to determine significant changes in bulk  $\text{NH}_4^+$  concentrations and organic- $^{15}\text{N}$  content.

One Si contact slide from each sample container was analyzed in transects across the slide using a TRIFT-II ToF-SIMS (Physical Electronics International, Eden Prairie, MN). Analysis areas ( $200 \times 200 \mu\text{m}$ ) were presputtered before analyzing  $100 \times 100 \mu\text{m}$  areas in the center of the sputter crater as reported previously (Cliff et al., 2002). Analyses were performed every  $500 \mu\text{m}$  so that the portion of the slide in contact with the labile N source was approximately in the middle of the transect. Organic- $^{15}\text{N}$  abundance was estimated by measuring  $\text{CN}^-$  isotopes of nominal masses 26 and 27 using a modification of the SS algorithm described by Cliff et al. (2004), in which the  $^{27}\text{Al}$  signal was deconvolved from the  $^{27}\text{CN}^-$  signal by fitting a Pearson four-parameter model (PeakFit<sup>®</sup> User's Manual, 2002, SYSTAT Software Inc., Richmond, CA).

Results from remaining bulk N analyses are presented in Figs. 2A and B. Bulk  $\text{NH}_4^+$  content was initially about  $40 \mu\text{g NH}_4^+ \text{-N g}^{-1}$  dry soil, increasing significantly between days 1 and 2 and between days 2 and 3, and stabilizing at about  $190 \mu\text{g NH}_4^+ \text{-N g}^{-1}$  dry soil. The high initial  $\text{NH}_4^+$  content indicates that either significant  $\text{NH}_4^+$  was present

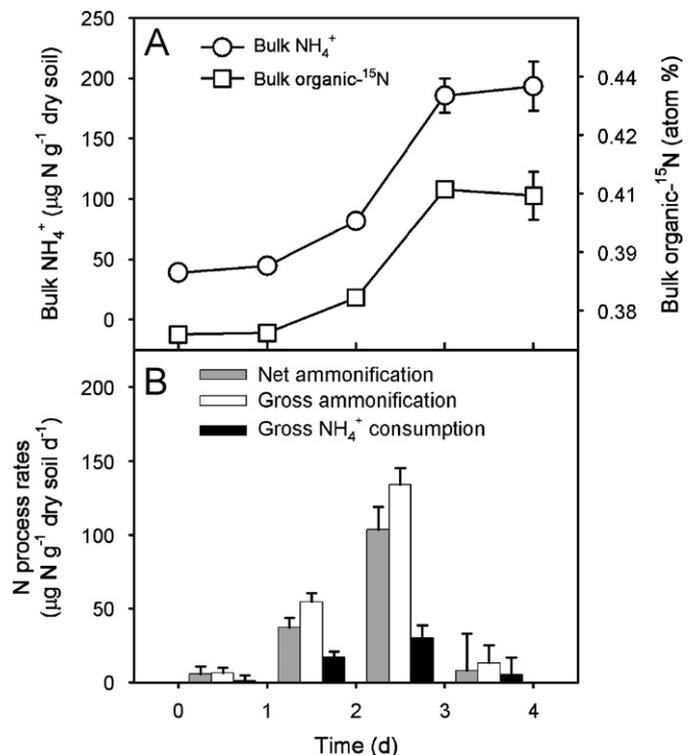


Fig. 2. (A) Bulk  $\text{NH}_4^+$  and bulk organic- $^{15}\text{N}$ , and (B) net ammonification, gross ammonification, and gross  $\text{NH}_4^+$  consumption as a function of incubation time. Error bars represent one standard error of the mean.

in the soil extract used as inoculum or that significant N mineralization had occurred during the period of moisture equilibration at 4 °C. The isotope ratio of the material left after removing inorganic N showed that significant amounts of  $^{15}\text{NH}_4^+$  were assimilated by the microbial biomass during the final 3 days of incubation (Fig. 2A) despite being accompanied by high rates of mineralization (Figs. 2A and B). Fig. 2B presents the rate estimates for net and gross ammonification and gross  $\text{NH}_4^+$  consumption. The rates of N mineralization are one to two orders of magnitude higher than those typically reported in the literature for natural soils (Booth et al., 2005). This is no doubt a consequence of the artificial experimental setup. Nevertheless, despite this rapid N mineralization,  $^{15}\text{NH}_4^+$  assimilation was detectable in the biomass N. The amount of  $^{15}\text{N}$  recovered in the biomass was consistent with the amount of  $^{15}\text{N}$  lost from the  $\text{NH}_4^+$  pool and to the cumulative N immobilization calculated from the gross  $\text{NH}_4^+$  consumption rates. Further, assuming that  $\text{NH}_3$  volatilization and abiotic  $\text{NH}_4^+$  consumption were negligible components of the total consumption rate, the quantity of inorganic N assimilated ranged from 12% to 40% of the quantity of  $\text{NH}_4^+$  liberated by mineralization (Fig. 2B).

Isotope tracer and dilution methods have advanced our understanding of nutrient cycling in soils. Nevertheless, the ToF-SIMS transect data reveal some of the limitations of the bulk methods. Fig. 3 presents examples of transects of organic- $^{15}\text{N}$  ratios acquired using ToF-SIMS analyses of Si wafers in contact with the soil microcosms. In each case, locations of natural abundance organic-N content exist in proximity to the N-rich organic layer, which is centered at  $Y = 0.0$  mm. As incubation time increased, steep gradients in the atom%  $^{15}\text{N}$  of organic N appeared within millimeters of the organic-N-rich hotspot, presumably associated with locations of increased  $^{15}\text{NH}_4^+$  assimilation relative to mineralized N. It is also interesting to note that in all transects analyzed, locations existed with higher organic- $^{15}\text{N}$  ratios than the values for bulk biomass presented in Fig. 2A. This is a consequence of the fact that the organic- $^{15}\text{N}$  content estimated by CF-IRMS presented in Fig. 2B reflects a spatial average that includes a high quantity of natural abundance  $^{15}\text{N}$  localized near  $Y = 0.0$  mm and a relatively small quantity of highly labeled organic  $^{15}\text{N}$  in close spatial proximity.

We have presented early results from a novel combination of experimental techniques that explore N processes at differing scales. These data serve to emphasize the ability of ToF-SIMS to analyze microsite heterogeneities of N assimilation in soil processes and the variable interpretations of soil process data possible when these data are acquired at differing scales. The potential exists to manipulate the physical and biochemical properties of these microcosms and to use ToF-SIMS in conjunction with bulk analyses to examine the impact of these variables on the spatial scale and relative rates of mineralization and immobilization of soil N.

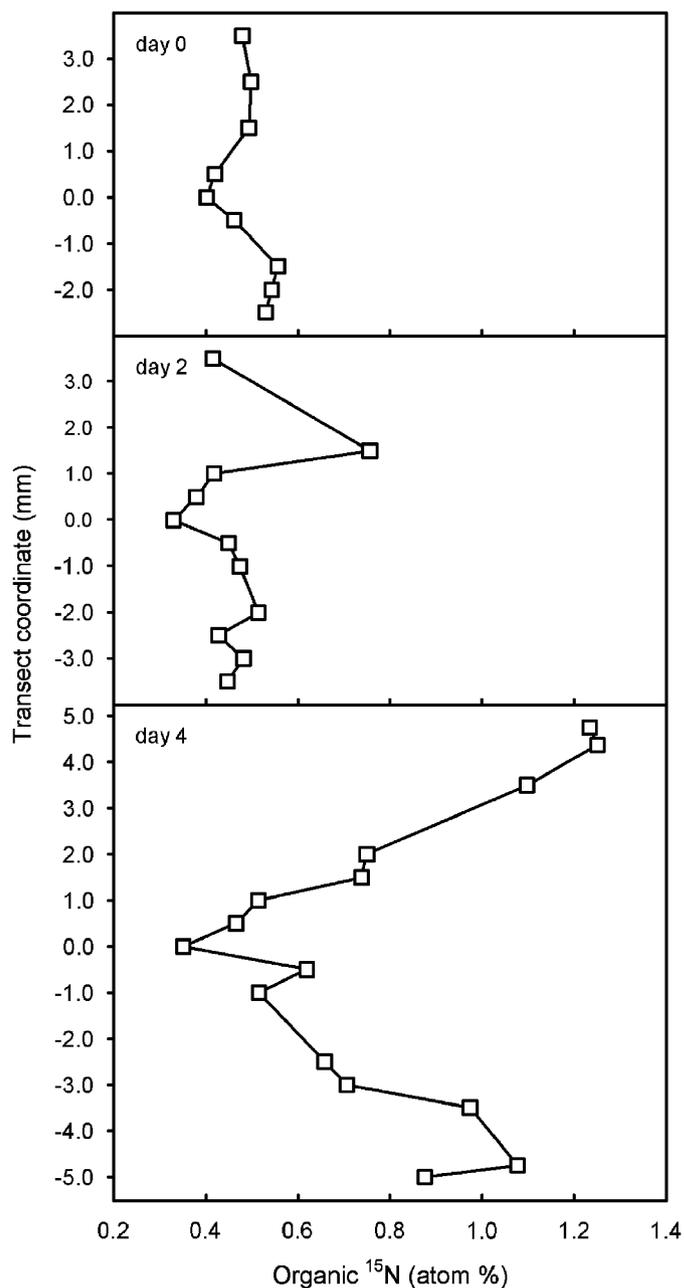


Fig. 3. Examples of ToF-SIMS organic- $^{15}\text{N}$  isotope ratio analyses of transects from Si wafers in contact with the experimental microcosms. Transects shown were taken from microcosms sacrificed at days 0, 2, and 4. Zero on the ordinate indicates the approximate center point of the highly labile organic-N source. Data points are plotted at four times the actual scale of the analyzed areas.

This research was supported by a grant from the Soils and Soil Biology program of USDA National Research Initiative. A portion of the research described in this paper was performed in the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory. We thank Matt Pohl for laboratory assistance and Vanessa Bailey for review comments.

## References

- Betlach, M.R., Tiedje, J.M., Firestone, R.B., 1981. Assimilatory nitrate uptake in *Pseudomonas fluorescens* studied using nitrogen-13. *Archives of Microbiology* 129, 135–140.
- Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on gross nitrogen cycling rates in terrestrial ecosystems: a synthesis and analysis of data from the literature. *Ecological Monographs* 75, 129–157.
- Brooks, P.D., Stark, J.M., McInteer, B.B., Preston, T., 1989. Diffusion method to prepare extracts for automated nitrogen-15 analysis. *Soil Science Society of America Journal* 53, 1707–1711.
- Chen, J., Stark, J.M., 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. *Soil Biology & Biochemistry* 32, 47–57.
- Cliff, J.B., Gaspar, D.J., Bottomley, P.J., Myrold, D.D., 2002. Exploration of inorganic C and N assimilation by soil microbes with Time-of-flight secondary ion mass spectrometry. *Applied and Environmental Microbiology* 68, 4067–4073.
- Cliff, J.B., Gaspar, D.J., Bottomley, P.J., Myrold, D.D., 2004. Peak fitting to resolve  $CN^-$  isotope ratios in biological and environmental samples using ToF-SIMS. *Applied Surface Science* 231–232, 912–916.
- Davidson, E.A., Stark, J.M., Firestone, M.K., 1990. Microbial production and consumption of nitrate in an annual grassland. *Ecology* 71, 1968–1975.
- Harris, R.F., 1981. Effect of water potential on microbial growth and activity. In: Parr, J.F., Gardner, W.R., Elliot, L.F. (Eds.), *Water Potential Relations In Soil Microbiology*. Soil Science Society of America, Madison, WI, pp. 23–95.
- Hoagland, D.R., Arnon, D.I., 1950. The water culture method for growing plants without soil. *California Agricultural Experiment Station Circular 347*, College of Agriculture, University of California, Berkeley, CA, 32pp.
- Kirkham, D., Bartholomew, W.V., 1954. Equations for following nutrient transformations in soil, utilizing tracer data. *Soil Science Society of America Proceedings* 18, 33–34.
- Luxhoi, J., Brockhoff, P.B., 2004. Analysis of variance on gross nitrogen mineralization data. *Soil Biology & Biochemistry* 36, 735–736.
- Rice, C.W., Tiedje, J.M., 1989. Regulation of nitrate assimilation by ammonium in soils and in isolated soil microcosms. *Soil Biology & Biochemistry* 21, 597–602.
- Schimmel, J.P., Firestone, M.K., 1989. Nitrogen incorporation and flow through a coniferous forest soil profile. *Soil Biology & Biochemistry* 53, 779–784.
- Van't Riet, J., Stouthammer, A.H., Plant, R.J., 1968. Regulation of nitrate assimilation and nitrate respiration in *Aerobacter aerogenes*. *Journal of Bacteriology* 96, 1455–1464.
- Vickerman, J.C., Briggs, D., (Ed.). 2001. *ToF-SIMS: Surface Analysis by Mass Spectrometry*. IM Publications, Chichester, UK, 789pp.