Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of $^{15}$N tracer field studies


¹Department of Biology, Boston University, 5 Cumming Street, Boston, Massachusetts 02215 USA
²Department of Biology, University of Florida, Gainesville, Florida 32611-8525 USA
³Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska 99775 USA
⁴Department of Biology, Vassar College, 124 Raymond Avenue, Poughkeepsie, New York 12604 USA
⁵U.S. Environmental Protection Agency, ORD-NHEERL-WED, 200 SW 35th Street, Corvallis, Oregon 97333 USA
⁶Natural Environment Research Council, Polar House, Swindon SN2 1EU United Kingdom
⁷School of Natural Resources and Environment, University of Michigan, 440 Church Street, Ann Arbor, Michigan 48109 USA
⁸Environmental Change Research Centre, Geography Department, Pearson Building, University College London, Gower Street, London WC1E 6BT United Kingdom
⁹Department of Plant, Soil and Environmental Sciences, University of Maine, Orono, Maine 04469 USA
¹⁰Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106-9620 USA
¹¹Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd LL57 2UW United Kingdom
¹²Department of Environmental Sciences, University of Virginia, P.O. Box 400123, Charlottesville, Virginia 22904-4123 USA
¹³Department of Ecology and Evolutionary Biology, Cornell University, E215 Corson Hall, Ithaca, New York 14853 USA
¹⁴Forest and Landscape Denmark, University of Copenhagen, Rolighedsvej 25 DK-1586 Frederiksberg C, Denmark
¹⁵Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, Minnesota 55108 USA
¹⁶Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309 USA
¹⁷Department of Biology, Western Washington University, Bellingham, Washington 98225-9160 USA
¹⁸Department of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona 86011 USA
¹⁹CSIRO Land and Water, Waite Campus, PMB 2 Glen Osmond, South Australia 5064 Australia
²⁰Department of Ecology and Evolutionary Biology, University of Michigan, 830 North University Avenue, Ann Arbor, Michigan 48109-1048 USA
²¹U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, Oregon 97331 USA
²²Swiss Federal Institute for Forest, Snow and Landscape Research, Zürcherstr. 111, CH-8903 Birmensdorf, Switzerland
²³James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH United Kingdom
²⁴Groundwater Quality and Assessment Section, National Water Research Institute, Environment Canada, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario L7R 4A6 Canada
²⁵Department of Earth and Environmental Sciences, University of Waterloo, 200 University Avenue W., Waterloo, Ontario N2L 3G1 Canada
²⁶Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94240, 1090 GE Amsterdam, The Netherlands

Abstract. Effects of anthropogenic nitrogen (N) deposition and the ability of terrestrial ecosystems to store carbon (C) depend in part on the amount of N retained in the system and its partitioning among plant and soil pools. We conducted a meta-analysis of studies at 48 sites across four continents that used enriched $^{15}$N isotope tracers in order to synthesize information about total ecosystem N retention (i.e., total ecosystem $^{15}$N recovery in plant and soil pools) across natural systems and N partitioning among ecosystem pools. The greatest recoveries of ecosystem $^{15}$N tracer occurred in shrublands (mean, 89.5%) and wetlands (84.8%) followed by forests (74.9%) and grasslands (51.8%). In the short term (<1 week after $^{15}$N tracer application), total ecosystem $^{15}$N recovery was negatively correlated with fine-root and soil $^{15}$N natural abundance, and organic soil C and N concentration but was positively correlated with mean annual temperature and mineral soil C:N. In the longer term (3–18 months after $^{15}$N tracer application), total ecosystem $^{15}$N retention was negatively correlated with foliar natural-abundance $^{15}$N but was positively correlated with mineral soil C and N concentration and C:N, showing that plant and soil natural-abundance $^{15}$N and soil C:N are good indicators of total ecosystem N retention. Foliar N concentration was not significantly related to ecosystem $^{15}$N tracer recovery, suggesting that plant N status is not a good predictor of total ecosystem N retention. Because the largest ecosystem sinks for $^{15}$N...
tracer were below ground in forests, shrublands, and grasslands, we conclude that growth enhancement and potential for increased C storage in aboveground biomass from atmospheric N deposition is likely to be modest in these ecosystems. Total ecosystem $^{15}\text{N}$ recovery decreased with N fertilization, with an apparent threshold fertilization rate of 46 kg N·ha$^{-1}$·yr$^{-1}$ above which most ecosystems showed net losses of applied $^{15}\text{N}$ tracer in response to N fertilizer addition.

Key words: atmospheric nitrogen deposition; carbon storage; data synthesis; meta-analysis; nitrogen retention and loss; stable isotopes.

INTRODUCTION

Nitrogen (N) is an essential element that often limits net primary productivity in terrestrial ecosystems (LeBauer and Treseder 2008). Human activities such as fossil fuel combustion, synthetic-fertilizer application, and animal agriculture have greatly increased emissions of reactive N and its deposition to terrestrial ecosystems (Vitousek et al. 1997, Galloway et al. 2008). Initially, N inputs can increase productivity (LeBauer and Treseder 2008); however, once inputs exceed biotic demand and abiotic sinks for N, additional anthropogenic N inputs can induce plant nutrient imbalances, reduced productivity, increased N export from terrestrial ecosystems, and soil- and stream-water acidification (Agren and Bosatta 1988, Aber et al. 1989, 1998). More recently, the potential effects of N inputs on terrestrial ecosystem carbon (C) retention and loss are receiving attention, due to concerns with the global C cycle. Terrestrial ecosystems represent major sources and sinks for atmospheric CO$_2$ (IPCC 2007), and because N is often the limiting nutrient in terrestrial ecosystems, N inputs from atmospheric deposition can increase C storage by increasing primary production and C storage within plants (Hungate et al. 2003, Thomas et al. 2010). In soils, N additions can increase C storage in some ecosystems by reducing decomposition and respiratory C loss (Fog 1988, Agren et al. 2001, Zak et al. 2008, Janssens et al. 2010); however, N inputs can also stimulate C loss via decomposition, which in some ecosystems can more than offset positive impacts on productivity (Mack et al. 2004).

Applications of N enriched in the stable isotope $^{15}\text{N}$ (hereafter “$^{15}\text{N}$ tracer”) have produced considerable data on fates and retention of N inputs to terrestrial ecosystems (Schlesinger 2009), as they can be used to track cohorts of N inputs into ecosystem components and to determine the fate of N additions across time scales (Currie and Nadelhoffer 1999). To date, there has been one synthesis of $^{15}\text{N}$ tracer data from agricultural systems (Gardner and Drinkwater 2009), but no comparable synthesis across natural ecosystems that span broad geographical areas, except for a recent synthesis by Curtis et al. (2011) focusing on pathways for NO$_3^-$ loss.

We used meta-analysis techniques to synthesize results from ecosystem-scale $^{15}\text{N}$ tracer experiments in natural ecosystems to compare N sinks across studies that varied in time scale of recovery, ecosystem types, and locations (Fig. 1). Our objectives were to identify: (1) variables controlling total ecosystem N retention, (2) impact of method and form of $^{15}\text{N}$ tracer application on measured N retention, (3) whether plant and soil $^{15}\text{N}$ natural abundances are useful indicators of total ecosystem N retention, (4) effects of N fertilization rates on partitioning of N among ecosystem pools and on total ecosystem N retention, and (5) implications for the N fertilization effect on C storage.

We predicted that most N would be retained in belowground ecosystem pools, but that grassland, tundra, and shrubland ecosystems would have a greater proportion of N retained below ground compared to forests due to greater C allocation to roots than shoots (De Deyn et al. 2008). Also, we expected that shrublands and evergreen forests, with typically low nutrient availability, would retain greater proportions of N additions than ecosystems such as deciduous forests and grasslands, which often have greater nutrient availability and are dominated by plant species with shorter leaf life spans (Aerts and Chapin 2000). We expected that sites with higher precipitation or temperature would exhibit less total ecosystem N retention because both of these factors can stimulate internal N-cycling rates and thereby enhance production and loss of mobile forms of N. However, as temperature and precipitation increase, so can rates of net ecosystem production, which can contribute to N retention. Furthermore, because others have shown strong positive correlations between natural-abundance $^{15}\text{N}$ of foliage and N losses (Meints et al. 1975, Gebauer and Schulze 1991, Garten 1993, Högborg 1997, Emmett et al. 1998, Pardo et al. 2006, Templer et al. 2007), we expected that foliar and soil $^{15}\text{N}$ natural abundances would correlate negatively with total ecosystem N retention. Foliar $^{15}\text{N}$ natural abundance is positively correlated with N losses due to fractionation during microbial processes, such as nitrification, which correlate with N losses, and enrichment of remaining soil N pools that plants take up. Foliar $^{15}\text{N}$ natural abundance has also been shown to negatively correlate with rates of N transfer from mycorrhizal fungi to plants (Hobbie and Ouimette 2009, Högborg et al. 2011), which can also indicate low N availability and relatively high ecosystem N retention. Finally, we predicted that ecosystems with higher N additions would retain smaller proportions of N inputs than ecosystems receiving low N inputs from $^{15}\text{N}$ tracer application alone, due to potential saturation of plant and microbial N demands.
METHODS

We assembled data from ecosystem-scale 15N tracer studies (Appendix) reported in peer-reviewed sources using Biosis and Web of Science and from participants in workshops conducted at the National Center for Ecological Analysis and Synthesis (NCEAS) in Santa Barbara, California (USA). Criteria for inclusion in our analysis included (1) following the fate of 15N tracers in nonagricultural ecosystems and (2) having been done in the field at a scale sufficiently large to examine plants and soils. We excluded studies using soil cores that did not include entire plants. We examined fates of 15N inputs among ecosystem pools across ecosystem types including forests (32 sites), grasslands (7 sites), shrublands (4 sites), tundra (6 sites), and wetlands (2 sites) from locations in North America (26 sites), South America (1 site), Europe (19 sites), and Oceania (2 sites). Sites varied in elevation, mean annual temperature and precipitation, total N deposition, and rate of N addition (Fig. 2; Appendix). In some cases a site was represented more than once in an analysis if the 15N-tracer recovery data were available from more than one treatment or ecosystem type. We averaged values for 15N recovery in cases where a site was represented more than once with the same type of plot. “Plot” represented the experimental unit in the majority of studies included in this analysis.

Our analysis included studies that reported total ecosystem 15N-tracer recovery, considered here as the sum of 15N retained in soils, fine and coarse roots, leaf litter (Oi horizon), and aboveground biomass. Organic soil horizons included Oe, Oa, and sometimes partial A horizons. Mineral soil depths varied by study; we could not constrain these data to a particular depth due to the wide variability of depths measured by investigators. In some cases, researchers reported recoveries within only some of these pools without reporting total ecosystem recovery. Therefore, more studies were included in our analyses of recovery within individual pools than in analyses of total ecosystem 15N recovery (Appendix). We compared total ecosystem 15N tracer recovery between ambient (unfertilized) and fertilized plots. Fertilization was defined as studies that added >2.5 kg N·ha⁻¹·yr⁻¹ beyond atmospheric deposition. Although 15N tracer applications alone involve N additions, this was not considered to be fertilization since 15N tracer masses are very small (<2.5 kg N·ha⁻¹·yr⁻¹).

15N recovery calculations

We used 15N tracer recoveries based on N mass, amount of 15N applied, and 15N enrichments of ecosystem pools. Amounts of 15N applied to plots ranged from 0.0069 to 18 kg 15N·ha⁻¹ (0.5–99 atom% 15N). We calculated the proportion of 15N tracer recovered (15Nrec) within each ecosystem pool as

\[
15N_{\text{rec}} = \frac{F_{\text{sample}} - F_{\text{ref}}}{F_{\text{tracer}} - F_{\text{ref}}} \times \frac{N_{\text{pool}}}{N_{\text{tracer}}}
\]

where \(F = \frac{15N}{15N + 14N}\) is the fractional abundance of 15N in the sample, in the non-labeled reference sample or the tracer; \(N_{\text{pool}}\) and \(N_{\text{tracer}}\) are the masses of N in the ecosystem pool at a point in time and in the tracer applied to that point in time, respectively (from Providoli et al. 2005). We used 15N tracer recoveries as estimates of ecosystem net N retention of the cohort of N added at specific times. Total ecosystem retention of applied 15N was calculated as the sum of 15N tracer recoveries within soils (including microbial biomass), roots, and aboveground plant biomass.
Total ecosystem $^{15}$N tracer recovery was reported over a range of time scales from less than one day to 11 years after $^{15}$N application. However, it was not possible to make meaningful comparisons among sampling periods due to the paucity of studies measuring $^{15}$N tracer recovery over multiple time scales. We categorized the duration of a study into five time scales based on time from application of the tracer to data collection: $<1$ week ($n = 30$ studies), 1 week to 1 month ($n = 11$ studies), $>1$ month to 3 months ($n = 32$ studies; hereafter 1–3 months), $>3$ to 18 months (hereafter 3–18 months; $n = 48$ studies) and $>18$ months ($n = 21$ studies). In a few studies data were reported from multiple times within one of these time scales as a series of $^{15}$N applications across a growing season or across more than one year. In each case, we averaged values of ecosystem pools.
within a study within a time scale. Values within a site were kept separate for distinct vegetation types, \(^{15}\)N application forms, and experimental treatment. For studies in which \(^{15}\)N was applied more than once, categories for time scale were based on the timing of the first \(^{15}\)N application. Data from ambient and fertilized plots were kept separate.

**Meta-analyses**

We chose percentage of \(^{15}\)N tracer recovery as the effect size of interest (Osenberg et al. 1999), and used an unweighted meta-analytic approach because not all studies were replicated and many (~25%) did not report variances. We examined fates of \(^{15}\)N tracer applications among ecosystem pools for two time periods: short term, <1 week to examine short-term partitioning of \(N\), and long term, 3–18 months to examine partitioning of \(^{15}\)N tracers after one growing season.

We sought to identify factors leading to variation in \(^{15}\)N recovery among studies using a mixed model for categorical classifications or correlation analyses for continuous descriptor variables. Categorical variables included ecosystem type (forest, grassland, shrubland, tundra, wetland), dominant plant species growth form (graminoid, evergreen tree plantation, evergreen tree, evergreen shrub, deciduous tree), mycorrhizal association (arbuscular mycorrhizae, ectomycorrhizae, and ericoid mycorrhizae), co-dominant growth form (graminoid, tree, shrub, forb, or other), previous disturbance to site (agriculture, grazing or pasture, hurricane, selective harvest [removal of particular plant species] and fire), form of \(^{15}\)N application (\(^{15}\)NH\(_4\), \(^{15}\)NO\(_3\), \(^{15}\)NH\(_4\)\(^+\), \(^{15}\)NO\(_3\)\(^-\)), and method of \(^{15}\)N application (application to canopy or to soil surface). A minimum of two studies per category was necessary to be included in the meta-analysis.

We used Spearman rank correlation analyses to examine correlations between total ecosystem \(^{15}\)N tracer recovery and continuous independent variables. Continuous variables included elevation, mean annual temperature and precipitation, annual rates of atmospheric deposition of \(N\) (NH\(_4\)^+ + NO\(_3\)^-); most reported as total deposition, but some used throughfall as a proxy), foliar \(N\) concentration and \(^{15}\)N natural abundance, fine-root \(^{15}\)N natural abundance, and organic and mineral soil C and N concentration, C:N concentration ratio, and \(^{15}\)N natural abundances. Natural-abundance samples were collected prior to or in separate plots from \(^{15}\)N tracer applications.

We examined impacts of fertilizer addition and form on total ecosystem \(N\) retention by comparing ambient to fertilized studies. We also conducted a separate analysis of fertilization studies that was constrained to studies using “low \(N\)” and “high \(N\)” experimental treatments (12 sites; Appendix). We calculated change in total ecosystem \(^{15}\)N tracer recovery in response to \(N\) fertilization and compared the effect size across studies. In most studies “low \(N\)” corresponds to ambient levels of atmospheric deposition, but in some cases \(N\) inputs were experimentally elevated (albeit at low levels). “High \(N\)” plots were always subject to additions well above ambient \(N\) inputs.

Studies using \(^{15}\)N tracer have methodological limitations that constrain our ability to make precise quantitative measures of ecosystem \(N\) retention, and these limitations should be considered in our comparisons of sites and studies. Viewing the measures reported here of ecosystem recovery of \(^{15}\)N as ecosystem \(N\) retention requires the assumption that \(^{15}\)N recovery in vegetation and soil samples are scaled to ecosystem pools correctly with no systematic bias and low uncertainty. In reality, accurate scaling of \(^{15}\)N tracer recoveries in soil and vegetation samples is difficult. Soil \(N\) pools, in particular, are large and heterogeneous, whereas sample numbers are typically small in comparison. Vegetation \(N\) pools are also difficult to scale because of difficulties in quantifying belowground biomass, uncertainties of relationships between aboveground and belowground allometry, and assumptions about tissue stoichiometry in large, variable tissues such as foliage, roots, and branches, which vary among vegetation types. Also, tracer experiments assume rapid mixing of the \(^{15}\)N tracer into ecosystem inorganic-\(N\) pools, which is not always the case. For example, tracers sprayed on vegetation and soil might only enter relevant pools after the next rain event, and mixing effectiveness could depend on pool size. Total ecosystem recoveries are best viewed as relative ecosystem retention across studies, keeping in mind that many explanatory variables are not necessarily independent. Therefore, we cannot rank the importance of different factors with great confidence, nor can we characterize their interactions.

We used MetaWin 2.1 software (Rosenberg et al. 2000) for meta-analyses using categorical data and calculated unweighted means and 95% bias-corrected bootstrapped confidence intervals based on 999 iterations for nonnormally distributed data. Normality was tested with the Shapiro-Wilk test using SAS JMP software (version 8.0.2; SAS Institute 2009). We considered groups with non-overlapping confidence intervals to be significantly different. To analyze relationships between the responses and continuous variables, we calculated Spearman rank correlations using SAS JMP software (version 8.0.2).

**Results**

*Ambient conditions: controls on total ecosystem recovery of \(^{15}\)N*

Terrestrial ecosystems retained on average 62.7% of \(^{15}\)N tracer applications. Mean (range) total ecosystem \(^{15}\)N recovery was 59.6% (48.3–72.3%) for <1 week, 80.1% (62.9–99.4%) for 1 week to 1 month, 50.7% (40.5–61.2%) for 1 to 3 months, 69.4% (60.4–78.5%) for 3 to 18 months, and 61.6% (48.5–73.9%) for >18 months. Total \(^{15}\)N recovery for the 1-week-to-1-month sampling
period was greater than the <1-week sampling period, but the range of values is large and overlaps between sampling periods. Also, we did not compare the different time periods because not all studies were included in each of the sampling periods.

The sum of $^{15}$N recoveries among ecosystem pools can exceed 100% (Fig. 3) but is not equivalent to the reported total ecosystem $^{15}$N recovery (Figs. 4 and 5). Some studies reported only a subset of ecosystem pools, while others reported all pools; therefore the two approaches are not directly comparable. Also, the sum of $^{15}$N recoveries was sometimes >100% because of non-mutually exclusive categories shown in Fig. 3, such as microbial biomass and soil pools.

Many continuous variables were significantly correlated with total ecosystem $^{15}$N tracer recovery in the short term (<1 week). Total ecosystem $^{15}$N tracer recovery was negatively correlated with site elevation, fine root and soil $^{15}$N natural abundance, and organic soil C and N concentration, but was positively correlated with mean annual air temperature and mineral soil C:N (Table 1). There were nearly significant negative relationships between total ecosystem $^{15}$N recovery and both mean annual precipitation ($P = 0.08$) and atmospheric N deposition rate ($P = 0.06$).

Fewer significant correlations between total ecosystem $^{15}$N tracer recovery and continuous variables emerged from the long-term (3 to 18 months) data set. However, most identified were fairly strong (Table 1). Total ecosystem $^{15}$N tracer recovery was negatively correlated only with foliar natural abundance $^{15}$N, but was positively correlated with mineral soil C, N, and C:N. There was a positive relationship between total ecosystem $^{15}$N recovery and precipitation, but it was not statistically significant ($P = 0.07$).

For the long-term data set, total ecosystem $^{15}$N recovery differed significantly among categorical variables including ecosystem type, dominant plant species growth form, co-dominant growth form, mycorrhizal association, and site history (Fig. 4). The greatest total ecosystem recovery occurred in shrublands (mean, 89.5%; $n = 6$ sites), followed by wetlands (84.8%; $n = 2$), forests (74.9%; $n = 23$), and grasslands (51.8%; $n = 16$). Ecosystems dominated by evergreen shrubs had the greatest total ecosystem recovery (89.5%; $n = 6$), followed by deciduous trees (77.9%; $n = 11$), evergreen trees (74.0%; $n = 9$), graminoids (72.4%; $n = 12$), and evergreen plantations (66.9%; $n = 3$). Ecosystems with ericoid mycorrhizae (89.5%; $n = 6$) had significantly greater total ecosystem recovery than ecosystems associated with ecto- (72.5%; $n = 11$) or arbuscular mycorrhizae (53.0%; $n = 18$). Because all six shrubland sites were dominated by evergreen shrubs and had ericoid mycorrhizae, distinguishing effects of vegetation vs. mycorrhizal type on total ecosystem $^{15}$N tracer recovery was not possible. The two sites (both at Harvard Forest) that had previously been disturbed by a hurricane had significantly greater total ecosystem $^{15}$N tracer recovery (85.1%; $n = 2$) than ecosystems previously disturbed by burning (75.0%; $n = 2$), grazing

![Graphs showing mean $^{15}$N tracer recovery among terrestrial ecosystem pools for short-term (<1 week) and long-term (3–18 months) time periods following $^{15}$N addition. Not all studies are represented among each ecosystem pool, which may result in recovery >100%. Not all ecosystem pools or ecosystem types are available for each sampling time.](image-url)
Form and method of 15N tracer application also affected total ecosystem 15N recovery (Fig. 5). Studies in which 15N tracers were applied as 15NH4+ had significantly lower total ecosystem 15N tracer recovery (53.4%, n = 23) than those where 15N was applied as 15NH415NO3 (85.3%, n = 15) or 15NO3/C0 (80.2%, n = 10). Studies in which 15N tracers were applied to the plant canopies had significantly greater total ecosystem 15N tracer recovery (81.7%, n = 16) than those studies where tracers were distributed onto the soil surface (63.1%)

(68.3%; n = 7), selective harvest (67.7%; n = 4), or agriculture (57.2%; n = 4).

Form and method of 15N tracer application also affected total ecosystem 15N recovery (Fig. 5). Studies in which 15N tracers were applied as 15NH4+ had significantly lower total ecosystem 15N tracer recovery (53.4%, n = 23) than those where 15N was applied as 15NH415NO3 (85.3%, n = 15) or 15NO3/C0 (80.2%, n = 10). Studies in which 15N tracers were applied to the plant canopies had significantly greater total ecosystem 15N tracer recovery (81.7%, n = 16) than those studies where tracers were distributed onto the soil surface (63.1%)

**Table 1. Nonparametric correlations for total ecosystem percentage 15N tracer recovery.**

<table>
<thead>
<tr>
<th>Site characteristic</th>
<th>Sampling time after 15N application</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>rS</td>
<td>P</td>
<td>N</td>
<td>rS</td>
</tr>
<tr>
<td>Mean elevation</td>
<td>30</td>
<td>-0.52</td>
<td>0.0036</td>
<td>48</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean annual temperature</td>
<td>30</td>
<td>0.52</td>
<td>0.0032</td>
<td>47</td>
<td>-0.23</td>
</tr>
<tr>
<td>Mean annual precipitation</td>
<td>30</td>
<td>-0.33</td>
<td>0.076</td>
<td>48</td>
<td>0.27</td>
</tr>
<tr>
<td>Total nitrogen atmospheric deposition</td>
<td>28</td>
<td>-0.36</td>
<td>0.064</td>
<td>46</td>
<td>0.22</td>
</tr>
<tr>
<td>Foliar N concentration</td>
<td>22</td>
<td>-0.21</td>
<td>0.34</td>
<td>33</td>
<td>0.026</td>
</tr>
<tr>
<td>Foliar natural-abundance 15N</td>
<td>20</td>
<td>-0.15</td>
<td>0.52</td>
<td>23</td>
<td>0.46</td>
</tr>
<tr>
<td>Fine-root natural-abundance 15N</td>
<td>26</td>
<td>-0.57</td>
<td>0.0022</td>
<td>29</td>
<td>0.11</td>
</tr>
<tr>
<td>Organic soil C concentration</td>
<td>26</td>
<td>-0.79</td>
<td>&lt;0.0001</td>
<td>25</td>
<td>0.17</td>
</tr>
<tr>
<td>Organic soil N concentration</td>
<td>28</td>
<td>-0.59</td>
<td>0.0011</td>
<td>35</td>
<td>0.025</td>
</tr>
<tr>
<td>Organic soil C:N</td>
<td>26</td>
<td>0.30</td>
<td>0.14</td>
<td>25</td>
<td>0.18</td>
</tr>
<tr>
<td>Organic soil natural-abundance 15N</td>
<td>26</td>
<td>-0.79</td>
<td>&lt;0.0001</td>
<td>33</td>
<td>0.14</td>
</tr>
<tr>
<td>Mineral soil C concentration</td>
<td>28</td>
<td>-0.28</td>
<td>0.14</td>
<td>18</td>
<td>0.72</td>
</tr>
<tr>
<td>Mineral soil N concentration</td>
<td>28</td>
<td>-0.29</td>
<td>0.14</td>
<td>24</td>
<td>0.55</td>
</tr>
<tr>
<td>Mineral soil C:N</td>
<td>28</td>
<td>0.67</td>
<td>&lt;0.0001</td>
<td>18</td>
<td>0.72</td>
</tr>
<tr>
<td>Mineral soil natural-abundance 15N</td>
<td>26</td>
<td>-0.56</td>
<td>0.0028</td>
<td>15</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

**Notes:** The table includes ambient plots sampled at both a short-term (<1 week) and a long-term (3–18 months) time period. Significant relationships (P < 0.05) are in bold. N is the number of studies; rS is the Spearman rank correlation coefficient. Nonparametric correlations are from JMP software, version 8.0.2 (SAS Institute 2009).

**Fig. 4.** Total ecosystem 15N tracer recovery (mean and 95% confidence intervals) among ecosystem types, dominant plant species growth form, mycorrhizal association, co-dominant growth form, and major previous site disturbance. Data are from ambient plots sampled during the long-term time period. Nonoverlapping 95% confidence intervals and data points with different lowercase letters indicate significant differences at P = 0.05. Sample size (the number of studies within each ecosystem category) is given just above the x-axis.
The form of $^{15}$N tracer applied varied with ecosystem type and therefore some of the effect of form of applied $^{15}$N tracer may reflect ecosystem differences. $^{15}$Nitrogen tracers were applied primarily as $^{15}$NO$_3^-$ or $^{15}$NH$_4^+$ to forest sites, but as $^{15}$NH$_4$-$^{15}$NO$_3$ to a mixture of grassland, shrub, and tundra sites (only one forest site). Where $^{15}$N was applied as $^{15}$NO$_3^-$, most researchers applied it to the soil surface. Application of $^{15}$N tracers to the canopy occurred in low-statured ecosystems such as tundra, shrublands, wetlands, or grasslands.

**Fertilization experiments: controls on total ecosystem recovery of $^{15}$N**

Fertilizer N additions reduced ecosystem $^{15}$N recoveries compared to sites receiving only N inputs from ambient deposition and $^{15}$N tracer applications (Fig. 5). This overall pattern, however, concealed significant differences in recoveries among fertilizer N forms where addition of NH$_4$NO$_3$ as fertilizer led to significantly greater total ecosystem $^{15}$N tracer recovery compared to ambient plots where the other N forms (urea, NO$_3^-$, NH$_4^+$) all had lower recoveries (Fig. 5). In experiments containing both “low” and “high” N treatments, there was a negative relationship between rate of N added and the difference in total ecosystem $^{15}$N tracer recovery between low and high N treatments (Fig. 6; $r = -0.46$; $P = 0.0013$).

**Ambient conditions: fate of nitrogen among ecosystem pools**

Overall, soil and litter retained more $^{15}$N tracer than did plants in short- and long-term studies (Fig. 3). For the short term the largest sinks for $^{15}$N tracers were below ground in (1) soil organic horizons, averaging 39.0% in forests ($n = 24$ studies), 16.9% in grasslands ($n = 8$), and 42.8% in tundra $n = 4$); (2) litter pools, averaging 31.6% in forests ($n = 20$), 10.6% in grasslands ($n = 8$), and 6.5% in tundra ($n = 2$); and (3) mineral soil horizons, averaging 29.9% in forests ($n = 22$) and 18.0% grasslands ($n = 10$). $^{15}$N tracer recovery in soil microbial biomass was low in forests (11.5%, $n = 26$) and grasslands (6.2%, $n = 4$). In contrast, $^{15}$N recovered in microbial biomass of tundra (36.1%, $n = 4$) was almost as high as in organic soil (42.8%, $n = 4$) and much greater than in litter (6.5%, $n = 2$) and roots (2.3% and 1.5% in fine and coarse roots, respectively, $n = 5$ and 5, respectively). Less than 10% of the recovered tracer was in plants for the short-term sampling period for all three ecosystem types, with most of that in roots. Fine-root biomass (6.3% and 2.3% in forests and tundra, respectively, $n = 14$ and 5, respectively) retained more $^{15}$N tracer than did coarse-root biomass (3.5% and 1.5% in forests and tundra, respectively, $n = 4$ and 5, respectively) or foliage (0.6% in forests, $n = 2$). There were insufficient data to examine sinks for tracer $^{15}$N in shrubland and wetland ecosystems for the short-term sampling period.

![Figure 5](image-url)
The largest sinks for $^{15}$N tracers among ecosystem types for the long-term data set were organic soil in forests (35.5%, $n=31$), litter in grasslands (25.5%, $n=9$), shrublands (33.8%, $n=6$), and wetlands (34.1%, $n=2$), and foliage in tundra (12.1%, $n=3$; Fig. 3). Total plant biomass (above- and belowground) retained less $^{15}$N than soils and litter in forests (10.4% in vascular plant biomass), grasslands (30%), shrublands (23.2%), wetlands (9.6%), and tundra (29.9%). Proportions of $^{15}$N tracer recovery in non-vascular plants (bryophytes and lichens; 20.1, 29.5 and 26% in forests, shrublands and wetlands, respectively, $n=6, 6$ and 2, respectively) were significantly larger than other vegetation sinks, but were not different from belowground pools such as soil and litter. $^{15}$Nitrogen tracer recovery in non-vascular plants of grasslands (14.2%, $n=9$) was smaller than the total amount found in soils, litter, or live vascular plant biomass.

**Fertilization experiments: fate of $^{15}$N among ecosystem pools**

Fertilizer N additions altered the partitioning of $^{15}$N tracers in the short and long term for forest and tundra ecosystems (Fig. 3). In the short term the addition of fertilizer N in forests led to decreased $^{15}$N recovery in organic soil, microbial biomass, and fine roots, but increased $^{15}$N recovery in litter. Fertilization led to greater $^{15}$N recovery in both fine and coarse roots in tundra for the short term. There were insufficient data to compare ambient to fertilized plots for soil and litter pools in tundra.

After one growing season, fertilizer N additions in forests also led to decreased $^{15}$N in organic soil, but not in mineral soil, microbial biomass, or fine roots. Total $^{15}$N recoveries in live plants of forests increased with fertilizer N addition, with most tracer moving into aboveground plant biomass, while decreasing in soils and litter. Fertilization in tundra ecosystems decreased $^{15}$N recovery in microbial biomass, but increased $^{15}$N in fine roots and foliage. There were insufficient data to examine the impact of N addition on partitioning of N in grasslands, shrublands or wetlands.

**DISCUSSION**

**Ecosystem nitrogen retention**

Our results show that additions of N from either atmospheric deposition (<1-week data; Table 1) or from fertilization (Figs. 5 and 6) decreased total ecosystem N retention with an apparent threshold fertilization rate of 46 kg N ha$^{-1}$ yr$^{-1}$. Above this rate, N addition decreased $^{15}$N retention, while N fertilization at lower rates enhanced $^{15}$N retention relative to unfertilized plots. The values for total ecosystem $^{15}$N retention overlap with ecosystem N retention estimated for whole-watershed N mass balances (Aber et al. 2003, Dise et al. 2009). Nitrogen retention rates varied across 121 forested sites in Europe, with high N retention (87%) associated with low atmospheric N deposition rates (<8 kg N ha$^{-1}$ yr$^{-1}$) and lower retention rates associated with higher N deposition (Dise et al. 2009). For the northeastern United States, N budgets from 83 forested watersheds show that N retention averages 76% of incoming atmospheric-N deposition, and decreases from >90% retention for sites receiving <7 kg N ha$^{-1}$ yr$^{-1}$ to <60% retention for sites receiving >11 kg N ha$^{-1}$ yr$^{-1}$.
Our estimates of mean ecosystem N retention may be lower than these watershed studies, which included gaseous losses as part of their estimates of N retention, whereas our estimates of N retention rely on 15N tracer recovered in plant biomass and soils only. The convergence of results of 15N tracer studies and watershed N input–output balances are suggestive of general patterns of the decreasing relative importance of biological sinks along gradients of increased N inputs. With greater N availability, it is possible that N sinks in plants or microbes, or on exchange sites on minerals or soil organic matter, became saturated when N fertilizer was added as urea, NH4+-N, or NO3--N, such that more NO3--N was lost due to leaching or lost as N gases (NO, N2O, or N2) from nitrification or denitrification. However, it is unclear why the same mechanisms did not occur following addition of fertilizer as NH4NO3. Ammonium nitrate fertilizer was added to a variety of ecosystems, including forests and tundra, suggesting that there was no bias between form of N fertilizer added and ecosystem type examined. Form of 15N application also does not explain the relationship between N addition and total ecosystem 15N retention as there was a mixture of forms of 15N tracers applied in those sites that resulted in less 15N retention following N fertilizer addition, as well as greater 15N retention following N fertilizer addition (Fig. 6).

Tundra sites had increased total ecosystem 15N retention following N fertilization compared to unfertilized controls. The three tundra sites included in this study (Niwot Ridge, Colorado, USA; Toolik Lake, Alaska, USA; Galbraith Lake, Alaska, USA) are N limited (Shaver and Chapin 1980, Chapin and Shaver 1985, Bowman et al. 1993), and may have been even after N fertilization. These sites may have retained greater amounts of applied 15N through enhanced biological activity when N was in greater supply. In contrast to tundra sites, forest responses to N addition were mixed. Total ecosystem 15N tracer recovery decreased with fertilization in the evergreen forests in Aber (Wales), the evergreen and deciduous forests in the Catskill Mountains (New York, USA), and deciduous stands that received 15NO3--N tracer application at Harvard Forest (Massachusetts, USA), but increased in the evergreen forests of Alptal (Switzerland) and some stands at Harvard Forest.

Studies that have quantified leachate 15N found that <10% of 15N tracer applied to non-fertilized plots (Tietema et al. 1998, Zak et al. 2004, Providoli et al. 2005) and 16% of 15N tracer applied to fertilized plots (Lamontagne et al. 2000) is lost via leaching. These results suggest that the remaining N in ecosystems evaluated in this study could have been transported below the sampled soil depth or lost due to translocation within roots to outside of the experimental plots, movement with arthropods outside of the plots, wind (e.g., pollen or litter being blown out of plots), NH3 volatilization, or by nitrification or denitrification converting it to gaseous products (NO, N2O and N2). Losses via denitrification could account for only a small proportion of total 15N applied. For example, Tietema et al. (1998) reported N2O fluxes <4 kg ha⁻¹ yr⁻¹ from European forests, and Christensen et al. (2002) estimated that only 2.6% of applied 15N tracer could have been lost as N2O in a mixed-hardwood forest in the United States. We are not aware of any studies that have determined 15N loss as NO or N2. Alternatively, we may have underestimated 15N incorporation into plant biomass.

Controls on ecosystem nitrogen retention

Results of our analyses demonstrate that a variety of factors influence ecosystem N retention. Total ecosystem N retention for the long-term sampling period varied significantly among ecosystem and mycorrhizal types, with shrublands and wetlands retaining significantly more tracer 15N than forests or grasslands, though the sample sizes for shrublands (n = 6) and wetlands (n = 2) were small. Plant and microbial traits, and their interactions, could explain the high retention of 15N tracers in shrublands. All shrubland sites included in this study were nutrient poor with high soil C:N (e.g., Johnson et al. 2003), likely contributing to relatively high N demand by plants and microbes. High mineral-soil C:N may promote microbial N immobilization and reduce net nitrification, which together could contribute to greater N retention. Such mechanisms may be operative in ecosystems in general, as mineral-soil C:N and total ecosystem retention of applied 15N were positively correlated across our entire data set (Table 1). Similarly, total ecosystem 15N recovery was negatively correlated with organic soil N concentration in the short-term (Table 1), suggesting that ecosystems are less likely to retain applied pulses of 15N when soils are N rich. However, total ecosystem 15N recovery was also negatively correlated with organic soil C concentration, suggesting a limited role of soil microbes in N retention given that microbial N immobilization is driven by soil C availability. This result is surprising given past work showing the importance of microbial uptake of N in ecosystem N retention (Vitousek and Matson 1984). Similar to soils, foliar N concentrations are considered indicators of N availability in terrestrial ecosystems (Aber et al. 1998). However, we found no relationship between foliar N concentration and ecosystem 15N tracer recovery, nor did we find significant relationships between foliar N concentration and plant 15N sink strength. Foliar N concentrations vary between species, masking potential changes due to N availability. Also, increases in foliar N content are often not accompanied by similar increases in N concentration due to a dilution effect caused by greater biomass. Overall, these findings suggest that compared to plant N concentrations, soil N concentrations or C:N are better predictors of ecosystem N retention. However, plants often control N retention through long-term C:N feedbacks such that lower
higher mean annual air temperatures (Table 1) suggests that higher temperatures may have led to greater rates of N uptake by plants and microbes. Greater uptake could have more than offset possible increases in rates of soil N-cycling processes, such as mineralization and nitrification, and losses via gas emissions or leaching. These results agree with watershed mass balances showing that N export decreases with increasing temperatures (Schaefer and Alber 2007). The decrease in 15N retention with increasing elevation is not surprising given that air temperature typically declines with increasing elevation and biological sinks may be attenuated. Also, as sites at higher elevations can be characterized by thinner soils, larger proportions of N additions could physically bypass biological sinks (Curtis et al. 2011).

Greater ecosystem recovery of 15N tracers when applied by spraying the canopy (81.7%) compared to the ground (63.1%) is not surprising because N can be taken up by foliage or absorbed by the canopy surface. In contrast, N deposited on soil can be lost via leaching or gaseous loss. 15Nitrogen tracers were applied as 15NH4+, 15NO3−, and 15NH415NO3, and to both the canopy and the ground in a variety of ecosystems, suggesting that there was not a bias between method of 15N tracer application and ecosystem type examined.

The smaller recovery of 15N tracer applied as 15NH4+ compared to 15NO3− under ambient conditions (Fig. 5) is surprising because soon after NH4+ enters soil it is likely retained on cation exchange sites in soil organic matter and clays or preferentially taken up by soil microbes or plants. In contrast, NO3− is more prone to leaching losses or gaseous losses during denitrification or volatilization. It is possible that the lower tracer recoveries after 15NH4+ applications were due to losses by 15NH3 volatilization (Nõmmik and Vahtras 1982). However, few soils (i.e., Grandvillard) in our study likely had pH values high enough to drive NH3 volatilization. Several reports have suggested that some NO3− may be incorporated into organic matter by abiotic reactions (Azhar et al. 1986, Berntson and Aber 2000, Dail et al. 2001, Davidson et al. 2003, Fitzhugh et al. 2003), but others suggest that this process is unlikely to occur in nature to an ecologically significant degree (Colman et al. 2008, Davidson et al. 2008, Morier et al. 2010). If NO3− incorporation was significant, this initial retention of 15N could have contributed to the relatively high total ecosystem 15N tracer recovery in those studies where 15N was applied as 15NO3− or 15NH415NO3. Volatilization of NO3− from plant canopies, as HNO3 and other forms of N, has been estimated to be significant in several forest ecosystems, possibly accounting for losses up to 10% of experimentally added N (Dail et al. 2009). When added as fertilizer, NH4NO3 also had the highest total ecosystem 15N retention. But unlike the ambient conditions, fertilizer with NH4+ had, as expected, a higher recovery than fertilizer with NO3− (Fig. 5). Thus, NO3− fertilizer may be more susceptible to leaching or gas loss than NH4+ and urea fertilizer, which may be retained by cation exchange.

15N natural abundances of foliage, roots, and soils were negatively correlated with total ecosystem 15N tracer recovery during the long-term sampling period. High plant 15N natural abundance can be an indicator of an ecosystem with relatively fast N-cycling rates, high N losses, and low rates of N transfer from mycorrhizal fungi in terrestrial ecosystems (e.g., Högb erg 1997). These ecosystems would be expected to retain smaller proportions of applied 15N tracer compared to ecosystems with relatively slower N cycling, smaller losses, and greater N transfer from mycorrhizal fungi. The significant negative correlation we found suggests that plant and soil 15N natural abundance are also good indicators of ecosystem N-retention capacities across different ecosystem types, including grasslands, forests, shrublands, and wetlands.

**Atmospheric nitrogen inputs and carbon storage**

This data set is the largest assembled to date of studies using 15N-labeled N inputs to identify patterns and
drivers of N partitioning among ecosystem pools within non-agricultural systems. As such, this data set is also a valuable resource for assessing possible impacts of N deposition on C uptake and storage. Our observation that most 15N tracer applications accumulated in belowground pools under ambient conditions and low levels of N-fertilizer input in forest, grassland, and shrubland ecosystems is consistent with results of a smaller study of plots in nine temperate forests in North America and Europe (Nadelhoffer et al. 1999), most of which are included within this meta-analysis. Carbon storage would be maximized if large proportions of N inputs were immobilized by aboveground plant tissues and contributed to increased rates of photosynthesis. However, this was not the case in our study; aboveground vegetation was a small sink for N additions relative to soils or to soils plus exports (whether measured by difference or directly). Similar to Nadelhoffer et al. (1999), we conclude that immediate growth enhancement from atmospheric N deposition should be modest in forests under ambient levels of N availability because little of the applied 15N is recovered in aboveground biomass (6% recovery total) within one year of 15N application. Compared to forests, a larger amount of 15N tracer went into aboveground biomass in shrublands and grasslands (i.e., 19% and 20%, respectively) at the long-term timescale. However, 15N tracer was applied to the canopies of the shrubland ecosystems in this study, enhancing the likelihood of N retention associated with aboveground biomass due to direct absorption immediately following 15N tracer application. Data were not available to determine above vs. belowground partitioning of N for tundra or wetland ecosystems.

Data were available to examine the impact of elevated fertilizer N inputs on movement of N between soil and plant pools in forests only. In these ecosystems, elevated fertilizer N inputs led to movement of N from soil to plant pools, which could increase plant growth due to increased rates of photosynthesis and less allocation to short-lived roots and mycorrhizal fungi, which together could result in a larger C sink in forests. If N fertilizer additions can be used as a proxy for greater rates of atmospheric N deposition or accumulation of N over time, these results suggest that the size of the C sink in forests could increase as a result of elevated levels of atmospheric N deposition. However, the magnitude of this effect is not well known at present and is likely to vary with dominant species (Thomas et al. 2010).

Suggestions for future work on the fate of nitrogen

Ecosystems within North America and Europe are well represented by 15N tracer studies, especially evergreen forests and arctic tundra. Additional 15N tracer studies are needed at lower latitudes, particularly in warm and wet sites, to understand the fate of N inputs within savanna and tropical ecosystems. Future 15N studies should explore N losses in the forms of NO3—dissolved organic N, NH3, N2O, NO, and N2—through lateral movement of N compounds across the landscape. We suggest that, at a minimum, researchers measure 15N tracer recovery in roots, organic soil, mineral soil, litter, and aboveground plant pools to aid in mechanistic understanding of the fate of N inputs in terrestrial ecosystems and for future cross-site comparisons. Finally, additional studies that sample fates of tracers at multiple timescales would help separate mechanisms of N retention among microbial and plant pathways to better understand how N inputs are likely to affect ecosystem C balance. Studies on the decadal scale would be particularly valuable in assessing issues such as C sequestration.

Conclusions

Our meta-analysis of 15N tracer studies demonstrates that local site characteristics such as ecosystem type, vegetation growth form, mycorrhizal type, soil C:N, and disturbance history, as well as the form of 15N application, all influence ecosystem 15N retention. Also, our results suggest that plant and soil natural-abundance 15N values can be used as a qualitative indicator of total ecosystem N retention. Nitrogen inputs are largely incorporated into the soils of forests, shrublands, and grasslands, consistent with previous assertions that most atmospherically deposited N moves belowground. Total ecosystem N retention increased with mineral soil C:N, consistent with the idea that factors controlling microbial N uptake can shape the long-term sink for N in soils and thus total ecosystem N retention. At high rates of N input we found that N uptake by plants increased, but the magnitude of 15N recovery in mineral soil decreased, with an apparent threshold fertilization rate of 46 kg·ha−1·yr−1 above which whole ecosystems had a net loss of 15N tracer relative to inputs. Given the importance of mineral soil as a nitrogen sink across the range of N inputs examined, we hypothesize that long-term increases in N input could lower mineral soil C:N (via direct N incorporation and/or lower litterfall C:N) and ultimately lead to decreased ecosystem N retention.

Acknowledgments

We thank Gail Stichler, Leslie Allfree, and the rest of the staff at NCEAS for their help in planning each of our workshops. We thank Lindsey Rustad and Tracey Walls for their helpful feedback and ideas about meta-analyses. We also thank Jill Baron, Scott Holub, Stephanie Juice, and three anonymous reviewers for helpful comments on drafts of the manuscript. This work was conducted as a part of the “Fate of Nitrogen Inputs in Terrestrial Ecosystems” Working Group supported by the National Center for Ecological Analysis and Synthesis, a Center funded by the National Science Foundation (Grant #EF-0553768), the University of California–Santa Barbara, and the State of California. Any use of trade names is for descriptive purposes and does not imply endorsement by the U.S. Government.

Literature Cited

IPCC [Intergovernmental Panel on Climate Change]. 2007. Climate change 2007: the physical science basis. Cambridge University Press, Cambridge, UK.


SAS Institute. 2009. JMP software, version 8.0.2. SAS Institute, Cary, North Carolina, USA.


SUPPLEMENTAL MATERIAL

Appendix

Site characteristics and experimental design for \(^{15}\)N tracer studies included in the meta-analysis (Ecological Archives E093-161-A1).