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Forest succession, soil carbon accumulation, and rapid nitrogen storage in poorly-remineralized soil organic matter

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Forest succession, soil carbon accumulation, and rapid nitrogen storage in poorly remineralized soil organic matter

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Abstract. Substantial nitrogen (N) retention by temperate terrestrial ecosystems results from the rapid storage of newly deposited N in stable soil organic matter. Yet, we poorly understand the ecosystem properties that regulate the kinetics of this process. We applied mineral ^{15}N to temperate hardwood forest soils to test the hypothesis that N stabilization is faster owing to greater stocks of soil carbon (C) in late-successional than in young forests. Within 26 minutes of addition, about 30% of tracer N was stored in stable form in organic-horizon soil with a median residence time of >29 years. About 5–10% of tracer N was stored in a soluble organic form. An additional 30% of tracer N was recovered within hours from organic-horizon soils in a remineralizable (labile) form, apparently derived from microbial biomass. Over the following year, tracer N storage in stable and soluble organic pools remained constant while recovery from labile and microbial pools declined. Tracer storage was greater in older forests with larger soil C pools, supporting our hypothesis that the accumulation of soil C with forest succession promotes ecosystem N retention. Rapid storage of stable soil N in the O horizon may create a source for chronic dissolved organic N losses from watersheds.

Key words: carbon; dissolved organic nitrogen; ecosystem; kinetics; mass balance; microbial biomass; nitrogen retention; Pennsylvania; soil organic matter; succession; temperate hardwood forest; watershed.

INTRODUCTION

Human activities have tripled the preindustrial global rate at which atmospheric nitrogen (N_2) is fixed into reactive N forms, triggering rapid N cycling throughout the biosphere (Galloway et al. 2008). These accelerated N fluxes degrade environmental quality and alter ecological systems with biota adapted to scarce nitrogen. Such outcomes have stimulated interest in identifying large sinks that might rapidly remove N from active cycling for long periods.

One such N sink in terrestrial ecosystems is soil organic matter (SOM; Templer et al. 2012). Nitrogen storage in SOM is rapid. Within just hours to days of being introduced to soil in reactive mineral forms, N is incorporated in soil organic molecules (Zogg et al. 2000, Morier et al. 2008). Furthermore, this N storage in SOM is stable. Mineral ^{15}N added to soil as a tracer (1) has subsequently been recovered in fractions thought to be recalcitrant based on their physical properties (Nõmmik 1970, Castellano et al. 2012), (2) remains in SOM for at least a year after it is initially incorporated (Perakis and Hedin 2001), and (3) exhibits limited potential for leaching and remineralization (Kaye et al. 2002, Hagedorn et al. 2005).

This rapid stabilization of N in soil organic matter is an important process because it could explain why ecosystems accumulate N even while there is no increase in the size of biological N pools such as live vegetation (Dise and Wright 1995, Goodale et al. 2000) and microbial biomass (Zogg et al. 2000, Corre et al. 2007). Owing to the significance of rapid N stabilization as a sink in the N cycle, we must now determine its pathways (e.g., microbial turnover, abiotic reactions), its kinetics, and the ecosystem properties that might regulate those kinetics. Ecosystem successional state may be one of these regulating properties. As noted above, net whole-ecosystem N retention results from the storage of N in soil organic molecules. And because soil organic C (SOC) accumulates as forests age (Pregitzer

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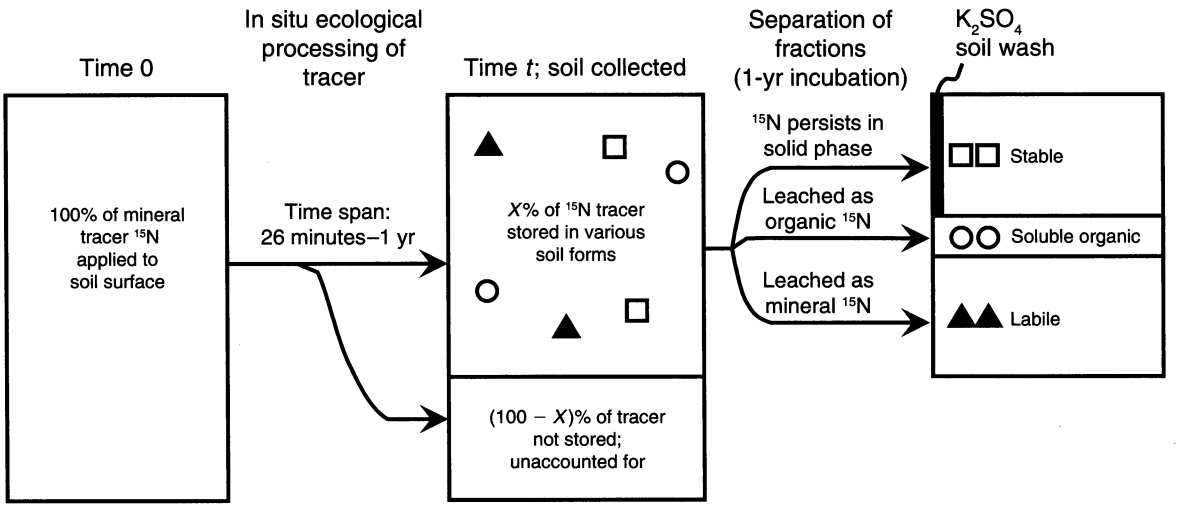

FIG. 1. A depiction of ^{15}N pulse-chase procedure and operational definition of stable, soluble, and labile organic tracer N pools.

and Euskirchen 2004), soil N storage and thus ecosystem N retention may be greater in older forests (Fisk et al. 2002).

While it is understood that organic C promotes N storage in soil (Hart et al. 1994), the importance of SOC in the kinetics of rapid N stabilization is not well established. Here, we test the hypothesis that N stabilization in soil is faster owing to greater SOC stocks in late-successional forest stands than in young stands recovering from recent clear cuts. In doing so, we document the kinetics of N transfer from mineral pools into stable, labile (remineralizable), microbial, and soluble organic N pools. By investigating how the accumulation of SOC may regulate rapid N storage in these pools, this work aims to elucidate the role of forest age in ecosystem nutrient retention, and further develop succession as an organizing concept in ecology.

METHODS

We combined a tracer application of inorganic ^{15}N with an experimental contrast in forest age. We sprayed ammonium nitrate as $^{15}NH_4{}^{15}NO_3$ (99 atom% ^{15}N) onto the surface of the soil organic horizon in temperate hardwood stands in central Pennsylvania, USA. We added NH_4^+ and NO_3^- to reflect the notable presence of both compounds in atmospheric N deposition. We used whole-stand manipulations of forest age that controlled for a common climate, forest composition, and soil type (a well-drained, loamy-skeletal, mixed Typic Dystrudepts). A solution of tracer ^{15}N was sprayed to three young stands (7–15 years since last harvest) and to three late-successional, second-growth stands (100–120 years old) dominated by red oak (*Quercus rubra*) and white oak (*Q. alba*). Tracer was added at 89 mg $^{15}N/m^2$, elevating bulk-soil NO_3^- and NH_4^+ pools by 15% each, perhaps temporarily stimulating microbial turnover and initial tracer N storage. We collected organic-horizon (O-horizon) and mineral (top 15 cm) soil to quantify tracer recovery at 11 time points ranging from 26 minutes to 363 days following tracer application.

Soil collected at each of the 11 time points immediately commenced a year-long laboratory incubation (25°C at field capacity moisture) to biologically assay the stability of SOC and any stored ^{15}N (Fig. 1). Soil was leached 19 times during the incubation to persistently remove mineral N. Leaching was more frequent initially (four times in the first two weeks) and tapered off to monthly. This leaching was done with a C-free and N-free water solution supplemented with millimolar and micromolar concentrations of phosphorus, base cations, and trace metals (hereafter, water). The soil was finally washed with a salt solution (5 mL of 0.5 mol/L K_2SO_4 for every g soil) at the end of the incubation to remove residual exchangeable N.

Stable tracer N was operationally defined as tracer N still present in solid-phase soil after the incubation and K_2SO_4 wash, and was detected by analyzing post-incubation soil with isotope-ratio mass spectrometry (IRMS). We interpret this operationally defined pool to include N that is bound and thus weakly or not cycling biologically, and N that is tightly cycled by microbial biomass with a relatively short residence time in free mineral and soluble organic forms. Labile tracer N was defined as tracer N present in soil upon field collection, but leached by water during the subsequent incubation as inorganic (mineralized) N. Additionally, we measured tracer N leached (by water) as dissolved organic N (DON), reflecting stored organic N that was soluble but not mineralized. Inorganic and organic tracer N were detected in incubation leachate by converting N to ammonia (NH_3), diffusing it from solution, and trapping it as NH_4^+ on an acid trap that was analyzed with IRMS. At three of the time points (12 h, 21 d, and 77 d) following ^{15}N application, we quantified tracer N

TABLE 1. Features of the vegetation and organic-horizon soils in investigated forest stands.

Stand	Stand age (yr)†	Basal area (m^2/ha)‡	Species composition (%)†	Areal O-horizon mass (kg/m^2)	Total soil C (g/m^2)	Total soil N (g/m^2)	Stable C (g/m^2)	Labile C (g/m^2)	Stable N (g/m^2)	Labile N (g/m^2)
A	7			3.4 (0.4)	769 (84)	42 (5)	521 (64)	248 (26)	40.0 (5.2)	2.5 (0.3)
C	7			3.7 (0.5)	864 (109)	45 (6)	604 (89)	261 (30)	42.7 (6.1)	2.6 (0.4)
E	15			3.7 (0.4)	718 (59)	39 (3)	542 (47)	176 (22)	36.5 (3.4)	2.7 (0.3)
B	100–120	0.62	46, 30	5.1 (0.5)	1301 (88)	61 (4)	909 (86)	392 (28)	55.1 (4.5)	6.0 (0.5)
D	100–120	0.35	20, 23	6.1 (0.6)	1659 (118)	92 (5)	1227 (107)	432 (20)	85.3 (4.9)	6.6 (0.8)
F	100–120	4.69	48, 15	6.4 (0.8)	1478 (124)	80 (7)	1117 (107)	360 (23)	71.9 (6.5)	6.4 (0.6)

Note: Values for soil information are means with SE in parentheses. Stands A, C, and E are recent clear-cuts. Stands B, D, and F are 100–120-year-old second-growth forests. Information on stand age, basal area, and species composition was provided by J. A. Harding.

† Exact age not known for stands B, D, and F.

‡ Values are given for *Quercus alba* and *Q. rubra*. Vegetation in stands A, C, and E was too young and small to inventory.

stored in microbial biomass using a chloroform fumigation-K_2SO_4 extraction procedure, followed by tracer N diffusion from the K_2SO_4 solution. Labile soil C was defined as C mineralized during the incubation, detected by monitoring CO_2 efflux with infrared gas analysis. Stable soil C was quantified as total C (detected with elemental analysis) minus labile C, so operationally includes soluble organic C. Full methods are in Appendix A.

Results and Discussion

Tracer N storage in stable and labile forms

In O-horizon soils collected 26 minutes after tracer ^{15}N application, total tracer recovery was 62%, 87%, and 84% of the applied tracer in the three young stands (stands A, C, and E in Table 1), and 81%, 87%, and 104% of the applied tracer in the three old stands (stand B, D, and F), with an overall average of 84% recovery. One year later, total tracer recovery had declined to 29%, 26%, and 25% in the young stands, and to 47%, 53%, and 74% in the old stands. This decline in total tracer recovery paralleled a decline in tracer storage in the labile fraction, while tracer storage in the stable and soluble organic fractions remained relatively constant (Fig. 2).

For O-horizon soils collected 26 minutes after tracer application, about 30% of applied tracer N was still present following a subsequent year of incubation with intensive leaching and extraction. We interpret this finding to indicate that within 26 minutes of application, approximately 30% of tracer N was stable (weakly or not actively cycling, or involved in tight mineralization–immobilization cycles). A similar amount of stable tracer N (about 30% of added tracer N) was found in all O-horizon soils collected thereafter, up to 363 d later, as evinced by no significant ($\alpha = 0.05$) time effect in repeated measures analysis of variance (Appendix B: Table B1). Tracer N was stored in the stable pool to a greater extent in late-successional O-horizon soil than in young O-horizon soil (39% vs. 28% of added tracer; $P < 0.05$, Appendix B: Table B2).

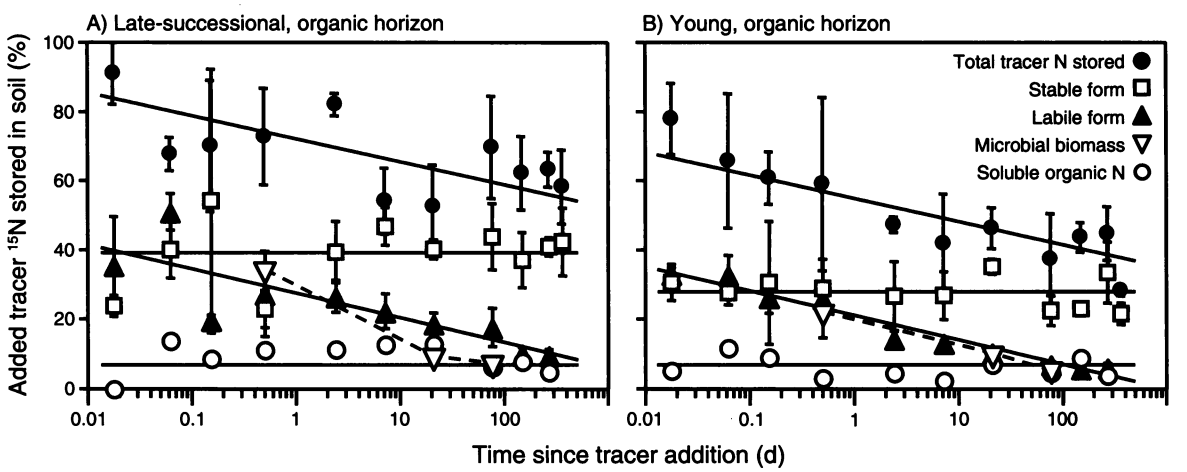

FIG. 2. Tracer ^{15}N storage in organic-horizon soil of (A) late-successional and (B) young temperate hardwood forests. Error bars are one standard error of the mean (error bars are omitted for storage in microbial biomass and soluble organic forms). Flat lines denote means across time for tracer storage in stable and soluble organic forms. Regression lines (see Appendix B: Table B2) denote effects of time since tracer addition on storage in total and labile pools. Dashed lines provide point-to-point connections for storage in microbial biomass.

Within 26 minutes of tracer application, about 30% of applied tracer N was present in the O horizon in mineralizable (labile organic) or mineral N form. That is, during the incubation of the soils collected 26 minutes after tracer application, about 30% of the applied tracer N was leached by water as mineral N. This labile + mineral (hereafter, labile) tracer N pool was consistently lower in subsequent soil collections (Fig. 2). This decline across sequential soil collections in labile tracer N occurred at similar rates in both late-successional and young O-horizon soils (Appendix B: Table B1), although labile pools in late-successional O-horizon soils held about 6% (of the added amount of tracer) more than did labile pools in young O-horizon soils ($P < 0.05$, Appendix B: Table B2). Labile tracer N, as a percentage of all tracer N recovered from O-horizon soil, declined across soil collections from 52% at 26 minutes after tracer application to 15% nine months later. Thus, the partitioning of O-horizon tracer N was trending toward the partitioning of O-horizon bulk-soil N, as labile bulk-soil N accounted for 5–10% of total bulk-soil N (Table 1).

For O-horizon soils collected 12 h and more after tracer application, tracer N leached as mineral N from the incubated soil had almost certainly been labile organic N (rather than never-cycled mineral tracer N) at the time of soil collection, given the dynamics of tracer N in microbial biomass. The amount of tracer N we recovered from microbial biomass at the time of soil collection was equivalent to the amount of tracer N that was leached as mineral N during the lengthy lab incubation that followed (t test for differences $P > 0.1$). Chloroform ($CHCl_3$) fumigation (our method for estimating microbial N) lyses microbial cells, which probably liberates mostly cytoplasmic materials, which in turn are thought to be readily mineralized (McGill et al. 1975). So the likelihood that $CHCl_3$-mobilized tracer N was in a readily mineralized form, coupled with the equivalent sizes of the $CHCl_3$-mobilized tracer N pool and the amount of mineral tracer N leached in the incubation, suggests that mineral tracer N leached in the incubation came from the mineralization of actively cycling (labile) N and not from a reservoir of unused tracer still in its initial mineral form. For soils collected within 3 h of tracer addition, it is conceivable that much of the mineral tracer N recovered during the 19 leaching events of the incubation actually came off in the first leaching event, which raises the possibility that it was never in a labile organic form. We do not know the time course of mineral tracer N release during the incubation.

The synchronous decline in labile and microbial tracer N across collections of O-horizon soil (Fig. 2) may indicate that labile tracer N cycled through microbial biomass while it was being depleted by loss to other sinks. These sinks were probably plant uptake, deep leaching, or gas loss, as the decline in labile tracer N in the O horizon did not correspond with an increase in stable or soluble organic tracer N in the O horizon (Fig. 2) nor with tracer N accumulation in the underlying 15 cm of mineral soil. After it was initially stored in the O horizon, any atom of tracer ^{15}N may have exchanged between recalcitrant detrital organic matter and active mineralization-immobilization cycles, and between the stable (long residence as immobilized) and leaky or labile components of those cycles. The relatively static sizes of stable and soluble organic tracer N pools in the O horizon (Fig. 2) do not mean that these pools are closed after initial storage. Rather, our results suggest that there is no net transfer of labile N to the stable and soluble refractory N pools after the initial allocation of newly added mineral N among these O-horizon pools. Thus, the contribution of O-horizon soil to net ecosystem N retention may be realized quite soon after N deposition.

Within mineral soil, storage of tracer in stable (4.1% of added N) and labile (5.5%) forms was much lower than was storage in O-horizon soil, and did not change through time nor differ between forest age groups (time and age effects $P > 0.05$, Appendix B: Table B3). Tracer abundance in mineral soils was inversely correlated with the mass of the overlying O horizon ($\sin^{-1}$[(fraction added N stored in mineral soil)$^{-0.5}$] vs. $\log_{10}$[overlying O-horizon mass], regression $P = 0.007$, $N = 60$, $r = -0.35$).

Roles of forest age and soil C in tracer N storage

Our data suggest that the storage in O-horizon soil of newly deposited N was greater in late-successional than in young forests because late-successional forests have more massive O-horizons. Storage of both labile and stable tracer N was greater where the areal content of O-horizon soil carbon was greater. Greater pools of stable and labile O-horizon carbon, in turn, were found where the O horizon was more massive (areal stable C vs. areal O horizon mass $r = 0.86$, areal labile C vs. areal O horizon mass $r = 0.63$, data $\log_{10}$-transformed). And, O horizon mass was greater in late-successional stands than in young stands (5.9 vs. 3.6 kg soil/m^2).

To integrate these pairwise relationships, we used path analysis to investigate whether effects of stand age on tracer ^{15}N storage in O-horizon soil might derive from effects of stand age on O-horizon mass and carbon content. The significance of these hierarchical relationships, and of time since tracer application for labile tracer N storage, was confirmed by this model after culling non-significant paths (Fig. 3). These relationships hold if either areal labile C or areal stable C is used to predict tracer N storage, reflecting the correlation between labile C and stable C ($r = 0.67$). The model was over fit and failed (zero lay within the 90% confidence intervals of many path coefficients) if labile C and stable C were both included in a single model as predictors of tracer N storage in O-horizon soil.

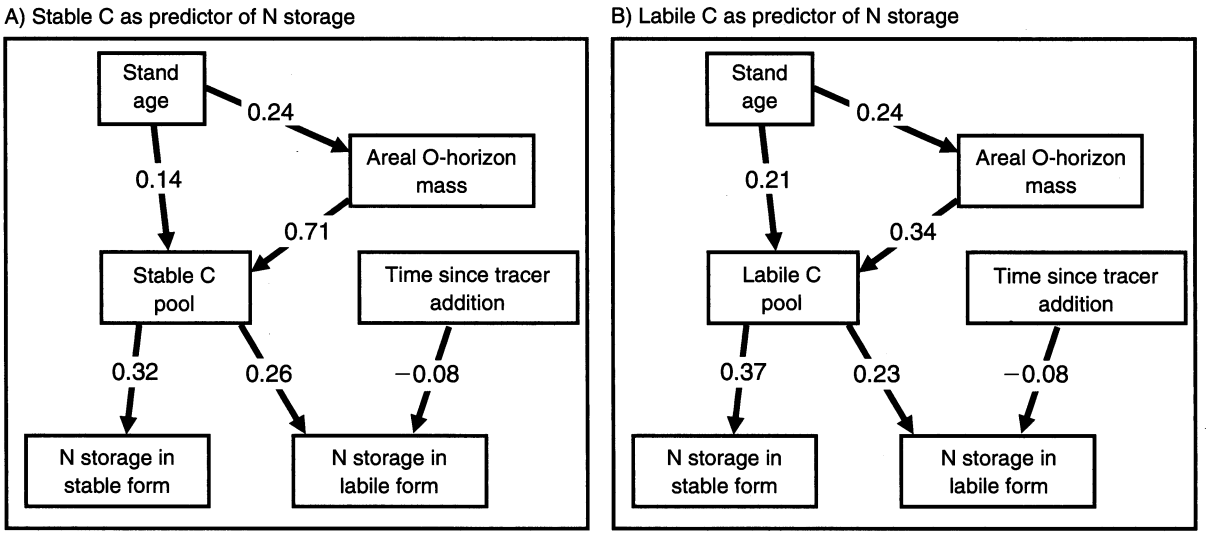

FIG. 3. Path analysis of tracer N storage in organic-horizon soil. Arrows show significant dependence paths and numbers on arrows denote path coefficients. (A) Model that uses O-horizon stable carbon as a predictor of tracer N storage. (B) Model that uses O-horizon labile C.

Larger soil C pools may promote greater soil N storage by fueling microbial N immobilization. If so, then labile C is probably the relevant soil C fraction because, on theoretical grounds, it should better fuel microbial turnover. Nitrogen immobilization in microbial biomass can happen within minutes to hours of N deposition (Zogg et al. 2000), and could stabilize N by placing it in tight mineralization-immobilization cycles (Stark and Hart 1997) that leave it scant opportunity for being exported, or by transferring the N to soil organic forms that persist as largely unmineralizable, recalcitrant necromass (McGill et al. 1975).

Soil C may also promote N storage by reacting abiotically with mineral N and producing organic N forms. Nitrite (NO_2^-) readily reacts abiotically with aromatic structures in soil organic matter (reviewed in Davidson et al. 2003), and storage in soil organic matter of N deposited as NO_2^- appears to be greater where SOC pools are larger (Lewis and Kaye 2012). Several carbon compounds (lignins, quinones, and phenols) associated with humus are thought to react abiotically with NH_3 (Schimel and Firestone 1989), although this process is unlikely to promote N storage in an organic horizon with low pH, where NH_3 is an unlikely N form (Nõmmik 1970). Another mechanism for stabilizing stored N is the binding and physical protection of partially degraded SOM, and its constituent N, with soil mineral particles (Kleber et al. 2007), although there were few if any mineral particles in the O horizon where we observed stable N storage.

We speculatively suggest that at least some of the rapid N stabilization resulted from microbial turnover transferring mineral N to microbial cell wall proteins. We have no data on the molecular forms of the stable tracer N we observed, but some of it was perhaps stored in amides, functional groups that serve as peptide bonds. Tracer ^{15}N is transferred to amides from quite different soil amendments, including mineral N (Clinton et al. 1995, Morier et al. 2008), plant residue N (DiCosty et al. 2003), and charred organic matter N (de la Rosa and Knicker 2011), so this transfer appears to be pervasive. Moreover, the amide signature appears stable, as it is present in the organic matter of all soil physical fractions, and remains constant through time even while the N concentrations of those fractions are changing (DiCosty et al. 2003). Any amides storing stable N are probably associated with microbial cell wall proteins, which are hypothesized to be more refractory than are cytoplasmic proteins (McGill et al. 1975).

Implications for watershed N cycles

Our results show that the storage in O-horizon soil of N deposited in mineral form is much faster than is the release of N back to free mineral form. A large fraction of inorganic N deposited to a forest O-horizon surface was stored within 26 minutes in a stable pool that resists leaching for at least a year (duration of our lab assay) and probably for decades. If the low soil respiration rates (median 20.9 μg C·[g soil]$^{-1}$·d^{-1}) observed at the end of our 1-yr assays could be maintained indefinitely (which is unlikely), the remaining soil organic C pool (median 225.8 mg C/g soil) would require a median of 29.6 years to fully mineralize. Presumably the tracer N stored with this C would similarly persist. In reality, mineralization rates would likely continue to diminish beyond one year, suggesting an even longer residence time for stabilized tracer N. Barring catastrophic N losses, this discrepancy between the kinetics of N storage

and N release necessitates the accrual of N in O-horizon soil, and perhaps in the whole ecosystem.

This kinetic discrepancy has implications for two aspects of our general understanding of watershed N cycles. First, early conceptual models proposed that more mature ecosystems were better at maintaining hold of their existing nutrient capital by means of efficient internal cycles, and that watersheds with mature ecosystems would export smaller nutrient loads than would their counterparts being reset (e.g., by clear-cutting) back to the initial stage of secondary succession (Odum 1969). Subsequent concepts emphasized plants as nutrient sinks, and maintained that slowdowns in the growth of older or chronically fertilized forests should curtail net (inputs > outputs) ecosystem N retention (Vitousek and Reiners 1975, Aber et al. 1989). We now recognize, as introduced above, that many temperate, terrestrial ecosystems exhibit net N retention in SOM even when biological N sinks are presumably not accruing. This sustained ecosystem N retention may be explained by the discrepancy between rapid soil N stabilization and slow soil N release (Hagedorn et al. 2005). Moreover, our results show that this discrepancy between rapid soil N stabilization and slow soil N release was of greater magnitude in older forests because one potential sink for N, namely the soil O horizon, accumulates with age. This finding suggests a mechanism by which older ecosystems might better maintain a tight hold on their nutrient capital, consistent with ideas from Odum (1969).

A second implication for our understanding of watershed N dynamics relates to DON export. Nitrogen losses from watersheds via DON export can be substantial (Perakis and Hedin 2002), feasibly perpetuating terrestrial ecosystem N limitation. Rapid storage of new N inputs in non-labile pools may maintain a soil N source that supplies a flux of DON. This suggestion is supported by the kinetics of soluble organic tracer N in O-horizon soil in our study. A mean of 7.0% (SE = 1.5) of applied tracer was present in soluble organic form at the time of soil collection, i.e., was leached as DON during the incubation after soil collection. As with tracer N stored in stable form, tracer N stored in soluble organic form did not differ across sequential collections of O-horizon soil, regardless of how long after tracer application the soil samples were collected (Fig. 2, Appendix B: Table B1). This observation suggests that a substantial fraction of newly deposited mineral N is rapidly immobilized into a pool that is not remineralized and leached (is non-labile), yet some of which can be leached in organic form owing perhaps to the release of soluble organic molecules from recalcitrant SOM or microbial biomass. The rapid storage of newly deposited N in a non-labile yet soluble form could provide a source for chronic ecosystem DON loss. This DON loss may be insensitive to ecosystem succession, as tracer N stored in soluble organic form in O-horizon soil did not differ between stand age classes (Appendix B: Table B1), consistent with observations that old-growth and aggrading (once-logged) watersheds export similar quantities of DON (Goodale et al. 2000).

Summary and conclusions

Our results show that the soil organic horizon in a temperate hardwood forest stored about 84% of newly added mineral N within 26 minutes of deposition. Initially, this storage was about evenly distributed between labile + mineral and stable forms, with most of the labile N probably cycling through microbial biomass as it was lost to other pools over a 9–12 month period. The fraction of tracer N stored in stable form was unchanged one year later and was greater in late-successional than in young forest stands, probably because older stands have more massive O horizons with greater C pools (Fig. 3). The link between soil C and N storage in the O horizon may reflect C-fueled microbial N uptake, abiotic reactions between mineral N and aromatic compounds, or both. Some fraction of the rapidly stored N appears to be recalcitrant yet soluble, and may represent a reservoir for chronic DON export from watersheds, or at least from O horizons. Our results indicate a positive relationship between ecosystem successional status and soil N storage capacity, and suggest that rapid N stabilization in O-horizon soil should inform models of soil and watershed N cycling.

Our study conclusively demonstrates that N moves quickly to a stable pool. Fruitful subsequent research might investigate how stable that pool is, what controls its capacity, and how quickly it saturates, and might also investigate the pathways by which N is rapidly stabilized and the molecular forms of stable soil N.

Acknowledgments

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Reports

Supplemental Material

Appendix A

Description of site selection, tracer ^{15}N application, soil sampling, fractionation of bulk-soil C and N and of tracer N, data analyses, and a photograph of field sites (*Ecological Archives* E095-233-A1).

Appendix B

Three tables displaying the output of statistical analyses reported in the main manuscript (*Ecological Archives* E095-233-A2).