Fast nitrate immobilization in N saturated temperate forest soils

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Abstract

Recent application of ^{15}N pool dilution techniques has suggested that gross nitrate immobilization rates in temperate forest soils with low N deposition may be significantly greater than previously thought. In contrast, there are some data that suggest forest soils, which have received high N-deposition, may not immobilize NO_3^-. Such studies do not include quantification of the relative importance of fast and slow immobilization of NO_3^-. We have examined the kinetics of NO_3^- immobilization in two temperate forest soils exposed to a range of experimental N deposition. We found that the greatest potential for NO_3^- immobilization in these soils was by a fast process of immobilization and only the hardwood forest with low (ambient) N deposition showed any significant slow NO_3^- immobilization (typically equated with microbial immobilization). High N additions have resulted in the loss of the slow immobilization process and a reduction in the amount of fast immobilization. The patterns of NO_3^- immobilization we report are important for two reasons. First, they demonstrate that immobilization of N, which exhibits rapid kinetics, may play an important part in regulating the N retention capacity of forests in response to N deposition. Second, they suggest that current models which include only the slower phase of N immobilization may be inaccurate representations of N immobilization processes in soils. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

The deposition of nitrogen on terrestrial ecosystems has the potential of altering patterns of productivity and nutrient cycling, in many cases not for the better (Aber et al., 1989, 1998; Wedin and Tilman, 1996). Negative effects are increasingly linked to the production, retention and loss of the mobile anion NO_3^-. While differences in land use history and community type appear to have strong effects on the sensitivity of terrestrial ecosystems to N deposition (Aber et al., 1995, 1998), the mechanisms of N retention which play a crucial role in regulating this sensitivity are not well understood. Studies in Europe and North America have shown that long-term immobilization of N in soils plays an essential role (Magill et al., 1997; Tietema et al., 1998). Microbial immobilization (Myrold and Tiedje, 1986; Paul and Clark, 1996) and abiotic fixation (Nommik and Vahtras, 1982; Schimel and Firestone, 1989; Strickland et al., 1992; Vitousek and Matson, 1985) of ammonium (NH_4^+) in soils have been well documented. While microbial immobilization of NO_3^- has been documented (Davidson et al., 1992; Stark and Hart, 1997), abiotic immobilization of the nitrite formed during nitrification (via nitrosation reactions) is the only known abiotic mechanism for NO_3^- immobilization (Azhar et al., 1986) and its significance in the field has not been demonstrated. Nonetheless, NO_3^- may comprise half or more of the N deposited on terrestrial ecosystems (Lovett, 1994) and may be produced by nitrification in N enriched stands.

Rapid internal cycling rates of NO_3^- within soils represents a potentially important pathway for incorporation of NO_3^- into soil organic matter (SOM). Using pool dilution methods, a growing number of studies in forest ecosystems with low N deposition have documented high rates of gross nitrification and NO_3^- immobilization, even though nitrate concentrations were

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low and net fluxes negligible (Davidson et al., 1992; Hart et al., 1994a; Stark and Hart, 1997). A notable exception to this pattern are some recent measurements in soils from mesic coniferous forests in Europe experiencing moderate to high rates of N deposition where no significant immobilization of NO_3^- was observed (Tietema, 1998) and therefore no evidence of sequestration of NO_3^- in soils. Together, these studies suggest that forest conditions (as affected by N deposition) may lead to significant alterations in NO_3^- immobilization.

However, neither of these groups of studies include quantification of the relative importance of fast and slow immobilization of NO_3^-. There can be a rapid phase of immobilization immediately following the addition of ^{15}N tracers to soils (Davidson et al., 1991) and standard methods remove this phase from characterizations of immobilization kinetics (Hart et al., 1994b). We present a detailed analysis of the kinetics of NO_3^- immobilization in two temperate forests which have received long-term (11 yr) of experimental N deposition. Using these data we demonstrate the importance of including fast kinetics of NO_3^- immobilization when analyzing forest potential for total N retention, the relative significance of fast versus slow immobilization processes and how these patterns are altered with long-term N deposition.

2. Materials and methods

Our study took place within the experimental chronic N deposition plots, which consist of a native mixed hardwood and plantation pine forests, at the Harvard Forest, Petersham, MA, USA (42°30′ N, 72°10′ W). Plots within each forest type have received 11 yr of continuous experimental N amendments of 0 (control), 5 (low) and 15 (high) g N m^{-2} yr^{-1} as NH_4NO_3 (Aber et al., 1993; Magill et al., 1997) (in addition to a background N deposition rate of 0.8 g m^{-2} yr^{-1}) (Ollinger et al., 1993). The two forest community types have shown considerable differences in their response to N deposition. The pine forest (dominated by *Pinus resinosa*) receiving 15 g N m^{-2} yr^{-1} is losing significantly more NO_3^- through groundwater leaching than the hardwood forest (dominated by *Quercus velutina*, *Q. rubra*, *Betula lenta*, *Acer rubrum* and *Fagus grandifolia*) receiving the same experimental N amendment. Neither forest is losing any NO_3^- via leaching in the control stands.

We quantified the dynamics of NO_3^- immobilization at three different times in September 1997, in the control and high N stands in each forest type. At each time, we took a set of six cores per stand (24 cores per collection). Cores were taken using sharpened PVC pipes measuring 3 cm (inner diam) by 15 cm (long).

Each individual set of six cores were taken from a single location, free of large tree roots and rocks, to minimize variability between the cores. However, sets of cores were taken from different locations within each experimental plot on different days. To inject the tracer, 4.8 ml of a 2.5 mM 98% ^{15}N KNO_3 solution was injected into 5 of each set of 6 cores with 6 individual injections using a 7.5 cm needle (three injections from each side of the core).

Following injection of the tracer, cores were capped and then placed within sand-filled tubs, which were buried just outside of the experimental plots. At fixed intervals of 0.25, 0.75, 2.75, 7.75 and 24 h following the injection of the tracer, soil cores were harvested. This sequence of sampling times was chosen to increase the precision with which nitrate immobilization could be separated into very rapid (< 1 h) and longer-term (< 1 d) processes. Harvesting soil cores consisted of extruding soil from the pipes, weighing it and then homogenizing it. From the homogenized soil, subsamples were taken for extraction in 2 M KCl (1:10 soil wet weight to extract volume) and determination of gravimetric water content. KCl extracts were placed on a shaker table for 24 h and then filtered. NH_4^+ and NO_3^- concentrations were measured using standard colorimetric methods (Maynard and Kalra, 1993) and ^{15}N–NO_3^- was measured using a diffusion procedure (Sørensen and Jensen, 1991) modified to achieve high recovery rates and thus accurate measurement of ^{15}N abundance (Sigman et al., 1997). Determination of ^{15}N abundance was performed using a Europa Scientific ANCA-sl coupled to a model 20-20 Stable Isotope Analyzer (Europa Scientific, Vandalia, OH).

One of the most important methodologies now used to characterize in situ gross N transformation rates is the pool dilution method (Hart et al., 1994b), a simple means of deriving gross production and consumption of NH_4^+ and NO_3^-. The method involves three steps: (1) adding ^{15}N enriched inorganic N (NH_4^+ or NO_3^-) to the soil; (2) estimating the amount of ^{15}N enrichment and pool size of the labeled pool at two times; and (3) using an analytical solution of a zero-order model (Hart et al., 1994b; Kirkham and Bartholomew, 1954) to derive gross N flux rates. A critical assumption within this approach is that actual N transformation rates exhibit zero-order kinetics. The zero-order kinetics of tracer loss can be described using a simple exponential decay function:

$$r = e^k \qquad (1)$$

where r is the fraction of ^{15}N–NO_3^- remaining and k is the rate constant for the immobilization process.

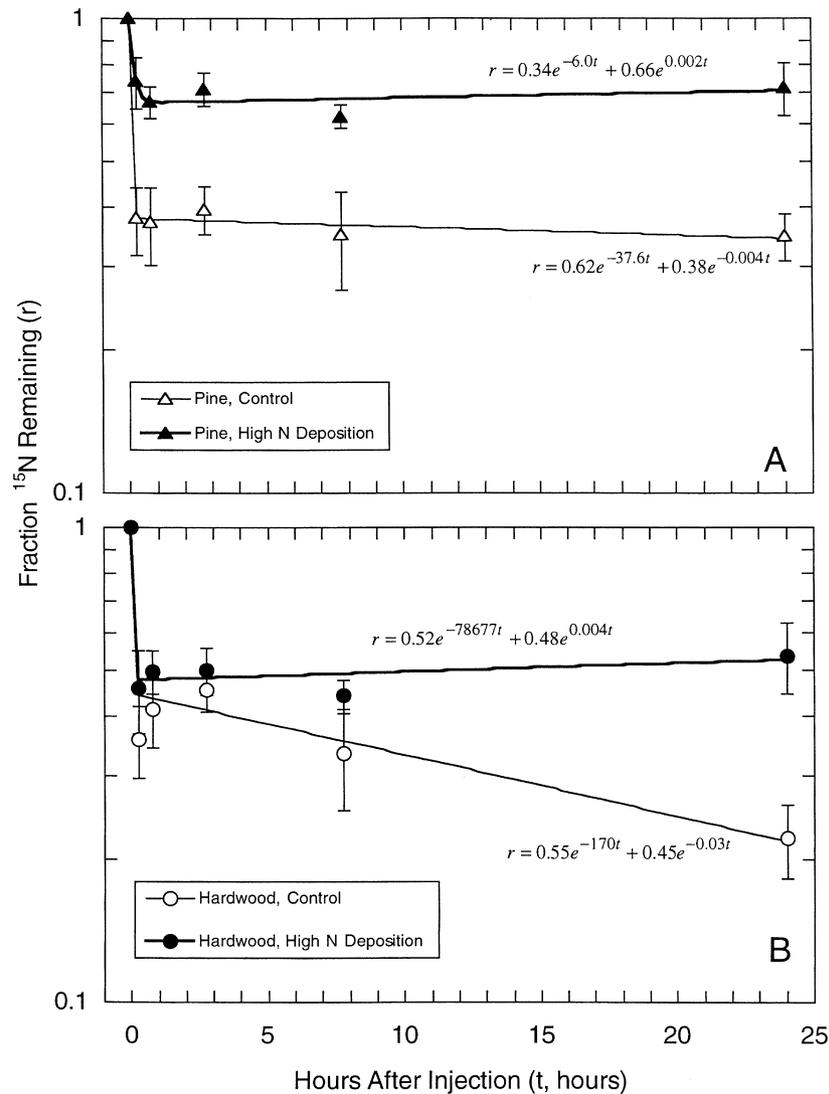It has been well-documented that immediately following the addition of ^{15}N tracers, a significant

Fig. 1. Kinetics of ^{15}N–NO$_3^-$ immobilization in field-exposed, intact soil cores at the Harvard Forest. The curves drawn for each forest by N-deposition combination are best-fit double exponential curve fits (Eq. (2)). Curve fits were performed on the raw data, not mean values for each time point ($n = 18$ per curve fit). Each point in the Fig. represents the mean of three replicate measurements ($\pm$one standard error of the mean). A = Conifer forest soil. B = Hardwood forest soil.

amount of tracer is rapidly immobilized (Davidson et al., 1991). Standard procedures for using ^{15}N tracers to quantify short-term internal cycling rates of N attempt to remove this initial immobilization because (1) it is thought to be the result of abiotic processes and (2) subsequent dynamics of microbial immobilization are assumed to follow zero order kinetics (Hart et al., 1994b). We suggest that removing the initial rapid phase of N immobilization from analyses of internal N cycling in soils may lead to an incomplete understanding of the potential for N immobilization in soils. Further, we demonstrate the utility of modeling tracer both fast and slow kinetics of immobilization using a double-exponential decay function:

$$r = P_a e^{k_a t} + P_b e^{k_b t} \qquad (2)$$

where r is the fraction of ^{15}N–NO$_3^-$ remaining; k_a is the rate constant for fast immobilization process; P_a is the proportion of ^{15}N–NO$_3^-$ immobilized by the fast process; k_b is the rate constant for the slow immobilization process; P_b is the proportion of ^{15}N–NO$_3^-$ immobilized by the slow process $(1-P_a)$. Using this double-exponential model, we demonstrate that it is possible to quantify the magnitude, time-course and relative significance of both rapid and slow N immobilization processes in soils.

3. Results

We observed strikingly different dynamics for ^{15}N–NO$_3^-$ immobilization in the different forest soil types
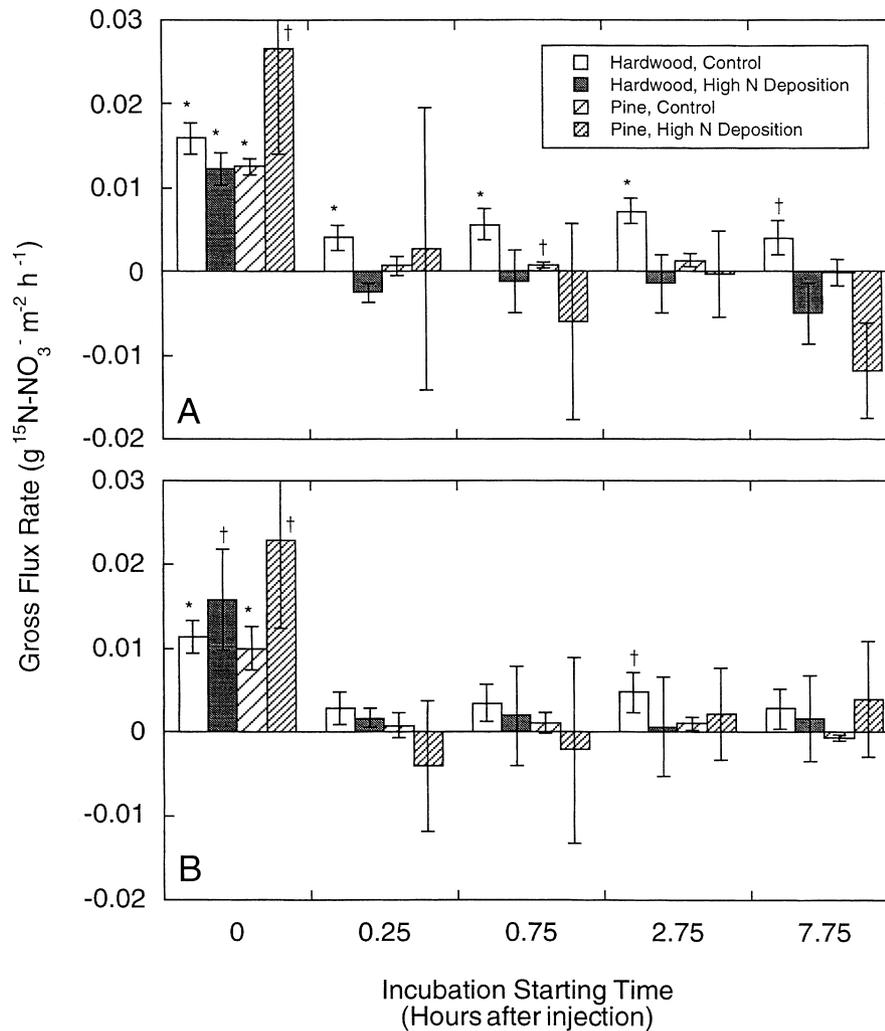
Fig. 2. Gross NO_3^- immobilization (A) and nitrification (B) rates derived using standard pool dilution equations (Hart et al. 1994b). Gross flux rates were calculated separately over periods starting from 0 to 7.75 h after injection and running to 24 h after injection. For the 0–24 h interval, the initial pool size measured in the core with no ^{15}N–NO_3^- added and the amount of ^{15}N–NO_3^- injected. Each bar represents the mean of three replicate calculations, with each estimate derived from a single set of cores ($\pm$ one standard error of the mean). Flux estimates which were significantly greater than zero (via a one-sided t-test) are indicated by * ($P < 0.05$) or † ($P < 0.10$).

and in response to the N deposition treatment (Fig. 1), with a large but variable fraction of added N immobilized over the shortest measured periods. In the conifer forest soil, ^{15}N–NO_3^- was immobilized within 15–45 min following the injection of the tracer, corresponding to a fast immobilization process (Fig. 1A). The fraction of added $^{15}NO_3^-$ immobilized varied from 34% in the High N stand to 62% in the control. In the hardwood forest soil, approx. 50% of the ^{15}N–NO_3^- tracer was immobilized within 15 min of injection for soils from both the control and high N deposition plots (Fig. 1B). For the high N deposition plot, no additional ^{15}N–NO_3^- was immobilized during the remaining 24 h. In contrast, the control plot showed slower but sustained ^{15}N–NO_3^- immobilization over the remaining 24 h.

We calculated gross NO_3^- immobilization and production rates (Kirkham and Bartholomew, 1954), for all periods starting from 0 to 7.75 h after injection and running to 24 h (Fig. 2). The only period that yielded estimates consistently greater than zero was from 0 to 24 h. However, the dynamics of ^{15}N–NO_3^- immobilization over this period do not follow zero order kinetics (Fig. 1), but show clear evidence of two distinct processes acting with different rate constants. The only gross flux estimates for intervals starting at 0.25 h or more after injection which were consistently greater than zero were the gross immobilization rates in the hardwood, control plot soil. The average gross NO_3^- immobilization rate for the slow immobilization process in the hardwood control plot soil was 125 mg NO_3^- m^{-2} d^{-1}, within the range reported by Stark and
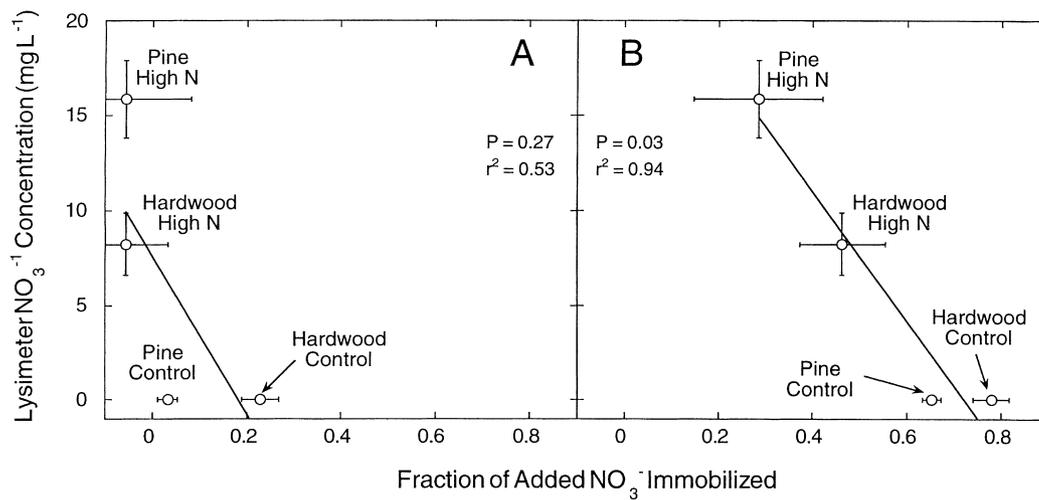
Fig. 3. Relationship between amount of ^{15}N–NO$_3^-$ immobilized during the 24 h exposure illustrated in Fig. 1 and the average soil lysimeter NO$_3^-$ concentration (at a depth of 60 cm) during the previous year (1996; A. Magill, J.D. Aber and G. Berntson, unpub.). (A) Fraction of ^{15}N–NO$_3^-$ tracer immobilized during the slow phase (estimated as $r - P_a$, where r is fraction immobilized after 24 h). (B) Fraction of ^{15}N–NO$_3^-$ tracer immobilized during both slow and rapid phases. Error bars are a single standard error of the mean. The thick line is the best fit linear relationship between the two variables.

Hart (1997) for a number of forests in the Western US. We did not observe significant gross NO$_3^-$ production rates in any of the soils we examined (Fig. 2).

4. Discussion

By examining the kinetics of ^{15}N–NO$_3^-$ immobilization over 24 h, we have demonstrated that the greatest potential for NO$_3^-$ immobilization over this interval in intact soil cores is by a fast process of immobilization which occurs within 15 to 30 min of the N addition. In the conifer forest, this rapid immobilization is the only significant process of NO$_3^-$ immobilization and long-term, chronic additions of N have resulted in a significant reduction (nearly 50%) in the capacity of these soils to immobilize NO$_3^-$. For the hardwood forest soil, rapid immobilization of NO$_3^-$ was similar regardless of the history of N deposition. However, NO$_3^-$ immobilization continued over the rest of the 24 h in the control stand, but not in the high N stand. Therefore, in the hardwood forest N deposition has also resulted in a large reduction (42%) in NO$_3^-$ immobilization over a 24 h, but for different reasons than in the conifer forest soil.

The relationship between the kinetics and amount of ^{15}N tracer immobilization in our intact cores and average soil NO$_3^-$ concentrations measured below the rooting zone (using tension lysimeters placed at a depth of 60 cm; Fig. 3;Magill et al., 1997), suggest that both the slow and rapid processes of NO$_3^-$ immobilization play a critical role in regulating ecosystem NO$_3^-$ mobility. The linear correlation between NO$_3^-$ immobilization capacity for the slow (presumably microbial) phase

and lysimeter NO$_3^-$ concentration is non-significant ($P = 0.47$; Fig. 3A), largely due to the three plots which showed insignificant slow immobilization (Fig. 2A). These data suggest that the loss of the slow phase of NO$_3^-$ immobilization is a sensitive and critical process regulating NO$_3^-$ immobilization in ambient conditions. It is only after this immobilization capacity is absent that NO$_3^-$ leaching starts. However, the three plots which exhibited no slow phase of NO$_3^-$ immobilization showed large variations in lysimeter NO$_3^-$ concentrations. This suggests that some other factor is playing an important role in regulating NO$_3^-$ retention in soils following the loss of the slow phase of immobilization. Including the amount of NO$_3^-$ immobilization during the initial rapid phase leads to a significant ($P = 0.06$), linear relationship between lysimeter NO$_3^-$ concentrations and NO$_3^-$ immobilization potential measured within intact cores (Fig. 3B). From these data we propose that the processes which comprise the rapid phase of immobilization play a critical role in regulating NO$_3^-$ retention in forest soils and that these processes appear to be sensitive to long-term N deposition once the more sensitive slow phase of immobilization is lost.

Our results demonstrate that the fast immobilization of NO$_3^-$ added to forest soils can represent a significant pathway for NO$_3^-$ immobilization and that this pathway is sensitive to long-term N deposition. These results, although preliminary, represent a novel hypothesis regarding how soil processes may control the sensitivity of terrestrial ecosystems to N saturation (Aber et al., 1998). In addition, these data suggest the importance of a rapid immobilization process which is absent from the most widely used models of N cycling

in terrestrial ecosystems (e.g. Parton et al., 1988; Rastetter et al., 1991; Townsend et al., 1996; Aber et al., 1997).

References

Aber, J.D., Magill, A., Boone, R., Melillo, J.M., Steudler, P., Bowden, R., 1993. Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecological Applications 3, 156–166.

Aber, J.D., Magill, A., McNulty, S., Boone, R., Nadelhoffer, K., Downs, M., Hallett, R., 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. Water, Air and Soil Pollution 85, 1665–1670.

Aber, J.D., McDowell, J., Nadelhoffer, K., Magill, A., Berntson, G.M., Kamakea, M., McNulty, S., 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. Bioscience 48, 921–934.

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. BioScience 39, 378–386.

Aber, J.D., Ollinger, S.V., Driscoll, C.T., 1997. Modeling nitrogen saturation in forest ecosystems in response to land use and atmospheric deposition. Ecological Modelling 101, 61–78.

Azhar, E.S., Verhie, M., Proot, M., Sandra, P., Verstraete, W., 1986. Binding of nitrite-N on polyphenols during nitrification. Plant and Soil 94, 369–382.

Davidson, E.A., Hart, S.C., Firestone, M.K., 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73, 1148–1156.

Davidson, E.A., Hart, S.C., Shanks, C.A., Firestone, M.K., 1991. Measuring gross nitrogen mineralization, immobilization and nitrification by ^{15}N isotope dilution in intact soil cores. Journal of Soil Science 42, 335–349.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994a. Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. Ecology 75, 880–891.

Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994b. Nitrogen mineralization, immobilization and nitrification. In: Weaver, R., Angle, C., Bottomley, P. (Eds.), Methods of Soil Analysis: Biochemical and Microbiological Properties. Soil Science Society of America, Madison, WI, pp. 985–1018.

Kirkham, D., Bartholomew, W.V., 1954. Equations for following nutrient transformation in soil, utilizing tracer data. Soil Science Society of America Proceedings 18, 33–34.

Lovett, G.M., 1994. Atmospheric deposition of nutrients and pollu-

tants in North America: an ecological perspective. Ecological Applications 4, 629–650.

Magill, A.H., Aber, J.D., Hendrichs, J.J., Bowden, R.D., Melillo, J.M., Steudler, P., 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological Applications 7, 402–415.

Maynard, D.G., Kalra, Y.P., 1993. Nitrate and exchangeable ammonium nitrogen. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, pp. 25–38.

Myrold, D.D., Tiedje, J.M., 1986. Simultaneous estimation of several nitrogen cycle rates using ^{15}N: theory and application. Soil Biology & Biochemistry 18, 559–568.

Nommik, H., Vahtras, K., 1982. Retention and fixation of ammonium and ammonia in soils. In: Stevenson, F.J. (Ed.), Nitrogen in Agricultural Soils. American Society of Agronomy, Madison, WI, pp. 123–171.

Ollinger, S.V., Aber, J.D., Lovett, G.M., Millham, S.E., Lathrop, R.G., Ellis, J.M., 1993. A spatial model of atmospheric deposition for the northeastern United States. Ecological Applications 3, 459–472.

Parton, W.J., Stewart, J.W.B., Cole, C.V., 1988. Dynamics of C, N, P and S in grassland soils: a model. Biogeochemistry 5, 109–132.

Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry, second ed. Academic Press, San Diego.

Rastetter, E.B., Ryan, M.G., Shaver, G.R., Melillo, J.M., Nadelhoffer, K.J., Hobbie, J.E., Aber, J.D., 1991. A general biogeochemical model describing the responses of the C and N cycles in terrestrial ecosystems to changes in CO_2, climate and N deposition. Tree Physiology 9, 101–126.

Schimel, J.P., Firestone, M.K., 1989. Inorganic nitrogen incorporation by coniferous forest floor material. Soil Biology & Biochemistry 21, 41–46.

Sigman, D.M., Altabet, M.A., Michener, R., McCorkle, D.C., Fry, B., Holmes, R.M., 1997. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry 57, 227–242.

Sørensen, P., Jensen, E.S., 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Analytica Chimica 252, 201–203.

Stark, J.M., Hart, S.C., 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385, 61–64.

Strickland, T.C., Sollins, P., Rudd, N., Schimel, D.S., 1992. Rapid stabilization and mobilization of 15N in forest and range soils. Soil Biology & Biochemistry 24, 849–855.

Tietema, A., 1998. Microbial carbon and nitrogen dynamics in coniferous forest floor material collected along a European nitrogen deposition gradient. Forest Ecology and Management 101, 29–36.

Tietema, A., Emmett, B.A., Gundersen, P., Kjønaas, O.J., Koopmans, C.J., 1998. The fate of ^{15}N-labelled nitrogen deposition in coniferous forest ecosystems. Forest Ecology and Management 101, 19–27.

Townsend, A.R., Braswell, B.H., Holland, E.A., Penner, J.E., 1996. Spatial and temporal patterns in potential terrestrial carbon storage resulting from deposition of fossil fuel derived nitrogen. Ecological Applications 6, 806–814.

Vitousek, P.M., Matson, P.A., 1985. Disturbance, nitrogen availability and nitrogen losses in an intensively managed loblolly pine plantation. Ecology 66, 1360–1376.

Wedin, D.A., Tilman, D., 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274, 1720–1723.