Ecosystems
https://doi.org/10.1007/s10021-018-0328-z

ECOSYSTEMS

Retention of Nitrate-N in Mineral Soil Organic Matter in Different Forest Age Classes

Colin B. Fuss,[1,2]* Gary M. Lovett,[1] Christine L. Goodale,[2] Scott V. Ollinger,[3] Ashley K. Lang,[4] and Andrew P. Ouimette[3]

[1]*Cary Institute of Ecosystem Studies, Millbrook, New York, USA;* [2]*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York, USA;* [3]*Complex Systems Research Center, University of New Hampshire, Durham, New Hampshire, USA;* [4]*Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire, USA*

Abstract

Conceptual models of nutrient retention in ecosystems suggest that mature forests receiving chronically elevated atmospheric nitrogen (N) deposition should experience increased nitrate (NO_3^-) losses to streams. However, at the Hubbard Brook Experimental Forest (New Hampshire, USA), recent stream NO_3^- concentrations have been unexpectedly low in mature watersheds. Poorly understood retention of NO_3^--N in soil organic matter (SOM) may explain this discrepancy. The relative availability of C and N in SOM influences NO_3^--N retention and may vary during succession due to processes of N mining and re-accumulation. To evaluate the strength of the SOM sink for NO_3^--N, we applied a $^{15}NO_3^-$ tracer to the mineral soil in eight stands spanning a forest chronosequence from about 20 years to old growth (≫ 200 years). We tracked ^{15}N recovery in SOM fractions in the upper 10 cm of B horizon over 5 weeks. Overall, forest age did not directly control the 5-week recovery of ^{15}N, but it had an indirect effect via its influence on SOM properties such as C/N. Old-growth forest soils had the lowest C/N, implying closer proximity to effective N saturation. Across sites, both the particulate- and mineral-associated SOM fractions rapidly incorporated ^{15}N, but recovery in each fraction generally declined with time, reflecting the dynamic nature of SOM. These results indicate that mineral horizons can provide an important N sink through the short term in forests of all ages, but that SOM-N remains subject to active cycling and potential loss from the soil pool over the longer term.

Key words: nitrogen; ^{15}N tracer; chronosequence; soil water; immobilization; Spodosol.

Highlights

- Low N losses from forests may be due to poorly understood soil retention.
- Nitrate is quickly retained in both particulate and mineral-associated soil organic matter.
- Ecosystem models should better consider controls on soil-microbial N retention.

Received 16 March 2018; accepted 25 November 2018

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10021-018-0328-z) contains supplementary material, which is available to authorized users.

Author Contributions Conceived of or designed study: CBF, GML, CLG, SVO, AKL, APO. Performed research: CBF, GML, AKL. Analyzed data: CBF, APO. Wrote the paper: CBF, GML, CLG, SVO, AKL, APO.

**Corresponding author; e-mail:* fussc@caryinstitute.org

Published online: 02 January 2019

Introduction

Human industrial and agricultural activities have greatly increased the quantity of actively cycling nitrogen (N) globally and its deposition to terrestrial ecosystems (for example, Galloway and others 2008). Although anthropogenic N deposition can initially increase plant productivity in N-limited terrestrial ecosystems (LeBauer and Treseder 2008), chronic N deposition in excess of biotic demand can lead to plant nutrient imbalances, soil and surface water acidification, and downstream eutrophication (Driscoll and others 2003). This phenomenon is of particular concern in the northeastern USA, which has a history of elevated N deposition (Driscoll and others 2003) and has large areas of forests reaching mature age (Pan and others 2011), and therefore would be expected to have declining net N accumulation in biomass. At the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, aboveground biomass has been relatively stable since the forest reached maturity in the mid-1980s (Fahey and others 2005). Although biogeochemical models generally predict increased NO_3^- leaching from soils in response to decreased plant demand and continued elevated deposition (Aber and others 2002; Gbondo-Tugbawa and Driscoll 2002; Pourmokhtarian and others 2012), stream NO_3^- losses have markedly declined during this period (Likens 2013; Fuss and others 2015).

Microbial immobilization is important in the immediate retention of deposited NO_3^- (for example, Stark and Hart 1997; Tahovská and others 2013; Weitzman and Kaye 2016), but microbial N turnover times are a few weeks or less (for example, Seely and Lajtha 1997; Zogg and others 2000, Perakis and Hedin 2001). Given that changes in microbial biomass are small on an interannual timescale (Holmes and Zak 1994), it is unlikely that microbial biomass directly provides a long-term N sink in mature forests. However, microbial N uptake could lead to a significant long-term sink for N in forests if that N is subsequently transferred to stable soil organic matter (SOM) (Zogg and others 2000; Curtis and others 2011).

Combined insights from watershed-scale N budget monitoring (Likens 2013; Yanai and others 2013) and biogeochemical modeling (Aber and Driscoll 1997) do indeed suggest that poorly understood mechanisms of soil N retention may be contributing to the pattern of low NO_3^- losses in forests like Hubbard Brook. Yanai and others (2013) specifically suggested that the mineral soil likely accounted for an undetermined N sink in recent years after repeated measurements indicated that forest floor N accumulation could only explain a small fraction of the measured N imbalance, although the rate of N accumulation in the forest floor is subject to considerable uncertainty. Mineral horizon SOM is typically a large pool of N in temperate forests. At Hubbard Brook, this N pool has been estimated to be 5900 $\pm$ 730 kg N/ha (Huntington and others 1988), or more than 70% of all N in the forest ecosystem (Yanai and others 2013). Consequently, detecting changes in soil N stocks over time is difficult due to the large size and heterogeneity of the soil pool. So although changes in SOM-N stocks cannot easily be quantified on the timescale of years or several decades, other methods point to a likely soil sink for N in temperate forests, including isotopic tracer studies (Nadelhoffer and others 2004; Hagedorn and others 2005; Goodale 2017), measurements of dissolved N fluxes with depth in the soil profile (Dittman and others 2007), and models of long-term recovery from historical disturbance (Bernal and others 2012). This increased awareness of mineral soil accumulation of N inputs in stable pools (Kaye and others 2003) ties into a recent recognition of the dynamic—rather than static—nature of SOM (Lehmann and Kleber 2015). The dynamic nature of SOM is reflected in variable C/N and natural abundance enrichment of ^{13}C and ^{15}N, both of which depend on the composition of organic matter inputs from plants and the degree of microbial processing (Nadelhoffer and Fry 1988; Högberg 1997; Rumpel and Kögel-Knabner 2011; Craine and others 2015). Additional insights into SOM cycling have been gained from analyzing SOM fractions separated physically based on soil particle size (for example, Hagedorn and others 2005; Castellano and others 2012) or density (for example, Sollins and others 2006; Wagai and others 2015; Kramer and others 2017; Pries and others 2017), where findings generally suggest that particulate or light fractions of SOM are more labile and accessible to microbes, whereas the mineral-associated or heavy fractions of SOM are more stable over a longer term. Consequently, SOM fractions may influence soil biogeochemical cycling at different timescales. Although much of the increased attention to SOM dynamics is due to interest in controls on soil carbon (C) sequestration and stability (for example, Schrumpf and others 2013; Beare and others 2014; Keiluweit and others 2016), it also recognizes the importance of soil for N storage (Castellano and others 2012; Kopáček and others 2013; Bingham and Cotrufo 2016).

Accumulation of N in mineral soils may help explain why anticipated N saturation effects,

including increased inorganic N leaching, have not occurred in many forests with historically elevated N deposition (Aber and others 2003). The conceptual model of N saturation by Lovett and Goodale (2011) emphasizes that added N can simultaneously flow to sinks in the vegetation or soil, as well as to leaching or gaseous losses. This model differentiates between *kinetic* and *capacity* saturation and suggests that the relative importance of N flows depends on the proximity of the sinks to capacity saturation and the levels of N inputs relative to uptake kinetics. The balance of N sinks and pathways is likely to vary as a function of net plant N demand (that is, biomass accumulation during forest succession, Vitousek and Reiners 1975) and microbial N demand in part determined by soil C/N (Riha and others 1986; Tahovská and others 2013).

Our primary objectives in this study were to evaluate the strength of the shallow mineral horizon SOM sink for NO_3^--N and determine the extent to which retention is influenced by SOM characteristics that may depend on forest age and plant–microbe dynamics, such as C/N ratio and the natural abundance of ^{13}C and ^{15}N. Lovett and others (2018) recently presented a conceptual model of N retention over the course of forest succession (the ''N Bank hypothesis'') and suggested that processes of N mining from mineral soil during forest regrowth followed by re-accumulation upon maturing would lead to an enhanced SOM sink for N in recently mature forests relative to old growth. Our study tests whether patterns of soil NO_3^--N retention are consistent with that theory. We hypothesized that the retention of NO_3^--N in old-growth forest soil would be lower than in soil of recently mature forests. The N Bank model also suggests that rapid accumulation of N in biomass should limit the amount of N retained in the soil of aggrading forests due to strong plant uptake and N mining. We consequently hypothesized that net retention of NO_3^--N in SOM would be low in young stands. An additional objective of our study was to determine how much of the ^{15}N tracer was retained in stable vs. labile fractions of the mineral soil over a 5-week period. We hypothesized that initially the ^{15}N tracer recovery would be highest in the relatively labile particulate organic matter (POM) fraction and would gradually move into the more stable mineral-associated organic matter (MAOM) due to microbial turnover and SOM stabilization. We tested our hypotheses by measuring NO_3^- retention through the use of ^{15}N-labeled isotopic tracer in a chronosequence of eight northern hardwood forest stands that ranged in age from just over 20 years to old growth.

Methods

Site Description

This study was conducted in the western White Mountain region of New Hampshire, USA, within and near the Hubbard Brook Experimental Forest (Figure 1). The forest composition of this region is dominated by northern hardwood species, including sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). Other common species found in this region and within our plots are red maple (*Acer rubrum* L.), white ash (*Fraxinus americana* L.), aspen (*Populus grandidentata* Michx. or *Populus tremuloides* Michx.), paper birch (*Betula papyrifera* var. cordifolia Marsh), and the occasional conifers red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* (L.) Mill). In the youngest stand, the early successional species pin cherry (*Prunus pensylvanica* L.f.) is common. The climate is cool-temperate and humid continental, with mean July and January temperatures of 18.8 and − 8.5°C, respectively (at the HBEF, 450 m elevation). Annual precipitation averages approximately 1400 mm and is distributed nearly evenly throughout the year. The soils of this region are largely Spodosols (Haplorthods) derived from glacial basal till and covered by a relatively thick (3–15 cm) forest floor (Likens 2013). The typical horizonation of mineral soils consists of an eluviated (E) horizon up to several cm thick, underlain by spodic horizons (Bhs and Bs), which are characterized by an accumulation of organic matter and iron and aluminum oxides (Johnson and others 1991). A thin A horizon is sometimes present at the top of the mineral soil, especially where E horizons have not fully developed. The thickness and development of soil horizons are highly variable spatially (Bailey and others 2014).

To study NO_3^- retention in mineral soils of differently aged forest stands, we used an eight-plot chronosequence, with two 900 m^2 plots in each of four age classes: early successional (very young) 20–30-year-old stands, mid-successional (young) 40–50-year-old stands, late successional (recently mature) approximately 100-year-old stands, and old-growth stands older than 200 years old (Table 1). The successional forests had been clearcut harvested, whereas the old-growth forests have no record of major disturbance (Goodale and others 2000). To estimate the ages of the two sites for which conclusive historical records were not available (CR, RL; Table 1), several of the largest early successional trees (aspen, paper birch) were

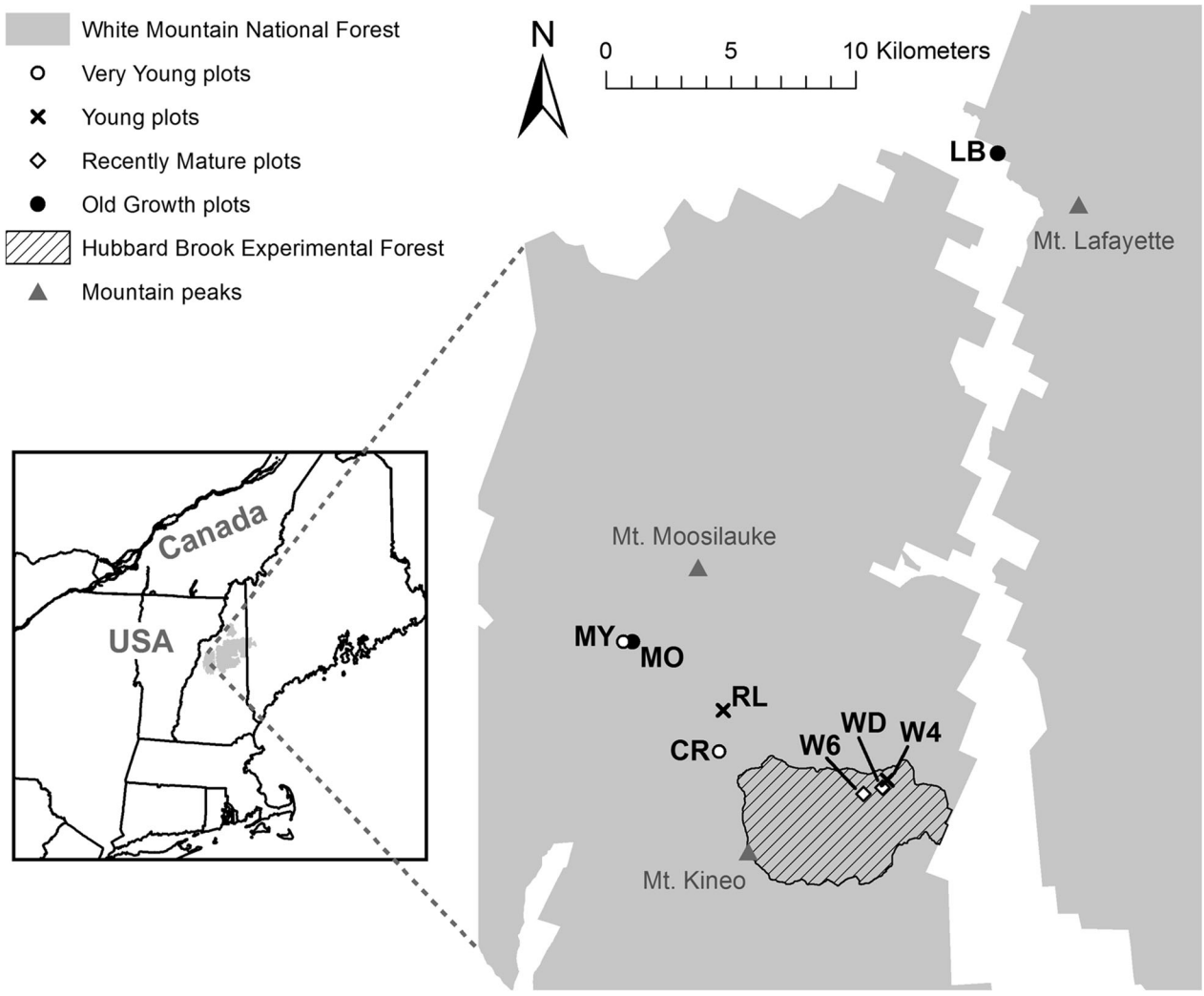

Figure 1. Location of study sites in the western White Mountain region of New Hampshire (NH), USA.

Table 1. Site Descriptions

Site name	Age (in 2015)	Age class	Location (lat-long)	Elevation (m)	Aspect
Moosilauke Young (MY)	21	Very Young	N44.0018°, W71.8631°	726	W
Cabin Road (CR)	27	Very Young	N43.9634°, W71.8146°	517	NW
HB-W4 (W4)	43	Young	N43.9544°, W71.7300°	568	S
Ravine Lodge (RL)	45	Young	N43.9776°, W71.8130°	646	SE
HB-West W6 (W6)	100	Mature	N43.9496°, W71.7405°	635	SE
HB-Wedge (WD)	100	Mature	N43.9517°, W71.7318°	574	S
Moosilauke Old (MO)	$\gg$ 200	Old Growth	N44.0008°, W71.8591°	823	W
Lafayette Brook (LB)	$\gg$ 200	Old Growth	N44.1818°, W71.6848°	668	NW

cored with an increment borer and their age was determined by counting the growth rings, under the assumption that the trees sprouted or germinated immediately after the harvest.

Field and Laboratory Methods

In August, 2015, nine mineral soil ''microplots'' were established within each plot of the chronosequence, in three groups of three microplots. One microplot in each group of three was pre-designated for sampling at each of three col-

lection times (2, 9, and 35 days) after tracer addition. For each microplot, a 15 × 15 cm block of forest floor was cut and lifted to expose the upper surface of the mineral soil. Because the tracer was added to the top of the B horizon, the A and E horizons were removed if they were present. For most of the microplots, the E horizon was not developed or was relatively thin (1–3 cm). To facilitate tracer application and subsequent sampling, a square section of window screen was placed on top of the mineral soil before the block of forest floor was replaced.

The isotopic tracer was applied as potassium nitrate ($K^{15}NO_3$, 99% atom percentage excess) at a rate of 100 mg $^{15}NO_3^-$-N m^{-2} to the top of the B horizon on the 18 and 19 August 2015 (4 sites per day). This addition was equivalent to approximately 20% of annual N deposition for this area in recent years. After removing the forest floor blocks, 45 ml of 3.33 mM $K^{15}NO_3$ solution was dripped from a syringe evenly across each 15x15 cm microplot. This application was equivalent to 2 mm of solution depth, an amount chosen to ensure both infiltration into the soil and an even spatial application. The experiment was timed so that the tracer application and initial soil sampling would be completed at all sites prior to any rain.

Sampling was conducted over the course of 5 weeks and consisted of two methods—small soil cores taken at various intervals to provide information about how the ^{15}N tracer was distributed by depth and between SOM fractions with time, and a quantitative removal of soil beneath microplots at the end of the experiment to provide an accurate measure of total ^{15}N recovery in SOM. Soil cores of 2 cm diameter were collected from two depths in the upper B horizon (0–3 cm and 3–10 cm) at each sampling interval: 0 days (from unlabeled soil just prior to tracer addition), 2 days, 9 days, and 35 days. The first 10 cm of B horizon soil was of interest both because it is a zone of high organic matter accumulation in Spodosols (Buurman and Jongmans 2005) and has a relatively high density of roots (Fahey and others 1988). Thus, we considered these surface mineral soil layers to be those most likely to reflect differences in SOM-N dynamics over successional timescales. For the 0-, 2-, and 9-day sample collections, the soil cores from each of the three replicate microplots were composited in the field to yield one sample per site per collection at each depth. The cores from microplots for the 35-day collection were kept and analyzed separately (3 samples per site per depth). This sampling design reduced the number of samples for the earlier collections where we were most interested in tracking the average movement of tracer to depth and association with soil fractions. The separate replicates from the final collection allowed for characterization of variability between microplots. On the 35th day after tracer application, three microplots at each site were quantitatively sampled by collecting the soil for the entire 15x15 cm block at both depth intervals.

After collection, the soil samples were dried at 60°C until they reached a constant weight. The soils were passed through a 2-mm sieve to remove rocks and roots. Soil core samples were physically fractionated into two organic matter pools: POM associated with the sand size class (> 53 μm) and MAOM associated with clay and silt classes (< 53 μm) (Castellano and others 2012). Approximately 4 g of dry soil was vigorously agitated in deionized water and poured onto a 53-μm sieve placed over a collection tray. The soil slurry on the sieve was alternatively rinsed with a stream of deionized water and massaged by hand to disrupt soil macroaggregates and allow the MAOM fraction to pass through the sieve. This process was continued until the water flowing through the sieve appeared clear against a white background. The < 53 μm fraction was centrifuged to concentrate the mineral particles, and the excess rinse water was discarded. Both fractions were redried at 60°C and weighed to determine the proportions of each size fraction in the whole soil. The > 53 μm fraction containing the POM was ground with a ball mill (Kleco) to a fine powder prior to analysis. Similarly, the samples for total ^{15}N tracer recovery (at 35 days), consisting of all the soil in each depth increment from the 15 × 15 cm microplot, were subsampled and ground with the ball mill.

Isotopic and elemental analyses were conducted at the University of New Hampshire Stable Isotope Lab using an Elementar Americas Pyrocube elemental analyzer coupled to a GeoVision isotope ratio mass spectrometer. The C and N isotope composition was expressed in standard delta notation ($\delta^{13}C$, $\delta^{15}N$) in per mil (‰) relative to V-PDB standard for C and atmospheric N_2 for N (see supplementary material for a detailed protocol of isotope analysis).

Computational Methods and Statistical Analyses

The recovery of tracer ^{15}N in SOM fractions from the small soil core samples from each collection was computed by first determining the atom% of ^{15}N coming from the tracer (atom% $^{15}N_{tracer}$) in each size fraction (> 53 μm or < 53 μm) through sub-

traction of the atom% ^{15}N of the fraction's natural abundance ^{15}N (atom% $^{15}N_{pre}$) from the total atom% ^{15}N in the soil fraction sample (atom% $^{15}N_{post}$) (equation 1), and then multiplying that value by the total %N of the soil size fraction, and dividing it by the size fraction's proportion of total soil mass ($M_{soil\ fraction}/M_{soil}$) and multiplying it by 10^3 to express the value in mg $^{15}N_{tracer}$/g soil found in each SOM fraction (equation 2). The soil concentrations of tracer in each size fraction from Equation (2) were then multiplied by the total < 2 mm soil mass for each depth, divided by the mass of the initial ^{15}N tracer application, and multiplied by 100 to express tracer recovery as a percent of the total added (equation 3).

$$\begin{aligned} &\text{atom\%}^{15}N_{\text{tracer/SOM fraction}} \\ &= \left(\text{atom\%}^{15}N_{\text{post}} - \ \text{atom\%}^{15}N_{\text{pre}}\right) \end{aligned} \tag{1}$$

$$\begin{aligned} &{}^{15}N_{\text{tracer/SOM fraction}}\left(\text{mg g soil}^{-1}\right) \\ &= (\text{atom\%}^{15}N_{\text{tracer/SOM fraction}} \\ &\times \ \%N_{\text{soil fraction}}/(M_{\text{soil fraction}}/M_{\text{soil}})) \times 10^3 \end{aligned} \tag{2}$$

$$\begin{aligned} &{}^{15}N_{\text{Rec/SOM fraction}}(\%) \\ &= \left({}^{15}N_{\text{tracer/SOM fraction}} \times M_{\text{soil}}/M_{15N\text{added}}\right) \times 100 \end{aligned} \tag{3}$$

The total recovery of tracer ^{15}N in SOM after 35 days was calculated by multiplying the N mass of each soil depth increment by the increase in measured atom% ^{15}N after 35 days relative to the natural abundance values for the given depth at each site (Table 2), dividing the mass of the tracer addition ($M_{15N\text{added}}$, mg $^{15}N/m^2$), and then multiplying by 100 (equation 4) (Nadelhoffer and others 2004; Goodale 2017).

$$\begin{aligned} {}^{15}N_{\text{Rec}}(\%) = &\left(\left(\text{atom\%}^{15}N_{\text{post}} - \text{atom\%}^{15}N_{\text{pre}}\right)\right. \\ &\left.\times M_{\text{pool}N}/M_{15N\text{added}}\right) \times 100 \end{aligned} \tag{4}$$

Paired t tests were used to compare the elemental and isotopic composition of the POM and MAOM fractions. Linear mixed-model analysis was used to explore relationships between final (35 days) ^{15}N tracer recovery and fixed-effect soil characteristics such as C/N, %N, %C, background $\delta^{15}N$, and $\delta^{13}C$, with site as a random effect. These soil variables for the total 0–10 cm depth were calculated as the mass-weighted average of the values from the 0–3 cm and 3–10 cm depths. Values for total N and total C were also tested as predictors of ^{15}N tracer retention in case soil bulk density variability masked relationships with %N or %C. To test the hypothesis that the SOM sink for NO_3^--N in old-growth forests is lower than in aggrading or recently mature forests, a planned contrast one-way ANOVA was used to test a linear mixed-effects model in which forest age was a fixed effect and site was a random effect. Student's t tests were used to test specific hypotheses regarding whether SOM composition (C/N, $\delta^{15}N$, and $\delta^{13}C$) differed between recently mature forests and old-growth stands.

Table 2. Soil Characteristics by Site and Depth

Site	Age class	Depth (cm)	Bulk density (< 2 mm; g/cm^3)	%C	%N	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
MY	Very young	0–3	0.63	5.56	0.38	14.5	− 26.3	5.4
		3–10	0.66	5.11	0.31	16.3	− 26.0	6.3
CR	Very young	0–3	0.45	9.31	0.43	21.7	− 26.6	4.9
		3–10	0.46	7.32	0.30	24.3	− 26.1	6.5
W4	Young	0–3	0.44	5.71	0.29	19.4	− 26.4	6.2
		3–10	0.64	3.80	0.24	15.8	− 26.4	6.5
RL	Young	0–3	0.44	8.84	0.48	18.6	− 26.8	5.4
		3–10	0.60	5.34	0.26	20.2	− 26.2	6.5
W6	Mature	0–3	0.35	6.03	0.30	20.0	− 26.1	6.3
		3–10	0.50	5.05	0.25	20.1	− 26.1	6.6
WD	Mature	0–3	0.41	6.16	0.31	20.2	− 26.8	6.5
		3–10	0.67	5.37	0.23	23.3	− 25.9	7.0
MO	Old growth	0–3	0.59	7.06	0.52	13.6	− 25.9	6.1
		3–10	0.60	5.77	0.41	13.9	− 25.8	6.8
LB	Old growth	0–3	0.65	3.72	0.26	14.4	− 26.0	7.1
		3–10	0.98	2.44	0.18	13.8	− 25.8	8.1

Results

Pretreatment Characteristics of Soils

Characteristics of B horizon SOM varied with forest age, with higher concentrations of N in the old-growth forest compared to younger-aged stands. The overall concentration of organic matter in soil (as %C) did not vary significantly with forest age. The C/N of SOM at both 0–3 and 3–10 cm depths decreased approximately 25% from the youngest sites to the oldest, while the natural abundance δ^{15}N increased by a similar magnitude with increasing stand age (Figure 2, Table 2). The soil C/N was lower in the old-growth forests compared to recently mature forests at both depths (0–3 cm: $P < 0.02$; 3–10 cm: $P < 0.005$). Natural abundance isotopic values were not significantly different between soils of old-growth forests and recently mature forests in either depth for δ^{15}N (0–3 cm, $P > 0.51$; 3–10 cm: $P > 0.45$) or δ^{13}C (0–3 cm: $P > 0.16$; 3–10 cm: $P > 0.26$).

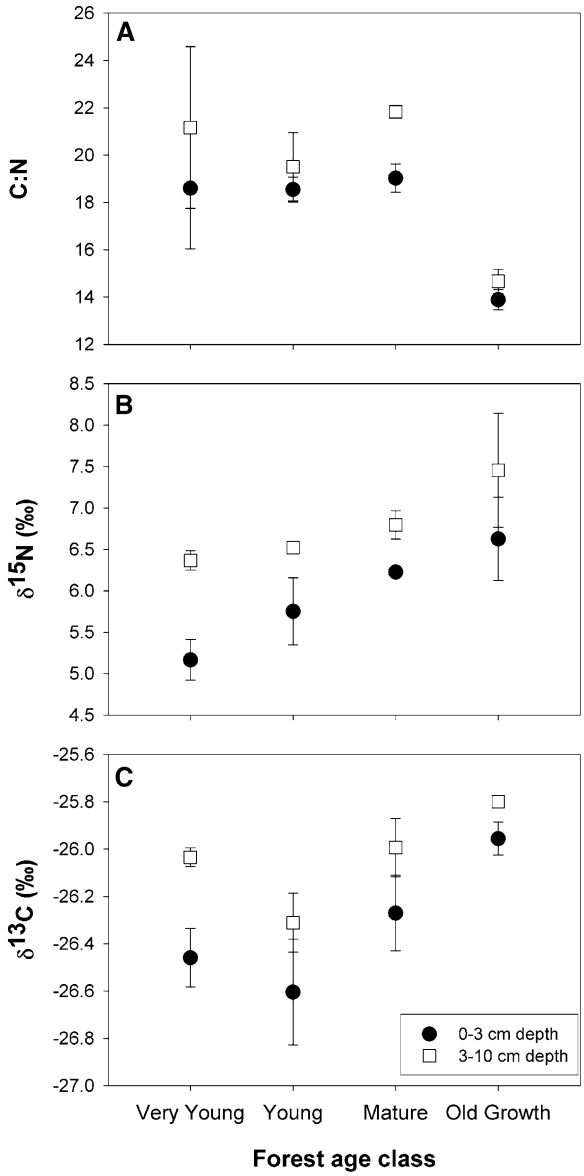

Figure 2. Mineral soil organic matter **A** C/N, **B** δ^{15}N, and **C** δ^{13}C by depth in B horizon in four forest age classes. Vertical bars indicate standard errors (n = 2 sites per age class).

The > 53 μm fraction of the soil (sand + POM) accounted for an average of 82% of the soil mass and varied minimally (80–86%) across sites. The POM contained in the > 53 μm fraction accounted for the majority of total SOM (mean of 70% of C in the 0–3 cm layer and 60% in the 3–10 cm layer), with the remaining 30–40% classified as MAOM in the < 53 μm fraction. The MAOM fraction had a lower C/N ratio (mean ± SE = 17.6 ± 0.9) than the POM fraction (C/N = 19.9 ± 1.1) ($P < 0.0004$; Table 3). The MAOM fraction was also more enriched in the heavy isotopes for both C (δ^{13}C = -26.0 ± 0.1‰ for MAOM; δ^{13}C = -26.4 ± 0.1‰ for POM, $P < 0.003$; Table 3) and N (δ^{15}N = 6.9 ± 0.2‰ for MAOM; δ^{15}N = 5.7 ± 0.2‰ for POM, $P < 0.00001$; Table 3).

Patterns of ^{15}N Recovery in SOM Fractions with Depth and Time

The ^{15}N tracer recovery was highest in the 0–3-cm layer at the initial 2-day sample collection and was detectable in both the POM and MAOM fractions, with roughly 75% of the recovered tracer (6–22% of the applied tracer) found in the POM fraction and the remainder recovered in the MAOM (1–6% of applied) (Figure 3). Overall, the depth and temporal patterns of ^{15}N tracer recovery were similar across the chronosequence. The distribution of the tracer between the soil size fractions remained relatively constant with time (Figure 3) and was nearly proportional to the distribution of total SOM among the fractions. The tracer recovery in the 0–3 cm depth was highest at the 2-day collection and declined overall with time. Among the forest age classes, the mean recovery of tracer ^{15}N in the 0–3 cm layer after 2 days ranged from 6.1 to 14.7% in the POM fraction and 2.0 to 4.3% in the MAOM fraction (Figure 3). Nine days after tracer application, the recovery of tracer ^{15}N in the 0–3-cm layer's composited samples declined in each age class, with means ranging from 3.1 to 8.6% in POM (30–49% reductions) and 0.7 to 2.4% in the MAOM (40–65% reductions). At the same time, the recovery of tracer ^{15}N in the 3–10-cm layer increased from a mean of 5.2 to 15.0% in POM and 1.6–3.5% in MAOM, indicating a movement of some of the tracer from the shallower to deeper

Table 3. Soil C and N Characteristics in the > 53 μm and < 53 μm Fractions

Site	Age class	Depth (cm)	%C	%N	C:N	δ^{13}C (‰)	δ^{15}N (‰)
(*a*) > *53* μm (*sand + POM)*							
MY	Very young	0–3	5.80	0.35	16.7	− 26.2	5.2
		3–10	4.59	0.23	20.3	− 25.9	5.8
CR	Very young	0–3	7.55	0.33	22.8	− 27.0	4.2
		3–10	5.57	0.20	28.3	− 27.3	5.0
W4	Young	0–3	4.54	0.28	16.1	− 26.8	5.5
		3–10	2.20	0.13	16.6	− 26.5	6.2
RL	Young	0–3	6.77	0.32	21.5	− 27.4	4.6
		3–10	1.74	0.07	25.7	− 26.7	4.8
W6	Mature	0–3	4.52	0.22	20.3	− 26.1	6.3
		3–10	3.96	0.18	21.8	− 26.0	6.7
WD	Mature	0–3	2.63	0.13	20.9	− 26.8	5.2
		3–10	3.50	0.13	27.0	− 26.4	6.2
MO	Old growth	0–3	7.91	0.53	14.9	− 26.2	5.7
		3–10	5.40	0.37	14.6	− 25.8	6.6
LB	Old growth	0–3	1.76	0.21	14.0	− 25.5	6.5
		3–10	0.98	0.17	16.6	− 25.7	7.1
(*b*) < *53* μm (*clay + silt + MAOM)*							
MY	Very young	0–3	7.01	0.45	15.5	− 26.2	6.1
		3–10	7.54	0.46	16.5	− 25.9	6.5
CR	Very young	0–3	11.53	0.58	19.8	− 26.5	6.4
		3–10	13.13	0.56	23.5	− 26.1	6.8
W4	Young	0–3	7.60	0.49	15.6	− 26.4	6.7
		3–10	6.87	0.42	16.5	− 26.1	7.1
RL	Young	0–3	11.31	0.64	17.6	− 26.6	6.2
		3–10	9.83	0.49	19.9	− 26.1	7.3
W6	Mature	0–3	9.44	0.47	20.2	− 26.2	6.3
		3–10	10.64	0.47	22.4	− 25.9	7.1
WD	Mature	0–3	9.71	0.50	19.3	− 26.3	6.7
		3–10	10.22	0.45	22.8	− 26.0	7.3
MO	Old growth	0–3	7.48	0.56	13.4	− 25.6	7.1
		3–10	7.08	0.50	14.1	− 25.5	7.4
LB	Old growth	0–3	8.67	0.69	12.5	− 25.9	7.9
		3–10	9.24	0.71	12.9	− 25.2	8.4

depth during the week between samplings. Between the 9-day and 35-day sample collections, the concentration of the ^{15}N tracer was generally stable in the 0–3-cm layer, while concentrations declined in the 3–10-cm layer. The ^{15}N tracer recovery in small soil cores was highly variable among the microplots, as evidenced by the 35-day collection of replicate soil cores (Figure 3).

Overall Recovery of ^{15}N in SOM

After 5 weeks, the total recovery of tracer ^{15}N in individual microplots ranged from a low of 7.2% in one microplot in the MO old-growth stand to 37.8% in one microplot in the recently mature stand WD. Retention of the $^{15}NO_3^-$ tracer in the combined 0–10 cm depth was not correlated with soil %N ($P > 0.48$), total N (%N x soil mass, $P > 0.91$), or natural abundance δ^{15}N ($P > 0.86$), but trended toward an increase with %C ($P > 0.077$) and total C ($P > 0.11$), and was significantly positively correlated with the soil C/N ($P < 0.051$; Figure 4), and negatively correlated with δ^{13}C ($P < 0.008$). The mean rate of $^{15}NO_3^-$-N retention in SOM was 46% lower in the old-growth stands than in the recently mature stands, but variability among microplots meant that the difference was not statistically significant ($P > 0.17$; Figure 5).

Discussion

Factors Affecting NO_3^--N Retention in SOM

Our initial prediction that forest successional status would control the retention of $^{15}NO_3^-$-N in the upper B horizon (that SOM would provide an enhanced N sink in recently mature forests) was not

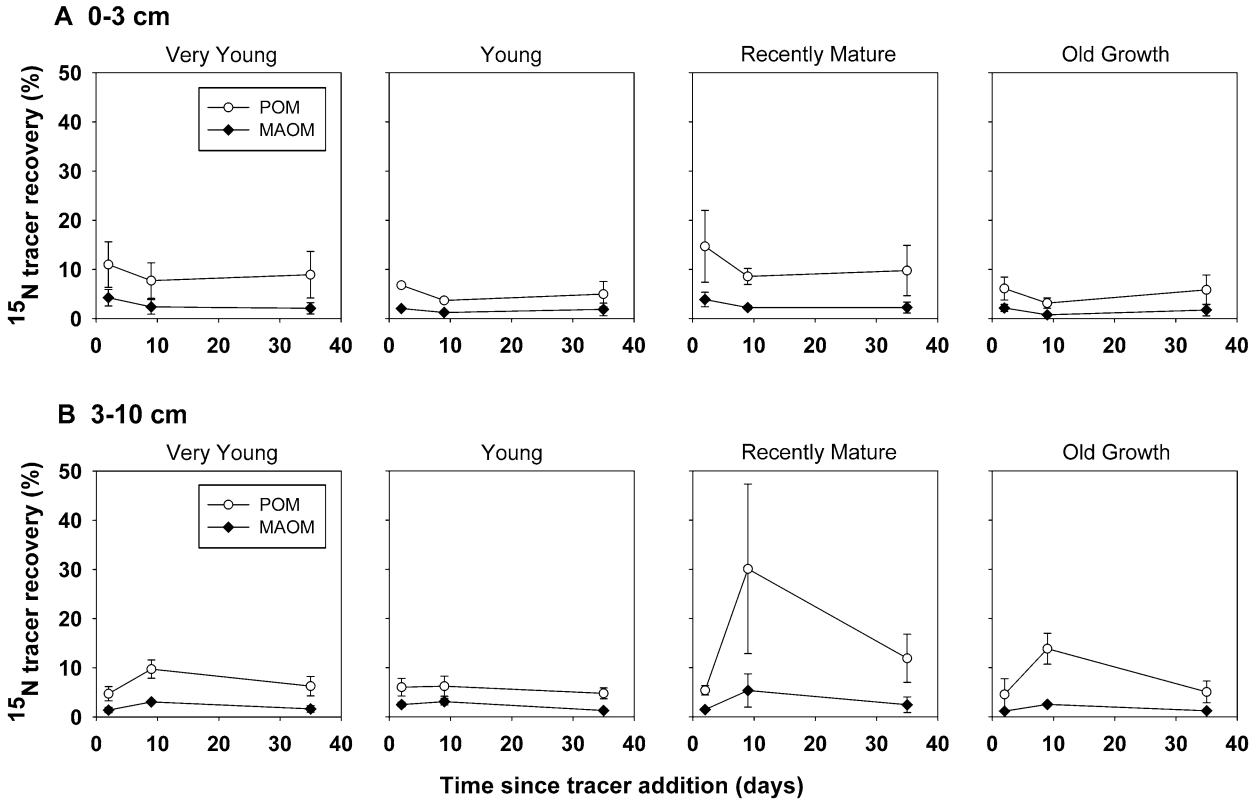

Figure 3. Changes in tracer recovery over time in POM and MAOM pools at **A** 0–3 cm and **B** 3–10 cm depths in the B horizon based on composites of three 2-cm cores per site. Vertical bars indicate standard errors (n = 2 sites per age class).

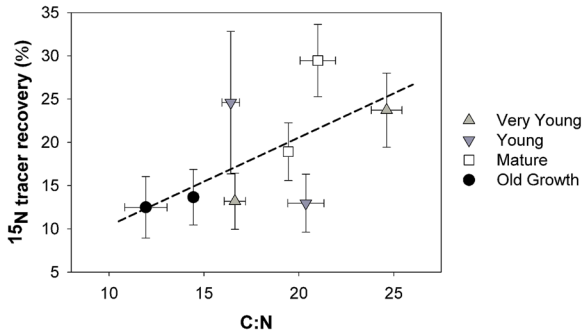

Figure 4. Tracer ^{15}N recovery in 0–10-cm B horizon soil as a function of soil C/N ($P < 0.051$). Vertical and horizontal bars indicate standard errors (n = 3 microplots per site). Dashed line is the fit from a linear mixed-effects regression model.

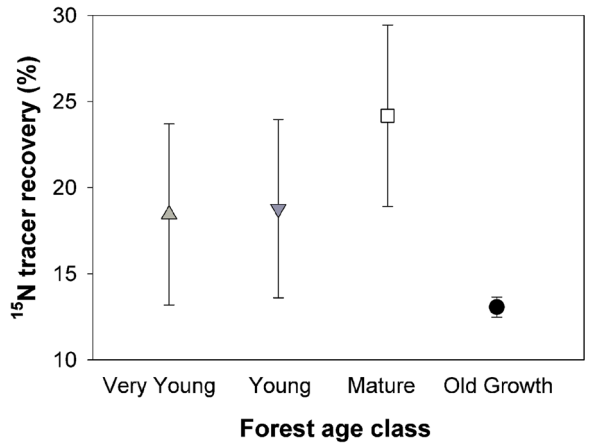

Figure 5. Total retention of added nitrate in the SOM pool of the top 10 cm of B horizon soil (excavated 15 × 15 cm blocks) after 35 days ($P > 0.17$, planned contrast ANOVA). Vertical bars represent standard errors (n = 2 sites per age class).

strongly supported by the evidence: The old-growth stands retained roughly half the ^{15}N as the mature stands, but this result was not statistically significant because of variability in ^{15}N recovery among samples within the same plot. However, we did find that SOM properties such as C/N and natural abundance δ^{13}C were important predictors of ^{15}N retention and those properties correlated with forest age, thus lending support to the notion that forest succession may control soil N retention indirectly.

Higher retention of $^{15}NO_3^-$-N occurred in soils with higher C/N (Figure 4) and those with lighter ^{13}C. Our results are largely consistent with the findings of other ^{15}N tracer studies. A meta-analysis of terrestrial ecosystem ^{15}N tracer experiments revealed that total recovery of ^{15}N is strongly and positively correlated with mineral soil C/N in the both short term (< 1 week) and long term (3–18 months) and negatively correlated with mineral soil ^{15}N natural abundance in the short term (Templer and others 2012). We found considerably higher soil C/N ratios in the three successional age classes compared to the old-growth stands (Figure 2). The higher C availability in these soils of younger forests should lead to enhanced N retention by microbes (Evans and others 2006; Curtis and others 2011; Tahovská and others 2013; Lewis and others 2014, Groffman and others 2018), especially when net plant demand for N is low (Kuzyakov and Xu 2013). Additionally, we found a negative correlation between ^{15}N tracer retention and the $\delta^{13}C$ of SOM, which may suggest that plant-derived C that is less processed by microbes (Nadelhoffer and Fry 1988; Boström and others 2007) was an important factor determining retention of NO_3^--N.

Our total recovery of ^{15}N in SOM of the top 10 cm of mineral soil after 5 weeks ranged from approximately 10–30%. These recovery rates are consistent with other studies that involved addition of $^{15}NO_3^-$ to mineral soils. In a central Pennsylvania forest, $^{15}NO_3^-$ was applied by syringe into the 0–15-cm mineral soil layer and approximately 17% of ^{15}N was recovered in either stable or labile fractions of the SOM pool after 20 days (Weitzman and Kaye 2016). Following $^{15}NO_3^-$ addition to a sugar maple-dominated forest in Michigan, less than 20% of ^{15}N was recovered in SOM or microbial pools after 4 weeks (Zogg and others 2000). Our results underscore the potential of mineral soils to serve as a strong sink for N in forests. While the 10–30% recovery in SOM may not appear particularly high, we note it is only in the top 10 cm of mineral soil. We applied the $^{15}NO_3^-$ at a relatively high concentration, and it is likely that the uptake capacity was exceeded initially and a substantial amount of the tracer remained as $^{15}NO_3^-$ when rain fell shortly after the 2-day sample collection, leading it to be flushed it deeper into the soil profile. Forest soils in this region have large amounts of C-rich soil deeper than 10 cm (Huntington and others 1988), so we presume total recovery would be greater if deeper soils were accounted for. Although we did not measure tracer recovery in roots or aboveground plant parts, we speculate that root retention was high, further explaining the relatively low SOM recovery. Most tracer studies have found relatively low recovery of ^{15}N in roots (typically < 15%; Lamontagne and others 2000; Perakis and Hedin 2001; Nadelhoffer and others 2004; Templer and others 2005; Goodale and others 2015), but these studies have traced $^{15}NO_3^-$ that was applied to the forest floor where immobilization is high due to an abundance of microbes and labile C. Probably the most analogous study to our mineral soil $^{15}NO_3^-$ addition was reported by Weitzman and Kaye (2016), who found that roots in the upper 15 cm of mineral soil retained up to 50% or more of the $^{15}NO_3^-$-N after 20 days. Over a longer term (several years), we would expect tracer ^{15}N in roots to decline due to turnover while concentrations in the mineral soil would potentially increase (Goodale 2017).

We did not find a significant effect of forest age on soil $^{15}NO_3^-$-N retention, but we did find that retention was correlated with SOM properties like C/N and $\delta^{13}C$, and those properties were generally correlated with forest age (Figure 2), including the C/N ratio (negative correlation) and natural abundances of ^{15}N and ^{13}C (positive correlations). The SOM with lowest C/N and highest background $\delta^{15}N$ was found at the old-growth stands (Figure 2, Tables 2–3). While the patterns of C/N and background $\delta^{15}N$ in SOM with forest age were evident, the reasons for such a relationship are not completely clear. However, multiple documented processes could help drive these patterns. Mineral soil N concentrations can decrease in younger successional stands due to N mining in support of plant growth (Richter and others 2000; Lovett and others 2018), and mineral soil C concentrations can increase from recent clearcuts to mid-successional to mature hardwood forests (Tang and others 2009). The low C/N and high $\delta^{15}N$ of SOM in the old-growth forests could also be a manifestation of increased microbial processing of SOM (Sollins and others 2006) in the absence of strong plant demands associated with net biomass accrual.

Distribution of ^{15}N Recovery Between SOM Fractions, with Depth and Time

Although we predicted that the POM pool would be the dominant initial sink for ^{15}N in SOM because of its presumed greater accessibility to microbes compared to MAOM, we found a substantial fraction of the tracer (~ 25% of the fraction recovered) in the MAOM pool, within 2 days after the tracer application. This relative distribution of tracer fate mirrored the relative distribution of

POM and MAOM in soil, as our fractionations indicate that approximately 30–40% of soil N is found in the MAOM. This result emphasizes that SOM associated with clay or silt particles is an important initial sink for N. Similarly, using density fractionation to separate SOM pools and track ^{15}N fates in the soil of an Oregon conifer forest, Holub and Lajtha (2004) found a strong initial sink for tracer ^{15}N (from ammonium, DON, and tannin-complexed organic N) in the heavy fraction, which is typically considered to be a less active and more recalcitrant pool of organic matter. Likewise, Goodale and others (2015) found slightly higher recovery of a ^{15}N-NO_3^- tracer in the heavy fraction than in the light fraction of a deciduous hardwood forest soil (Arnot Forest, New York), within 1 day of tracer application. In contrast, a $^{15}NO_3^-$ tracer study at Harvard Forest in Massachusetts found that a smaller portion ($< 5\%$) of the tracer was recovered in the heavy fraction after 18 h, whereas 17% was found in the light fraction (Compton and Boone 2002). It is unclear exactly what drives these differences in tracer recovery between SOM fractions, but could be related to variations in soil texture, water, and microbial communities. In general, ^{15}N tracer studies show that more tracer rapidly goes to ''inert'' SOM (heavy or MAOM fractions) than would be expected based on presumed microbial C availability.

Several studies have suggested that rapid abiotic immobilization of NO_3^- can be an important mechanism of N addition to stable SOM (for example, Dail and others 2001; Davidson and others 2003; Fitzhugh and others 2003), although others contend that abiotic immobilization is unlikely to occur or is ecologically insignificant (Colman and others 2008; Schmidt and Matzner 2009; Morier and others 2010). Because our earliest sampling point was two days after $^{15}NO_3^-$ application, a time when we could not differentiate between biotic and abiotic immobilizations, we cannot speculate on whether abiotic immobilization could have influenced our results.

Retention of $^{15}NO_3^-$ in POM and MAOM followed similar temporal patterns (Figure 3), suggesting that both pools provide transient sinks for actively cycling N. We had initially hypothesized that the sink for ^{15}N in MAOM would increase in importance with time as residues and byproducts resulting from microbial decomposition in the POM fraction became stabilized in MAOM. Separation of POM and MAOM is a coarse fractionation and is likely subject to some confounding issues. Although organic matter associated with the clay and silt size fraction of soil is thought to represent organic matter sorbed to minerals or physically protected in microaggregates (Jastrow and others 1996; Six and others 2002; Schrumpf and others 2013), it also could include a portion of active microbial biomass if microbes pass through the 53-μm sieve and are not discarded with the rinse water. Inclusion of a small amount of ^{15}N-labeled microbes could artificially inflate our estimate of tracer in the ''mineral-associated'' pool, especially in the days immediately following tracer addition.

There is also good reason to believe that the similar temporal patterns between ^{15}N in the POM and MAOM truly indicate that both fractions contain actively cycling SOM-N. Although SOM associated with minerals has traditionally been considered older and more stable (Eusterhues and others 2003; Kleber and others 2005; von Lützow and others 2006), Swanston and others (2005) were able to trace a pulse of ^{14}C (from industrially released $^{14}CO_2$ taken up by plants) to rapid incorporation into the strongly mineral-associated dense fraction following turnover of labeled roots and leaf litter. They reasoned this represented a fast-cycling C pool in MAOM. The MAOM fraction consists of organic matter adsorbed to mineral surfaces (Hassink 1997), although new organic matter adsorption largely occurs where organic matter is already present (Vogel and others 2014), and is found in layers (Sollins and others 2006; Kleber and others 2007). Evidence suggests the outer layers of organic matter may be involved in active cycling (Swanston and others 2005). Holub and Lajtha (2004) found ^{15}N moved from the heavy to the light fraction with time and suggested that microbes may transport the N from the heavy fraction to use in decomposition of plant material in the light fraction. We propose that both the MAOM and POM fractions both contain rapidly cycling pools that are subject to turnover and loss (through leaching or root uptake).

The decrease in ^{15}N we recovered in SOM with time is consistent with other N tracer studies (Seely and Lajtha 1997; Zogg and others 2000; Weitzman and Kaye 2016) and suggests that a considerable portion of the SOM is subject to active cycling rather than in a form stable over the long term. Similarly, while a substantial portion of the tracer was retained in the shallow 0–3-cm layer after 2 days, much of that moved deeper in subsequent days as the shallow soil ^{15}N concentration decreased and the 3–10 cm ^{15}N concentration increased (Figure 3). This movement can be explained by multiple processes, including rapid assimilation followed by fast remineralization (Curtis and others 2011), hydrologic transport of

soluble microbially derived compounds (Kalbitz and others 2003; Kaiser and Kalbitz 2012), or microbially derived N-rich hydrophilic organic compounds displaced from weak sorption by plant-derived DOM (Scott and Rothstein 2014). Interestingly, in most of our soils the 0–3-cm layer ^{15}N recovery appeared to stabilize or even increase between nine and 35 days (Figure 3). This may be due to increased sequestration of N in SOM fractions with longer-term stability or reflect a near net balance of downward hydrologic N transport and upward transport by fungal hyphae, as is the case with N found in decomposing surface leaf litter (Hart and Firestone 1991; Fahey and others 2011).

Relevance to Conceptual and Quantitative Ecosystem Models

Vitousek and Reiners (1975) hypothesized that forest succession should tightly control losses of a limiting nutrient such as N, with high retention of the nutrient in aggrading stands, and higher losses in old-growth forests that have lower rates of net biomass accumulation. In the case of N, these patterns should be expressed in the losses of plant-available inorganic N, though continued losses of plant-unavailable dissolved organic N (DON) can theoretically maintain ecosystem N limitation over long periods, especially in unpolluted ecosystems (Perakis and Hedin 2002). In the northeastern USA, where atmospheric N deposition has been chronically elevated, inorganic N leaching has been high in old-growth forests relative to younger forests (Vitousek and Reiners 1975; Gorham and others 1979; Goodale and others 2000, 2003). In contrast, Fisk and others (2002) did not observe elevated inorganic N leaching from old-growth stands compared to mature second-growth stands in the western Upper Peninsula of Michigan, where N deposition has historically been lower than in the Northeast.

Lovett and others (2018) proposed a revision of the Vitousek and Reiners (1975) hypothesis, suggesting that aggrading forests with N demands exceeding inputs must ''mine'' N from SOM, creating a relative deficit of N in the soil. Later in succession, as plant biomass accrual rates decline and net plant N accumulation approaches zero, N will re-accumulate in the mineral soil until it is closer to capacity and inorganic N leaching increases. The Lovett and others (2018) model (the N Bank hypothesis) proposes that the mineral soil would provide an enhanced sink for N in recently mature forests that would not be present in old-growth forest soils. This pattern could at least partly explain the current low leaching losses of N in mature forests such as the reference watershed at the HBEF. Additionally, the N Bank hypothesis leads to the expectation that in young forests the N sink in SOM would reflect a balance between a deficit in SOM-N created by recent N mining and strong vegetation sink due to rapid biomass accumulation. While we did not find statistically significant differences in soil $^{15}NO_3^-$ retention among the forest age classes, our results qualitatively follow the expected pattern, with the highest NO_3^- retention in mature forests and the lowest in old-growth forests (Figure 5), and thus provide some support for the Lovett and others' (2018) model. We note that chronosequence studies are notoriously challenging in mixed forests on heterogeneous landscapes (Yanai and others 2000), and our study only represented a five-week trace, which may or may not correspond to net retention patterns over a longer term.

The results of our study suggest the need for quantitative simulation models to better address the factors affecting SOM accumulation or stability during all stages of forest succession (Kaye and others 2003), as well as how they may change over the course of succession. It is possible that SOM provides both short- and long-term sinks for N that increases in importance as forests mature (Lovett and others 2018), but the ability of current models to simulate this may be limited by factors such as the quality of the parameters describing C/N effects on N turnover, estimates of total SOM accumulation and its partitioning into fractions of differing stability, controls on DON leaching, or their lack of processes of SOM-N mining or direct representation of microbial N cycling. In particular, our results relating NO_3^--N retention to mineral soil C/N and SOM fractions can be used to help test or parameterize models in their simulation of NO_3^- retention in mineral horizons. The similar temporal patterns of ^{15}N recovery in POM and MAOM fractions emphasize the need to study in more detail the longer-term dynamics of N in various soil fractions and how that relates to the active and passive SOM pools in simulation models.

Summary and Conclusions

Our results suggest that SOM in the upper mineral horizon can be an important sink for N in temperate northern forests throughout succession. We also demonstrate the importance of both particulate- and mineral-associated SOM as dynamic sinks for N. In mature forests at the HBEF, inorganic N leaching losses have been consistently lower than

expected based on traditional theory and current models. Retention of N in mineral soil likely explains some of this mismatch, but more extensive or longer-term studies are needed to determine whether the SOM sink for N is indeed stronger in recently mature forests before declining in old-growth forests. Short-term ^{15}N tracer studies like ours cannot quantify net ecosystem N balances, but they do provide useful tools for tracking the fate of N over particular timescales (Lovett and Goodale 2011) and can aid models designed to predict N balances over longer periods.

ACKNOWLEDGEMENTS

We thank Nick Jakubek, Wade Simmons, Liza Tetley, Charlene Tarsa, and Mikayla Jacobs for assistance with field or laboratory work. We also thank two reviewers that provided helpful comments to improve the manuscript. The HBEF is administered by the US Department of Agriculture Forest Service, Northern Forest Research Station, Newtown Square, PA. Hubbard Brook is a National Science Foundation supported Long-Term Ecological Research site. Support for this project was provided by the National Science Foundation (DEB 1257956—Ecosystem Studies; and DEB 1633026 and DEB 1637685—Long-Term Ecological Research).

REFERENCES

Aber JD, Driscoll CT. 1997. Effects of land use, climate variation, and N deposition on N cycling and C storage in northern hardwood forests. Glob Biogeochem Cycles 11:639–48.

Aber JD, Goodale CL, Ollinger SV, Smith M-L, Magill AH, Martin ME, Hallett RA, Stoddard JL. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience 53:375–89.

Aber JD, Ollinger SV, Driscoll CT, Likens GE, Holmes RT, Freuder RJ, Goodale CL. 2002. Inorganic nitrogen losses from a forested ecosystem in response to physical, chemical, biotic, and climatic perturbations. Ecosystems 5:0648–58.

Bailey SW, Brousseau PA, McGuire KJ, Ross DS. 2014. Influence of landscape position and transient water table on soil development and carbon distribution in a steep, headwater catchment. Geoderma 226–227:279–89.

Beare MH, McNeill SJ, Curtin D, Parfitt RL, Jones HS, Dodd MB, Sharp J. 2014. Estimating the organic carbon stabilisation capacity and saturation deficit of soils: a New Zealand case study. Biogeochemistry 120:71–87.

Bernal S, Hedin LO, Likens GE, Gerber S, Buso DC. 2012. Complex response of the forest nitrogen cycle to climate change. PNAS 109:3406–11.

Bingham AH, Cotrufo MF. 2016. Organic nitrogen storage in mineral soil: implications for policy and management. Sci Total Environ 551–552:116–26.

Boström B, Comstedt D, Ekblad A. 2007. Isotope fractionation and ^{13}C enrichment in soil profiles during the decomposition of soil organic matter. Oecologia 153:89–98.

Buurman P, Jongmans AG. 2005. Podzolisation and soil organic matter dynamics. Geoderma 125:71–83.

Castellano MJ, Kaye JP, Lin H, Schmidt JP. 2012. Linking carbon saturation concepts to nitrogen saturation and retention. Ecosystems 15:175–87.

Colman BP, Fierer N, Schimel JP. 2008. Abiotic nitrate incorporation, anaerobic microsites, and the ferrous wheel. Biogeochemistry 91:223–7.

Compton JE, Boone RD. 2002. Soil nitrogen transformations and the role of light fraction organic matter in forest soils. Soil Biol Biochem 34:933–43.

Craine JM, Elmore AJ, Wang L, Augusto L, Baisden WT, Brookshire ENJ, Cramer MD, Hasselquist NJ, Hobbie EA, Kahmen A, Koba K, Kranabetter JM, Mack MC, Marin-Spiotta E, Mayor JR, McLauchlan KK, Michelsen A, Nardoto GB, Oliveira RS, Perakis SS, Peri PL, Quesada CA, Richter A, Schipper LA, Stevenson BA, Turner BL, Viani RAG, Wanek W, Zeller B. 2015. Convergence of soil nitrogen isotopes across global climate gradients. Sci Rep 5:8280.

Curtis CJ, Evans CD, Goodale CL, Heaton THE. 2011. What have stable isotope studies revealed about the nature and mechanisms of N saturation and nitrate leaching from semi-natural catchments? Ecosystems 14:1021–37.

Dail DB, Davidson EA, Chorover J. 2001. Rapid abiotic transformation of nitrate in an acid forest soil. Biogeochemistry 54:131–46.

Davidson EA, Chorover J, Dail DB. 2003. A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. Glob Change Biol 9:228–36.

Dittman JA, Driscoll CT, Groffman PM, Fahey TJ. 2007. Dynamics of nitrogen and dissolved organic carbon at the Hubbard Brook Experimental Forest. Ecology 88:1153–66.

Driscoll CT, Whitall D, Aber J, Boyer E, Castro M, Cronan C, Goodale CL, Groffman P, Hopkinson C, Lambert K, Lawrence G, Ollinger S. 2003. Nitrogen pollution in the northeastern United States: sources, effects, and management options. BioScience 53:357–74.

Eusterhues K, Rumpel C, Kleber M, Kögel-Knabner I. 2003. Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. Org Geochem 34:1591–600.

Evans CD, Caporn SJM, Carroll JA, Pilkington MG, Wilson DB, Ray N, Cresswell N. 2006. Modelling nitrogen saturation and carbon accumulation in heathland soils under elevated nitrogen deposition. Environ Pollut 143:468–78.

Fahey TJ, Hughes JW, Pu M, Arthur MA. 1988. Root decomposition and nutrient flux following whole-tree harvest of northern hardwood forest. For Sci USA. http://agris.fao.org/agris-search/search.do?recordID=US8860983. Last accessed 08/02/2018.

Fahey TJ, Siccama TG, Driscoll CT, Likens GE, Campbell J, Johnson CE, Battles JJ, Aber JD, Cole JJ, Fisk MC, Groffman PM, Hamburg SP, Holmes RT, Schwarz PA, Yanai RD. 2005. The biogeochemistry of carbon at Hubbard Brook. Biogeochemistry 75:109–76.

Fahey TJ, Yavitt JB, Sherman RE, Groffman PM, Fisk MC, Maerz JC. 2011. Transport of carbon and nitrogen between litter and soil organic matter in a northern hardwood forest. Ecosystems 14:326–40.

Fisk MC, Zak DR, Crow TR. 2002. Nitrogen storage and cycling in old- and second-growth northern hardwood forests. Ecology 83:73–87.

Fitzhugh RD, Likens GE, Driscoll CT, Mitchell MJ, Groffman PM, Fahey TJ, Hardy JP. 2003. Role of soil freezing events in interannual patterns of stream chemistry at the Hubbard Brook experimental forest, New Hampshire. Environ Sci Technol 37:1575–80.

Fuss CB, Driscoll CT, Campbell JL. 2015. Recovery from chronic and snowmelt acidification: long-term trends in stream and soil water chemistry at the Hubbard Brook Experimental Forest, New Hampshire, USA. J Geophys Res Biogeosci 120:2360–74.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–92.

Gbondo-Tugbawa SS, Driscoll CT. 2002. Retrospective analysis of the response of soil and stream chemistry of a northern forest ecosystem to atmospheric emission controls from the 1970 and 1990 amendments of the Clean Air Act. Environ Sci Technol 36:4714–20.

Goodale CL. 2017. Multiyear fate of a 15N tracer in a mixed deciduous forest: retention, redistribution, and differences by mycorrhizal association. Glob Change Biol 23:867–80.

Goodale CL, Aber JD, McDowell WH. 2000. The long-term effects of disturbance on organic and inorganic nitrogen export in the white mountains, New Hampshire. Ecosystems 3:433–50.

Goodale CL, Aber JD, Vitousek PM. 2003. An unexpected nitrate decline in New Hampshire streams. Ecosystems 6:0075–86.

Goodale CL, Fredriksen G, Weiss MS, McCalley CK, Sparks JP, Thomas SA. 2015. Soil processes drive seasonal variation in retention of 15N tracers in a deciduous forest catchment. Ecology 96:2653–68.

Gorham E, Vitousek PM, Reiners WA. 1979. The regulation of chemical budgets over the course of terrestrial ecosystem succession. Annu Rev Ecol Syst 10:53–84.

Groffman PM, Driscoll CT, Durán J, Campbell JL, Christenson LM, Fahey TJ, Fisk MC, Fuss C, Likens GE, Lovett G, Rustad L, Templer PH. 2018. Nitrogen oligotrophication in northern hardwood forests. Biogeochemistry 141:523–39.

Hagedorn F, Maurer S, Bucher JB, Siegwolf RTW. 2005. Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO2: a ^{15}N and ^{13}C tracer study. Glob Change Biol 11:1816–27.

Hart SC, Firestone MK. 1991. Forest floor-mineral soil interactions in the internal nitrogen cycle of an old-growth forest. Biogeochemistry 12:103–27.

Hassink J. 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant Soil 191:77–87.

Högberg P. 1997. Tansley Review No. 95 ^{15}N natural abundance in soil–plant systems. New Phytol 137:179–203.

Holmes WE, Zak DR. 1994. Soil microbial biomass dynamics and net nitrogen mineralization in northern hardwood ecosystems. Soil Sci Soc Am J 58:238–43.

Holub SM, Lajtha K. 2004. The fate and retention of organic and inorganic 15N-nitrogen in an old-growth forest soil in Western Oregon. Ecosystems 7:368–80.

Huntington TG, Ryan DF, Hamburg SP. 1988. Estimating soil nitrogen and carbon pools in a northern hardwood forest ecosystem. Soil Sci Soc Am J 52:1162.

Jastrow JD, Miller RM, Boutton TW. 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Sci Soc Am J 60:801–7.

Johnson CE, Johnson AH, Huntington TG, Siccama TG. 1991. Whole-tree clear-cutting effects on soil horizons and organic-matter pools. Soil Sci Soc Am J 55:497–502.

Kaiser K, Kalbitz K. 2012. Cycling downwards—dissolved organic matter in soils. Soil Biol Biochem 52:29–32.

Kalbitz K, Schwesig D, Schmerwitz J, Kaiser K, Haumaier L, Glaser B, Ellerbrock R, Leinweber P. 2003. Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biol Biochem 35:1129–42.

Kaye JP, Binkley D, Rhoades C. 2003. Stable soil nitrogen accumulation and flexible organic matter stoichiometry during primary floodplain succession. Biogeochemistry 63:1–22.

Keiluweit M, Nico PS, Kleber M, Fendorf S. 2016. Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils? Biogeochemistry 127:157–71.

Kleber M, Mikutta R, Torn MS, Jahn R. 2005. Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. Eur J Soil Sci 56:717–25.

Kleber M, Sollins P, Sutton R. 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85:9–24.

Kopáček J, Cosby BJ, Evans CD, Hruška J, Moldan F, Oulehle F, Šantrůčková H, Tahovská K, Wright RF. 2013. Nitrogen, organic carbon and sulphur cycling in terrestrial ecosystems: linking nitrogen saturation to carbon limitation of soil microbial processes. Biogeochemistry 115:33–51.

Kramer MG, Lajtha K, Aufdenkampe AK. 2017. Depth trends of soil organic matter C: N and 15N natural abundance controlled by association with minerals. Biogeochemistry 136:237–48.

Kuzyakov Y, Xu X. 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol 198:656–69.

Lamontagne S, Schiff SL, Elgood RJ. 2000. Recovery of ^{15}N-labelled nitrate applied to a small upland boreal forest catchment. Can J For Res 30:1165–77.

LeBauer DS, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–9.

Lehmann J, Kleber M. 2015. The contentious nature of soil organic matter. Nature 528:60–8.

Lewis DB, Castellano MJ, Kaye JP. 2014. Forest succession, soil carbon accumulation, and rapid nitrogen storage in poorly remineralized soil organic matter. Ecology 95:2687–93.

Likens GE. 2013. Biogeochemistry of a forested ecosystem. New York: Springer.

Lovett GM, Goodale CL. 2011. A new conceptual model of nitrogen saturation based on experimental nitrogen addition to an oak forest. Ecosystems 14:615–31.

Lovett GM, Goodale CL, Ollinger SV, Fuss CB, Ouimette AP, Likens GE. 2018. Nutrient retention during ecosystem succession: a revised conceptual model. Front Ecol Environ 16:532–8.

Morier I, Schleppi P, Saurer M, Providoli I, Guenat C. 2010. Retention and hydrolysable fraction of atmospherically de-

posited nitrogen in two contrasting forest soils in Switzerland. Eur J Soil Sci 61:197–206.

Nadelhoffer KJ, Colman BP, Currie WS, Magill A, Aber JD. 2004. Decadal-scale fates of ^{15}N tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA). For Ecol Manag 196:89–107.

Nadelhoffer KJ, Fry B. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci Soc Am J 52:1633–40.

Pan Y, Chen JM, Birdsey R, McCullough K, He L, Deng F. 2011. Age structure and disturbance legacy of North American forests. Biogeosciences 8:715–32.

Perakis SS, Hedin LO. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, Southern Chile. Ecology 82:2245–60.

Perakis SS, Hedin LO. 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. Nature 415:416–19.

Pourmokhtarian A, Driscoll CT, Campbell JL, Hayhoe K. 2012. Modeling potential hydrochemical responses to climate change and increasing CO_2 at the Hubbard Brook Experimental Forest using a dynamic biogeochemical model (PnET-BGC). Water Resour Res 48:W07514.

Pries CEH, Bird JA, Castanha C, Hatton P-J, Torn MS. 2017. Long term decomposition: the influence of litter type and soil horizon on retention of plant carbon and nitrogen in soils. Biogeochemistry 134:5–16.

Richter DD, Markewitz D, Heine PR, Jin V, Raikes J, Tian K, Wells CG. 2000. Legacies of agriculture and forest regrowth in the nitrogen of old-field soils. For Ecol Manag 138:233–48.

Riha SJ, Campbell GS, Wolfe J. 1986. A model of competition for ammonium among heterotrophs, nitrifiers, and roots 1. Soil Sci Soc Am J 50:1463–6.

Rumpel C, Kögel-Knabner I. 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant Soil 338:143–58.

Schmidt BHM, Matzner E. 2009. Abiotic reaction of nitrite with dissolved organic carbon? Testing the ferrous wheel hypothesis. Biogeochemistry 93:291–6.

Schrumpf M, Kaiser K, Guggenberger G, Persson T, Kögel-Knabner I, Schulze E-D. 2013. Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences 10:1675–91.

Scott EE, Rothstein DE. 2014. The dynamic exchange of dissolved organic matter percolating through six diverse soils. Soil Biol Biochem 69:83–92.

Seely B, Lajtha K. 1997. Application of a 15N tracer to simulate and track the fate of atmospherically deposited N in the coastal forests of the Waquoit Bay Watershed, Cape Cod, Massachusetts. Oecologia 112:393–402.

Six J, Conant RT, Paul EA, Paustian K. 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241:155–76.

Sollins P, Swanston C, Kleber M, Filley T, Kramer M, Crow S, Caldwell BA, Lajtha K, Bowden R. 2006. Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. Soil Biol Biochem 38:3313–24.

Stark JM, Hart SC. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385:61.

Swanston CW, Torn MS, Hanson PJ, Southon JR, Garten CT, Hanlon EM, Ganio L. 2005. Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. Geoderma 128:52–62.

Tahovská K, Kaňa J, Bárta J, Oulehle F, Richter A, Šantrůčková H. 2013. Microbial N immobilization is of great importance in acidified mountain spruce forest soils. Soil Biol Biochem 59:58–71.

Tang J, Bolstad PV, Martin JG. 2009. Soil carbon fluxes and stocks in a Great Lakes forest chronosequence. Glob Change Biol 15:145–55.

Templer PH, Lovett GM, Weathers KC, Findlay SE, Dawson TE. 2005. Influence of tree species on forest nitrogen retention in the Catskill Mountains, New York, USA. Ecosystems 8:1–16.

Templer PH, Mack MC, Iii FSC, Christenson LM, Compton JE, Crook HD, Currie WS, Curtis CJ, Dail DB, D'Antonio CM, Emmett BA, Epstein HE, Goodale CL, Gundersen P, Hobbie SE, Holland K, Hooper DU, Hungate BA, Lamontagne S, Nadelhoffer KJ, Osenberg CW, Perakis SS, Schleppi P, Schimel J, Schmidt IK, Sommerkorn M, Spoelstra J, Tietema A, Wessel WW, Zak DR. 2012. Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of ^{15}N tracer field studies. Ecology 93:1816–29.

Vitousek PM, Reiners WA. 1975. Ecosystem succession and nutrient retention: a hypothesis. BioScience 25:376–81.

Vogel C, Mueller CW, Höschen C, Buegger F, Heister K, Schulz S, Schloter M, Kögel-Knabner I. 2014. Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nat Commun 5:2947.

von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions—a review. Eur J Soil Sci 57:426–45.

Wagai R, Kajiura M, Asano M, Hiradate S. 2015. Nature of soil organo-mineral assemblage examined by sequential density fractionation with and without sonication: is allophanic soil different? Geoderma 241–242:295–305.

Weitzman JN, Kaye JP. 2016. Variability in soil nitrogen retention across forest, urban, and agricultural land uses. Ecosystems 19:1345–61.

Yanai RD, Arthur MA, Siccama TG, Federer CA. 2000. Challenges of measuring forest floor organic matter dynamics: repeated measures from a chronosequence. For Ecol Manag 138:273–83.

Yanai RD, Vadeboncoeur MA, Hamburg SP, Arthur MA, Fuss CB, Groffman PM, Siccama TG, Driscoll CT. 2013. From missing source to missing sink: long-term changes in the nitrogen budget of a northern hardwood forest. Environ Sci Technol 47:11440–8.

Zogg GP, Zak DR, Pregitzer KS, Burton AJ. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81:1858–66.