Measuring ecosystem nitrogen status: a comparison of proxies

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Abstract. There are many proxies used to measure nitrogen (N) availability in watersheds, but the degree to which they do (or do not) correlate within a watershed has not been systematically addressed. We surveyed the literature for intact forest or grassland watersheds globally, in which several metrics of nitrogen availability have been measured. Our metrics included: foliar $\delta^{15}N$, soil $\delta^{15}N$, net nitrification, net N mineralization, and the ratio of dissolved inorganic to organic nitrogen (DIN:DON) in soil solution and streams. We were particularly interested in whether terrestrial and stream based proxies for N availability were correlated where they were measured in the same place. Not surprisingly, the strongest correlation (Kendall's tau) was between net nitrification and N mineralization (τ=0.71, p<0.0001). Net nitrification and N mineralization were each correlated with foliar and soil $\delta^{15}N$ (p<0.05). Foliar and soil $\delta^{15}N$ were more tightly correlated in tropical sites (τ=0.68, p<0.0001), than in temperate sites (τ=0.23, p=0.02). The only significant correlations between terrestrial- and water-based metrics were those of net nitrification (τ=0.48, p=0.01) and N mineralization (τ=0.69, p=0.0001) with stream DIN:DON. The relationship between stream DIN:DON with both net nitrification and N mineralization was significant only in temperate, but not tropical regions. To our surprise, we did not find a significant correlation between soil $\delta^{15}N$ and stream DIN:DON, despite the fact that both have

been used to infer spatially or temporally integrated N status. Given that both soil $\delta^{15}N$ and stream DIN:DON are used to infer long-term N status, their lack of correlation in watersheds merits further investigation.

1.0 Introduction

Nitrogen (N) limitation to primary production is widespread in both terrestrial and aquatic ecosystems, and variation in N availability drives differences in ecosystem properties across space and time (Vitousek and Howarth, 1991; Elser et al., 2007; LeBauer and Treseder, 2008). Yet quantifying N availability over timescales that are relevant to ecosystems is non-trivial. Short timescale measurements of N availability in soil are common (e.g. inorganic N pools, N mineralization and nitrification rates; Binkley and Hart, 1989; Sparks et al., 1996), but such short-term proxies are influenced by both short and long-term drivers, and thus it is difficult to know whether short-term proxies can be used to infer N status (i.e. the relative abundance of plant available N) over long timescales in relatively undisturbed ecosystems. For example, measured net mineralization and nitrification rates in arctic tundra are commonly less than annual rates of plant uptake (Schimel et al., 1996; Schmidt et al., 1999), and annual N budgets based on short-term measurements are difficult to balance (e.g. Magill et al., 1997). While long-term N status is relevant to many ecosystem properties and their responses to global change, it is more difficult to measure.

Land-based investigations of N cycling commonly measure soil extractable N, N mineralization, and nitrification, which give a snapshot of N status over minutes to days (Binkley and Hart, 1989; Robertson et al., 1999). Some researchers also use lysimeters to quantify dissolved N losses from below the rooting zone (Hedin et al., 2003; McDowell et al., 2004; Lohse and Matson, 2005) on a similar timescale. Repeated measurements give longer timescale information, but even the longest studies are short relative to ecosystem development.

In addition to these short-term proxies, there are two relatively common measurements of available N that are thought to average over space and/or time. The first is the ratio of dissolved inorganic (DIN) to organic (DON) N concentration lost from ecosystems. Losses of DIN are considered controllable by biota, and thus should be low if soil N is in short supply. In contrast, most DON is not accessible to plants, and thus represents a loss beyond biotic control (Hedin et al., 1995; Figure 1). Thus low DIN:DON in streams has been used to infer relative N-poverty in watersheds (e.g. McDowell and Asbury, 1994; Perakis and Hedin, 2002; Brookshire et al., 2012). The few sites where such measurements have been made over decades (e.g. the Luquillo Mountains of Puerto Rico, Harvard Forest in Massachusetts, Hubbard Brook LTER in New Hampshire; McDowell et al., 1992, McDowell et al., 2004, Bormann and Likens 2012) suggest stream DIN:DON is not particularly variable over this timescale, and thus this metric may integrate N availability over time as well as space (W.C. McDowell, pers. comm.). It is common for researchers measuring stream DIN:DON to infer ecosystem N status implicitly and to assume that a few measurements are indicative of longer-term patterns (e.g. Perakis and Hedin, 2002; Brookshire et al,. 2012).

In contrast to stream DIN:DON, soil $\delta^{15}N$ integrates soil N availability solely over time, and at steady state reflects the isotopic signature associated inputs (N fixation and/or deposition) and fractionation associated with outputs (Handley and Raven, 1992). The major N loss pathways (primarily denitrification, and to a lesser extent nitrate leaching) discriminate against ^{15}N, which thus remains in relative abundance in N-rich soils (Hogburg, 1997; Martinelli et al., 1999; Craine et al., 2009; Houlton and Bai, 2009, Craine et al., 2015; Figure 1). To some degree foliar $\delta^{15}N$ reflects soil $\delta^{15}N$ (Amundson et al., 2003), but there can be fractionation during nitrification, between bulk and soil solution N pools (Hogburg, 1997), during N uptake and assimilation by mychorrhizae and plant tissue (Hobbie et al., 2009; Dawson et al. 2002), and even during xylem transport (Soper et al., 2015). For this reason, foliar $\delta^{15}N$ may display greater variability between species in a single site than the bulk soil $\delta^{15}N$ (Vitousek et al., 1989; Nadlehoffer et al., 1996).

Given that proxies for N availability function over different spatial and temporal scales, we asked which proxies correlate in watersheds where several measurements have been made in the same place and at roughly the same time. We were particularly interested in whether short-timescale measurements (nitrification, mineralization) correlated with the more temporally (foliar and soil $\delta^{15}N$) and spatially (stream DIN:DON) integrated proxies. Unlike previous reviews (Sudduth et al., 2013) we focus solely on unmanaged systems where we were able to compare plant, soil, soil solution and stream proxies. This review assesses the relatinships between common foliar, surface soil (i.e. $\delta^{15}N$, nitrification and mineralization), and nutrient loss (i.e. soil solution and stream N concentrations) metrics of N availability from unmanaged ecosystems globally (Figure 2).

2.0 Methods

2.1 Literature Review

We surveyed the literature (through 2012) and contacted individual investigators to gather data from forested and grassland watersheds where more than one proxy of long-term N availability had been measured. We focused on the most commonly-used proxies for N status: foliar (n=78) and surface soil $\delta^{15}N$ (n=104; <20 cm depth), net nitrification rates (n=86; <20 cm depth), net N mineralization rates (n=88; <20 cm depth), the ratio of dissolved inorganic to organic N forms (DIN:DON) in soil solution below the rooting zone (n=43; >20 cm depth), and stream DIN:DON (n=32). We chose these metrics because 1) other authors have suggested that they are indicative of soil nutrient status (Martinelli et al., 1999, Amundson et al., 2001, Brookshire et al., 2012; Figure 1), and 2) they are thought to integrate N fluxes on different timescales (e.g. soil $\delta^{15}N$ integrates N losses over decades while net N mineralization rates integrate inorganic N production over days; Binkley and Hart, 1989, Hogburg 1997).

We used the search engines Web of Science and Google Scholar and searched key words: "nitrogen", "15N", "natural abundance", "mineralization", and "dissolved organic nitrogen", "*watershed name*". References in papers that resulted from the keyword search were then used to gather additional data. We limited our search criteria to studies that took place in forest or grassland ecosystems that had not incurred any large disturbances that might impair their function.

We collected data from 154 watersheds across a broad climatic range (Figure 2), in which at least two of the six N proxies of interest had been measured (see *Supplemental Data*). We used DataThief II software (version 1.2.1) to extract data from figures when data were not available in text or tables. When necessary, data were converted to standardize units.

From each paper we collected the following site description data: country, site, watershed, biome, ecosystem type, latitude, longitude, elevation (m), mean annual temperature (MAT; °C), mean annual precipitation (MAP; mm yr^{-1}), N deposition rate (kg N ha^{-1} yr^{-1}), soil depth (cm), soil solution (lysimeter) depth (cm), and N mineralization method. Site description data were gathered from other sources when they were not in the original publication.

In order to control for methodological differences, we limited our net nitrification and N mineralization data to those which used intact soil core, buried bag, and laboratory incubations of unamended soils (Boone, 1992; Piccolo et al., 1994), and eliminated studies using methods such as ion resin exchange beads or ^{15}N tracer techniques (Binkley et al., 1986; Hart and Firestone, 1989; Davidson et al., 1991; Templer et al., 2008). We did not limit net nitrification and N mineralization data based on the length of the incubation, as there is little change in rates between 1-7 days (Tietema et al., 1998), however we recognize that longer incubations may result in lower net rates. Soil data from mineral soil horizon only were used, and were preferentially collected in the 0-10 cm range, however if soil samples were not in 10 cm increments, we selected the increment that was most similar (e.g. A horizon, 0-5 cm, 0-15 cm), and no deeper than 20 cm.

When data were missing, or we were uncertain about location or collection method, we contacted the authors to request unpublished data, elucidation of data collection, data reduction, or soil samples. Terrestrial metrics were typically gathered from different papers than that of water-based metrics, requiring validation of congruent watershed location. For five watersheds, including Puerto Rico's Pared, Sonadora, Bisley, Tronoja watersheds and Hubbard Brook's watershed 6, we collected soil that we analyzed for $\delta^{15}N$. In Puerto Rico, we collected five mineral soil samples (0-10 cm) using an open side soil sampler from locations that were >3 m away from the stream. Replicate samples were combined in a Ziploc bag, air-dried and shipped to the Marine Biological Laboratory for analysis. Colleagues at Hubbard Brook collected three replicate B horizon samples for us from several soil pits dug across an elevation gradient in watershed 6 (Christopher Neill, pers. comm.), which were air-dried at the Marine Biological Laboratory prior to analysis.

2.2 Soil Sample Analysis

The soils we analyzed in house for $\delta^{15}N$ were homogenized, sieved (2 mm) and ground using a mortar and pestle. We analyzed samples at the Marine Biological Laboratory Ecosystem

Center Stable Isotope Laboratory for $\delta^{15}N$ using a Europa 20-20 continuous-flow isotope ratio mass spectrometer interfaced with a Europa ANCA-SL elemental analyzer. The analytical precision based on replicate analyses of $\delta^{15}N$ of isotopically homogeneous international standards was ± 0.1 ‰.

2.3 Statistics

Five of our six variables were not normally distributed, so we used a non-parametric Kendall tau rank test in R (version 2.11.1), to determine the significance of all potential correlations. Kendall's tau evaluates the degree of similarity between two sets of ranked data and generates a smaller co-efficient as the number of discordant pairs between two ranking lists becomes greater (Abdi 2007). The Kendall tau rank test is well suited for these comparisons as it is not sensitive to missing data and outliers, it measures both linear and non-linear correlations, and generates a more accurate p-value with small sample sizes (Helsel and Hirsch, 1992; Raike et al., 2003). We corrected for multiple comparisons by reporting Bonferroni adjusted p-values for each of our 15 comparisons (Bland and Altman, 1995). We used the watershed as our experimental unit. When multiple data were available for a single site, we calculated an unweighted mean for the watershed. We removed a single stream DIN:DON value from Cascade Head, Oregon, as it was ~20 times higher than the mean of all other stream values (Compton et al., 2003); however removing this outlier had little effect on the relationships examined.

3.0 Results

All terrestrial-based proxies that integrate across long and short timescales were significantly correlated with each other. Soil $\delta^{15}N$ was positively correlated with both net nitrification (n=60, τ=0.37, p<0.0001) and N mineralization (n=64, τ=0.41, p<0.0001). Foliar $\delta^{15}N$ was also positively correlated with net nitrification (n=43, τ=0.49, p<0.0001), and N mineralization (n=46, τ=0.34, p=0.001; Figure 2).

Not surprisingly, we found significant correlations between terrestrial-based proxies that measure nutrient availability on similar timescales. Foliar $\delta^{15}N$ was positively correlated with soil $\delta^{15}N$ (n=78, τ=0.40, p<0.0001). There was also a positive correlation between net nitrification and N mineralization (n=88, τ=0.71, p<0.0001; Figure 3).

Despite the correlation between all terrestrial-based measurements of N availability, terrestrial metrics did not exhibit similarly robust relationships with water-based proxies. No metric was significantly correlated with soil solution DIN:DON (n=53, p>0.05). Net nitrification (n=15, τ=0.48, p=0.01) and N mineralization (n=17, τ=0.69, p=0.0001) were the only metrics to correlate with stream DIN:DON. Soil solution and stream DIN:DON data were not correlated (Figure 3). All of the data in Figure 3, and their original sources, are available in Supplemental Table 1.

The lack of relationship between water-based and terrestrial-based metrics lead us to ask questions about variability of soil solution and stream DIN:DON across environmental gradients. We found that neither soil solution or stream DIN:DON were correlated with mean annual temperature, precipitation, elevation or N deposition (p>0.05). To our surprise, solution DIN:DON was not correlated with lysimeter depth (p>0.05).

Some relationships between proxies differed with latitude. Soil and foliar $\delta^{15}N$ were more tightly correlated in the tropics (n=24, τ=0.68, p<0.0001) than in the temperate zone (n=49, τ=0.23, p=0.02). Soil $\delta^{15}N$ was correlated with net nitrification in tropical (n=17, τ=0.39, p=0.03), but not temperate regions. Conversely, soil $\delta^{15}N$ was correlated with net N mineralization (n=44, τ=0.34, p=0.001) in temperate but not tropical areas. Stream DIN:DON was correlated with net nitrification (n=10, τ=0.63, p=0.01) and N mineralization (n=10, τ=0.78, p=0.002) in the temperate zone, and not in the tropics (n=4, p>0.05). Because we only found multiple proxies measured at eleven boreal sites, this limited our ability to compare correlated data in boreal regions with correlations in temperate or tropical areas.

4.0 Discussion

The metrics presented here are typically interpreted to fall into one of three categories: 1) long-timescale (decades to centuries) integrators of soil N losses (foliar and soil $\delta^{15}N$; Martinelli et al., 1999, Craine et al., 2015), 2) short-timescale direct measures of N transformations (mineralization, nitrification; Vitousek et al., 1982), and 3) short-medium timescale (weeks to years) measures of hydrologic N losses that are influenced by N availability in a catchment (soil solution and stream DIN:DON; Hedin et al., 1995; Perakis and Hedin, 2001). Our data suggest that category 1 and 2 metrics are correlated, and that short-term soil assays may capture similar patterns as inferred by long-term plant and soil-based proxies. However, the lack of correlation between long-term terrestrial proxies (plant and soil $\delta^{15}N$) and both soil solution and stream DIN:DON is interesting, as several authors have suggested that both types of proxies give insight into ecosystem N status (Vitousek et al., 1982; Hedin et al., 1995; Martinelli et al., 1999; Perakis and Hedin, 2001; Amundson et al., 2003; Brookshire et al., 2012).

It is particularly interesting that stream DIN:DON was not correlated with soil $\delta^{15}N$ as both are proxies used to infer long-term N status. There is a wealth of literature that uses stream DIN:DON to infer large spatial and temporal scale patterns in N availability (Hedin et al., 1995; Perakis and Hedin, 2002; McDowell et al., 2004; Fang et al., 2008). Similarly, many studies interpret soil $\delta^{15}N$ as an integrator of coupled N cycling and N losses over time (Martinelli et al., 1999; Houlton et al., 2006; Houlton and Bai, 2009, Craine et al., 2015). These are the only two proxies for N status that integrate over relatively long timescales, and their lack of correlation with each other warrants more careful consideration. We note that stream DIN:DON is sensitive to N deposition, and that relatively pristine settings have a lower DIN:DON than polluted ones (Perakis and Hedin, 2001). In our dataset, N deposition was not correlated with stream DIN:DON (τ=0.03, p>0.05), or any other metric. Although 48% of our sites lacked N deposition data, our data do not support the idea that N deposition is responsible for the lack of correlation between these two long-term proxies.

Another surprise from our dataset is that soil solution DIN:DON was not significantly correlated with any other metric, not even with stream DIN:DON, despite ~40% of papers in our dataset reporting both soil solution and stream DIN:DON in the same watershed. While the correlation between soil solution DIN:DON below the rooting zone and N availability has been documented across gradients in soil age and fertility (Hedin et al., 1995), this correlation was not found across the range of sites examined here. We found no relationship between soil solution DIN:DON and lysimeter depth, suggesting that the majority of N transformations responsible for the discontinuity between soil solution DIN:DON and that of terrestrial metrics are likely occurring either within the rooting zone or in riparian zones. Neither soil solution or stream DIN:DON was sensitive to environmental variability (i.e. elevation, temperature, precipitation, N deposition), suggesting that processing along flow paths may be responsible for the disconnect between soil solution and stream N concentrations. From these data, at least, it does not seem that soil solution DIN:DON can be used to infer terrestrial N status across this suite of unmanaged sites. These data also do not support the idea that soil solution DIN:DON is representative of N forms that leach into streams (Binkley et al., 1992; Pregitzer et al., 2004; Fang et al., 2008).

While nitrate (NO_3^-) removal along flow paths can reduce stream NO_3^- (Vidon et al., 2010), with higher percent removal in forested watersheds (Sudduth et al., 2013), DON has been shown to be relatively resistant to removal by decomposition and biologic uptake along subsurface flow paths (Carreiro et al., 2000, Neff et al. 2003). We found no correlation between stream and soil solution DIN:DON, and suggest that variation in NO_3^- removal (relative to DON) along flow paths of undisturbed ecosystems may explain this lack of correlation. The extent to which riparian zones influence nutrients varies spatially with geomorphology, soil texture, vegetation, and riparian zone development (McDowell et al., 1992, Mayer et al., 2007); and soils with high rates of leaching to ground water may bypass riparian processing. As nutrients leach down the soil profile, denitrification, biologic uptake, and storage are all potential mechanisms that could alter soil solution and stream N species concentrations. Investigation of soil profile processes and riparian zone spatial variability may help determine where and when watershed-scale N status can be inferred from these proxies. Alternatively, varied land-use (e.g. pasture, N fixing plant species, etc.) upstream of undisturbed sites is typically not reported in the literature, but is another possible explanation for the break down between terrestrial and water-based proxies.

While most observed relationships were consistent across latitudes, a few differed between the tropics and the temperate zone. The relationships between soil $\delta^{15}N$ with foliar $\delta^{15}N$, foliar $\delta^{15}N$ with net nitrification, and net nitrification with N mineralization were consistent across both tropical and temperate regions. However, net nitrification and N mineralization were correlated with stream DIN:DON only in temperate regions. These data suggest that while terrestrial proxies may be a useful across biomes, stream DIN:DON requires further research to understand the extent of its applicability across space. The relationship between foliar and soil $\delta^{15}N$ also differs across latitudes, in that the correlation in the tropics was much tighter than in the temperate zone. Bias in the literature towards natural abundance isotopic data from the temperate zone may explain why previous research looking at this relationship has been noisy (Craine et al., 2009).

One commonly reported metric that was not included in our analysis is the bulk soil carbon to nitrogen ratio (C:N). The conception for this manuscript focused on the relationship between soil $\delta^{15}N$ and stream DIN:DON, because these are most commonly used as long term proxies of N availability (Martinelli et al., 1999; Amundson et al., 2000; Perakis and Hedin et al. 2001; Brookshire et al. 2012). Specifically, theory regarding spatial differences in N availability, especially between the tropics and temperate zone, focus on the metrics we report here. Conclusions about N richness at the global scale have yet to use C:N data to support the theory for latitudinal gradation in N availability (Brookshire et al. 2011; Smith et al.; 2014). Soil C:N has already been shown to be tightly correlated with soil $\delta^{15}N$ at the global scale (Craine et al., 2015), but has yet to be compared to the other metrics we present here. Its relationship with soil $\delta^{15}N$ leads us to believe that soil C:N will likely reflect the same trends as that of soil $\delta^{15}N$. The measurement of soil C:N is perhaps reported more so than any other biogeochemical metric, and certainly more so than those included in this meta-analysis. We suggest that future research utilize meta-analysis techniques to look at how soil C:N changes across ecosystem gradients, and whether or not it agrees with latitudinal patterns observed for soil $\delta^{15}N$ and stream DIN:DON (Martinelli et al., 1999; Brookshire et al. 2011).

Although we found that temporal (soil $\delta^{15}N$) and spatial (stream DIN:DON) integrators of watershed N were correlated with short-term proxies (net nitrification and net N mineralization), water-based metrics did not correlate very well with most of the soil-based metrics of N availability or each other. Explicit comparisons of these proxies to each other, with a focus on how they are influenced by hot-spots, hot-moments, biological diversity, and N transformation between the soil-stream interface, will enhance their utility for understanding N availability at the ecosystem scale.

5.0 Conclusions

Despite decades of research the N status of terrestrial ecosystems remains difficult to measure, and researchers typically employ several metrics of N availability. While nitrification and mineralization most frequently correlate with other metrics, they are short-term proxies that vary over short spatial and temporal scales. Soil $\delta^{15}N$ and dissolved N losses from streams are long-term integrators of N loss that have been relied on to advance our understanding of N cycling at the global scale (Martinelli et al., 1999; Amundson et al., 2003; Hedin et al., 2003; Brookshire et al., 2012), however the lack of correlation between these two commonly used proxies highlights the need to better understand how these terrestrial and stream-based metrics vary in relation to each other and with soil N availability.

Understanding ecosystem N status at the watershed and landscape scale is a first step towards projecting ecosystem responses to climate change and environmental pollution (Aber et al., 1998; Oren et al., 2001; Reich et al., 2004). Soil N status can determine the rate at which detrimental N losses occur, such as NO_3^- (a drinking water contaminant) and nitrous oxide (a potent greenhouse gas). Furthermore, it is becoming more evident that projections regarding the potential for a terrestrial CO_2 sink, and concomitant feedbacks to the trajectory of climate change, are dependent on the nutrient status of soils (Thornton et al., 2007; Zaehle et al., 2010;

Wieder et al., 2015). The health and environmental implications of soil N status heighten the need to develop methodology to adequately assess long-term soil N availability.

6.0 Author contribution

M. Almaraz and S. Porder conceived research and designed study. M. Almaraz collected data and performed statistical analyses. M. Almaraz and S. Porder wrote the manuscript.

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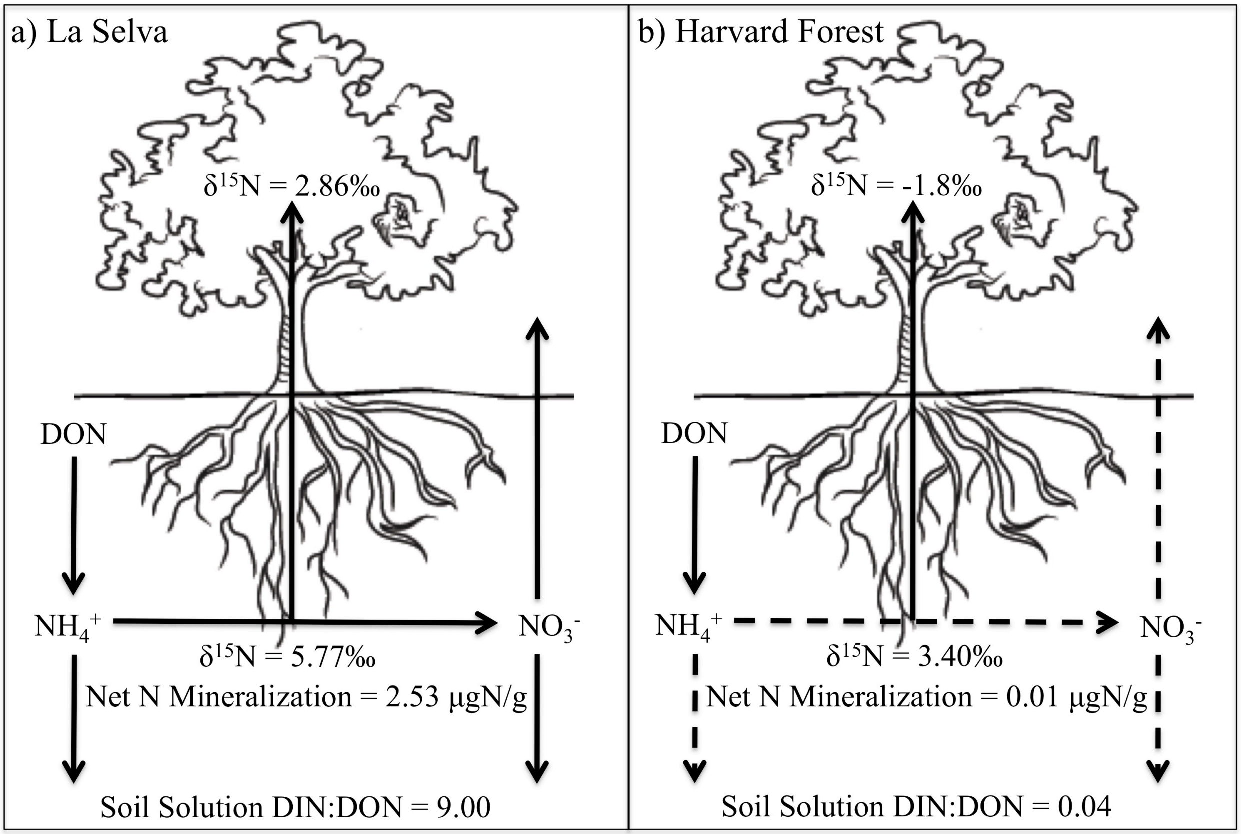

Figure 1. Nitrogen availability values for a) a nitrogen rich tropical forest at the La Selva field station in Costa Rica, and for b) a nitrogen limited temperate pine forest at Harvard Forest, Massachusetts. Solid and dotted lines represent the relative magnitude of fluxes (i.e. net N mineralization, denitrification to the atmosphere, dissolved organic and inorganic nitrogen leaching), which are contingent on ecosystem nitrogen status.

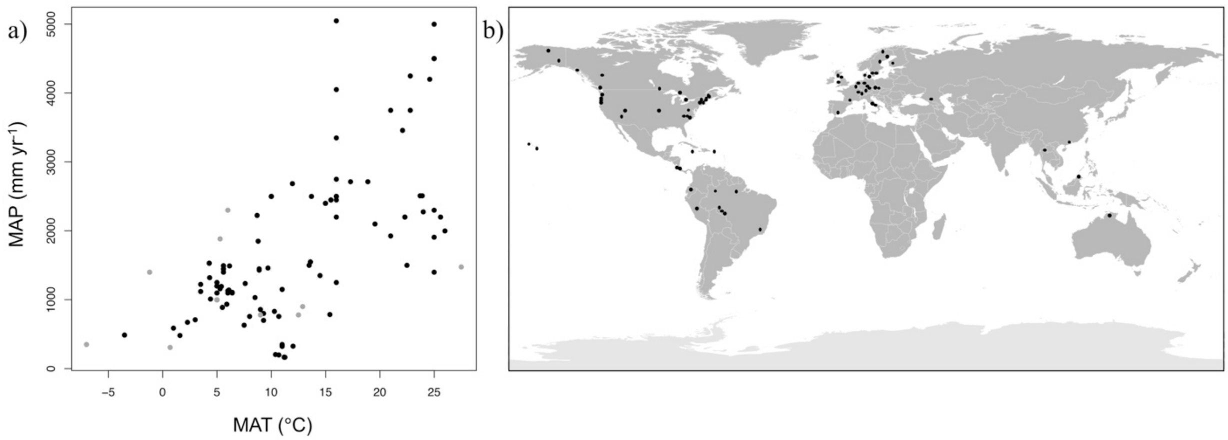

Figure 2. a) Distribution of grassland (grey) and forest (black) watershed mean annual temperature (MAT; °C) and mean annual precipitation (MAP; mm yr^{-1}) included in meta-analysis (left), and b) location of 154 sites (some black dots represent multiple watersheds; right).

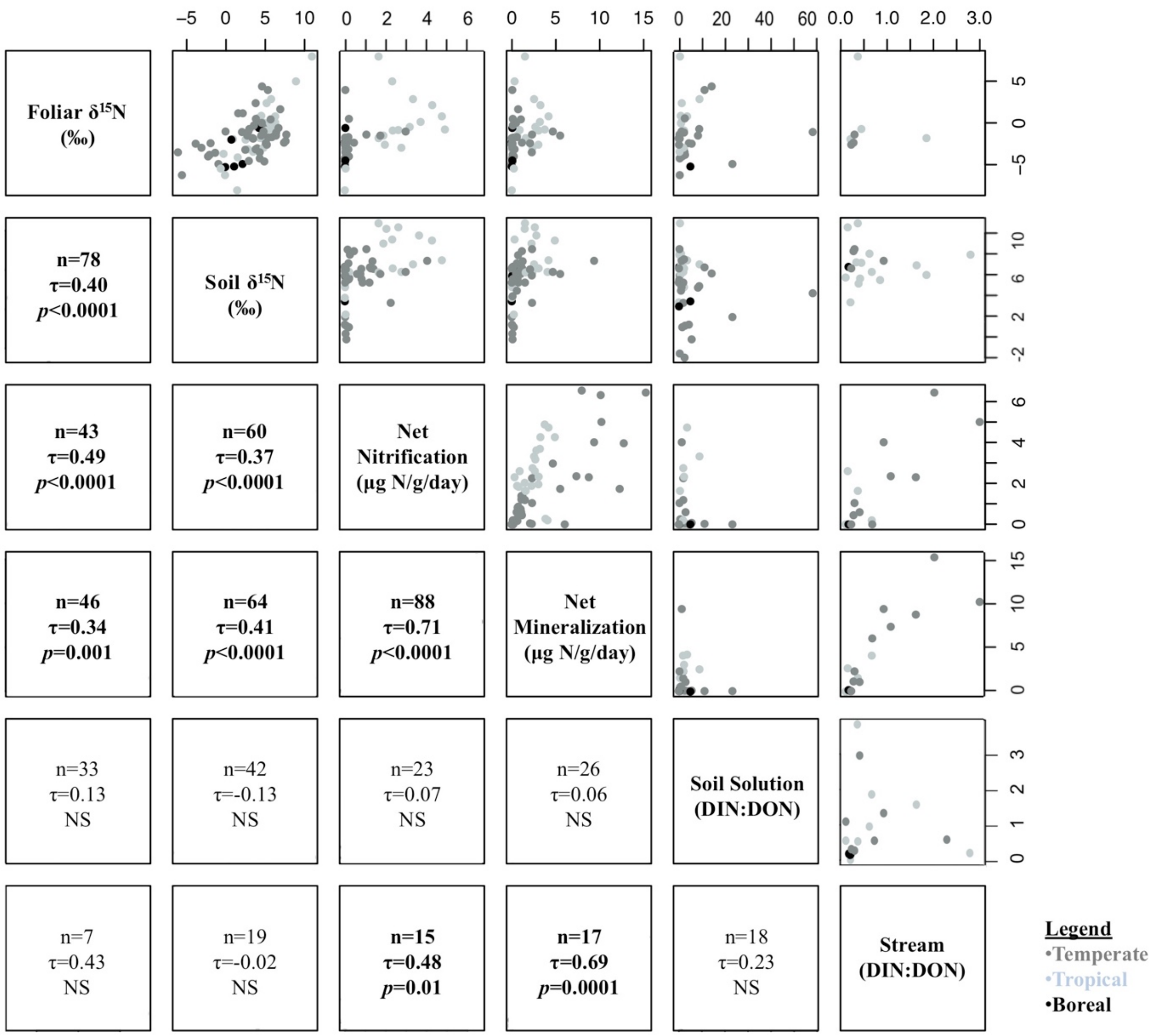

Figure 3. Correlation matrix of N status proxies (foliar and soil $\delta^{15}N$, net nitrification and N mineralization (<20 cm), the ratio of dissolved inorganic to organic N forms (DIN:DON) in soil solution below the rooting zone (>20 cm), and the DIN:DON in streams). Data are above the diagonal, summary statistics are below. NS signifies correlations that were not significant (*p*>0.05).

Supplemental Table 1. Site description data, full citations, foliar and surface soil $\delta^{15}N$ (<20 cm depth), net nitrification (<20 cm depth), net N mineralization (<20 cm depth), the ratio of dissolved inorganic to organic N forms (DIN:DON) in soil solution below the rooting zone (>20 cm depth), and stream DIN:DON.