

1 **Measuring ecosystem nitrogen status: a comparison of proxies**

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6 organic nitrogen

7

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

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

27 **1.0 Introduction**

28 Nitrogen (N) limitation to primary production is widespread in both terrestrial and  
29 aquatic ecosystems, and variation in N availability drives differences in ecosystem properties  
30 across space and time (Vitousek and Howarth, 1991; Elser et al., 2007; LeBauer and Treseder,  
31 2008). Yet quantifying N availability over timescales that are relevant to ecosystems is non-  
32 trivial. Short timescale measurements of N availability in soil are common (e.g. inorganic N  
33 pools, N mineralization and nitrification rates; Binkley and Hart, 1989; Sparks et al., 1996), but  
34 such short-term proxies are influenced by both short and long-term drivers, and thus it is difficult  
35 to know whether short-term proxies can be used to infer N status (i.e. the relative abundance of  
36 plant available N) over long timescales in relatively undisturbed ecosystems. For example,  
37 measured net mineralization and nitrification rates in arctic tundra are commonly less than  
38 annual rates of plant uptake (Schimel et al., 1996; Schmidt et al., 1999), and annual N budgets  
39 based on short-term measurements are difficult to balance (e.g. Magill et al., 1997). While long-  
40 term N status is relevant to many ecosystem properties and their responses to global change, it is  
41 more difficult to measure.

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

48 In addition to these short-term proxies, there are two relatively common measurements of  
49 available N that are thought to average over space and/or time. The first is the ratio of dissolved

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

63 In contrast to stream DIN:DON, soil  $\delta^{15}\text{N}$  integrates soil N availability solely over time,  
64 and at steady state reflects the isotopic signature associated inputs (N fixation and/or deposition)  
65 and fractionation associated with outputs (Handley and Raven, 1992). The major N loss  
66 pathways (primarily denitrification, and to a lesser extent nitrate leaching) discriminate against  
67  $^{15}\text{N}$ , which thus remains in relative abundance in N-rich soils (Hogburg, 1997; Martinelli et al.,  
68 1999; Craine et al., 2009; Houlton and Bai, 2009, Craine et al., 2015; Figure 1). To some degree  
69 foliar  $\delta^{15}\text{N}$  reflects soil  $\delta^{15}\text{N}$  (Amundson et al., 2003), but there can be fractionation during  
70 nitrification, between bulk and soil solution N pools (Hogburg, 1997), during N uptake and  
71 assimilation by mycorrhizae and plant tissue (Hobbie et al., 2009; Dawson et al. 2002), and  
72 even during xylem transport (Soper et al., 2015). For this reason, foliar  $\delta^{15}\text{N}$  may display greater

73 variability between species in a single site than the bulk soil  $\delta^{15}\text{N}$  (Vitousek et al., 1989;  
74 Nadlehoffer et al., 1996).

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

85

## 86 **2.0 Methods**

87

### 88 **2.1 Literature Review**

89         We surveyed the literature (through 2012) and contacted individual investigators to  
90 gather data from forested and grassland watersheds where more than one proxy of long-term N  
91 availability had been measured. We focused on the most commonly-used proxies for N status:  
92 foliar (n=78) and surface soil  $\delta^{15}\text{N}$  (n=104; <20 cm depth), net nitrification rates (n=86; <20 cm  
93 depth), net N mineralization rates (n=88; <20 cm depth), the ratio of dissolved inorganic to  
94 organic N forms (DIN:DON) in soil solution below the rooting zone (n=43; >20 cm depth), and  
95 stream DIN:DON (n=32). We chose these metrics because 1) other authors have suggested that

96 they are indicative of soil nutrient status (Martinelli et al., 1999, Amundson et al., 2001,  
97 Brookshire et al., 2012; Figure 1), and 2) they are thought to integrate N fluxes on different  
98 timescales (e.g. soil  $\delta^{15}\text{N}$  integrates N losses over decades while net N mineralization rates  
99 integrate inorganic N production over days; Binkley and Hart, 1989, Hogburg 1997).

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

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

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

115 In order to control for methodological differences, we limited our net nitrification and N  
116 mineralization data to those which used intact soil core, buried bag, and laboratory incubations of  
117 unamended soils (Boone, 1992; Piccolo et al., 1994), and eliminated studies using methods such  
118 as ion resin exchange beads or  $^{15}\text{N}$  tracer techniques (Binkley et al., 1986; Hart and Firestone,

119 1989; Davidson et al., 1991; Templer et al., 2008). We did not limit net nitrification and N  
120 mineralization data based on the length of the incubation, as there is little change in rates  
121 between 1-7 days (Tietema et al., 1998), however we recognize that longer incubations may  
122 result in lower net rates. Soil data from mineral soil horizon only were used, and were  
123 preferentially collected in the 0-10 cm range, however if soil samples were not in 10 cm  
124 increments, we selected the increment that was most similar (e.g. A horizon, 0-5 cm, 0-15 cm),  
125 and no deeper than 20 cm.

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

138

## 139 **2.2 Soil Sample Analysis**

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

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

146

### 147 **2.3 Statistics**

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

161

### 162 **3.0 Results**

163 All terrestrial-based proxies that integrate across long and short timescales were  
164 significantly correlated with each other. Soil  $\delta^{15}\text{N}$  was positively correlated with both net



165 nitrification ( $n=60$ ,  $\tau=0.37$ ,  $p<0.0001$ ) and N mineralization ( $n=64$ ,  $\tau=0.41$ ,  $p<0.0001$ ). Foliar  
166  $\delta^{15}\text{N}$  was also positively correlated with net nitrification ( $n=43$ ,  $\tau=0.49$ ,  $p<0.0001$ ), and N  
167 mineralization ( $n=46$ ,  $\tau=0.34$ ,  $p=0.001$ ; Figure 2).

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

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

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

184 Some relationships between proxies differed with latitude. Soil and foliar  $\delta^{15}\text{N}$  were more  
185 tightly correlated in the tropics ( $n=24$ ,  $\tau=0.68$ ,  $p<0.0001$ ) than in the temperate zone ( $n=49$ ,  
186  $\tau=0.23$ ,  $p=0.02$ ). Soil  $\delta^{15}\text{N}$  was correlated with net nitrification in tropical ( $n=17$ ,  $\tau=0.39$ ,  
187  $p=0.03$ ), but not temperate regions. Conversely, soil  $\delta^{15}\text{N}$  was correlated with net N

188 mineralization ( $n=44$ ,  $\tau=0.34$ ,  $p=0.001$ ) in temperate but not tropical areas. Stream DIN:DON  
189 was correlated with net nitrification ( $n=10$ ,  $\tau=0.63$ ,  $p=0.01$ ) and N mineralization ( $n=10$ ,  $\tau=0.78$ ,  
190  $p=0.002$ ) in the temperate zone, and not in the tropics ( $n=4$ ,  $p>0.05$ ). Because we only found  
191 multiple proxies measured at eleven boreal sites, this limited our ability to compare correlated  
192 data in boreal regions with correlations in temperate or tropical areas.

Comment [AM1]: Revision #2

#### 194 4.0 Discussion

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

207 It is particularly interesting that stream DIN:DON was not correlated with soil  $\delta^{15}\text{N}$  as both  
208 are proxies used to infer long-term N status. There is a wealth of literature that uses stream  
209 DIN:DON to infer large spatial and temporal scale patterns in N availability (Hedin et al., 1995;  
210 Perakis and Hedin, 2002; McDowell et al., 2004; Fang et al., 2008). Similarly, many studies

211 interpret soil  $\delta^{15}\text{N}$  as an integrator of coupled N cycling and N losses over time (Martinelli et al.,  
212 1999; Houlton et al., 2006; Houlton and Bai, 2009, Craine et al., 2015). These are the only two  
213 proxies for N status that integrate over relatively long timescales, and their lack of correlation  
214 with each other warrants more careful consideration. We note that stream DIN:DON is sensitive  
215 to N deposition, and that relatively pristine settings have a lower DIN:DON than polluted ones  
216 (Perakis and Hedin, 2001). In our dataset, N deposition was not correlated with stream  
217 DIN:DON ( $\tau=0.03$ ,  $p>0.05$ ), or any other metric. Although 48% of our sites lacked N deposition  
218 data, our data do not support the idea that N deposition is responsible for the lack of correlation  
219 between these two long-term proxies.

Comment [AM2]: Revision #3

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

234 representative of N forms that leach into streams (Binkley et al., 1992; Pregitzer et al., 2004;  
235 Fang et al., 2008).

236 While nitrate ( $\text{NO}_3^-$ ) removal along flow paths can reduce stream  $\text{NO}_3^-$  (Vidon et al., 2010),  
237 with higher percent removal in forested watersheds (Sudduth et al., 2013), DON has been shown  
238 to be relatively resistant to removal by decomposition and biologic uptake along subsurface flow  
239 paths (Carreiro et al., 2000, Neff et al. 2003). We found no correlation between stream and soil  
240 solution DIN:DON, and suggest that variation in  $\text{NO}_3^-$  removal (relative to DON) along flow  
241 paths of undisturbed ecosystems may explain this lack of correlation. The extent to which  
242 riparian zones influence nutrients varies spatially with geomorphology, soil texture, vegetation,  
243 and riparian zone development (McDowell et al., 1992, Mayer et al., 2007); and soils with high  
244 rates of leaching to ground water may bypass riparian processing. As nutrients leach down the  
245 soil profile, denitrification, biologic uptake, and storage are all potential mechanisms that could  
246 alter soil solution and stream N species concentrations. Investigation of soil profile processes and  
247 riparian zone spatial variability may help determine where and when watershed-scale N status  
248 can be inferred from these proxies. Alternatively, varied land-use (e.g. pasture, N fixing plant  
249 species, etc.) upstream of undisturbed sites is typically not reported in the literature, but is  
250 another possible explanation for the break down between terrestrial and water-based proxies.

251 While most observed relationships were consistent across latitudes, a few differed between  
252 the tropics and the temperate zone. The relationships between soil  $\delta^{15}\text{N}$  with foliar  $\delta^{15}\text{N}$ , foliar  
253  $\delta^{15}\text{N}$  with net nitrification, and net nitrification with N mineralization were consistent across both  
254 tropical and temperate regions. However, net nitrification and N mineralization were correlated  
255 with stream DIN:DON only in temperate regions. These data suggest that while terrestrial  
256 proxies may be a useful across biomes, stream DIN:DON requires further research to understand

257 the extent of its applicability across space. The relationship between foliar and soil  $\delta^{15}\text{N}$  also  
258 differs across latitudes, in that the correlation in the tropics was much tighter than in the  
259 temperate zone. Bias in the literature towards natural abundance isotopic data from the temperate  
260 zone may explain why previous research looking at this relationship has been noisy (Craine et  
261 al., 2009).

262 One commonly reported metric that was not included in our analysis is the bulk soil carbon  
263 to nitrogen ratio (C:N). The conception for this manuscript focused on the relationship between  
264 soil  $\delta^{15}\text{N}$  and stream DIN:DON, because these are most commonly used as long term proxies of  
265 N availability (Martinelli et al., 1999; Amundson et al., 2000; Perakis and Hedin et al. 2001;  
266 Brookshire et al. 2012). Specifically, theory regarding spatial differences in N availability,  
267 especially between the tropics and temperate zone, focus on the metrics we report here.  
268 Conclusions about N richness at the global scale have yet to use C:N data to support the theory  
269 for latitudinal gradation in N availability (Brookshire et al. 2011; Smith et al., 2014). Soil C:N  
270 has already been shown to be tightly correlated with soil  $\delta^{15}\text{N}$  at the global scale (Craine et al.,  
271 2015), but has yet to be compared to the other metrics we present here. It's relationship with soil  
272  $\delta^{15}\text{N}$  leads us to believe that soil C:N will likely reflect the same trends as that of soil  $\delta^{15}\text{N}$ . The  
273 measurement of soil C:N is perhaps reported more so than any other biogeochemical metric, and  
274 certainly more so than those included in this meta-analysis. We suggest that future research  
275 utilize meta-analysis techniques to look at how soil C:N changes across ecosystem gradients, and  
276 whether or not it agrees with latitudinal patterns observed for soil  $\delta^{15}\text{N}$  and stream DIN:DON  
277 (Martinelli et al., 1999; Brookshire et al. 2011).

278 Although we found that temporal (soil  $\delta^{15}\text{N}$ ) and spatial (stream DIN:DON) integrators of  
279 watershed N were correlated with short-term proxies (net nitrification and net N mineralization),

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280 water-based metrics did not correlate very well with most of the soil-based metrics of N  
281 availability or each other. Explicit comparisons of these proxies to each other, with a focus on  
282 how they are influenced by hot-spots, hot-moments, biological diversity, and N transformation  
283 between the soil-stream interface, will enhance their utility for understanding N availability at  
284 the ecosystem scale.

285

## 286 **5.0 Conclusions**

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

296 Understanding ecosystem N status at the watershed and landscape scale is a first step towards  
297 projecting ecosystem responses to climate change and environmental pollution (Aber et al.,  
298 1998; Oren et al., 2001; Reich et al., 2004). Soil N status can determine the rate at which  
299 detrimental N losses occur, such as  $\text{NO}_3^-$  (a drinking water contaminant) and nitrous oxide (a  
300 potent greenhouse gas). Furthermore, it is becoming more evident that projections regarding the  
301 potential for a terrestrial  $\text{CO}_2$  sink, and concomitant feedbacks to the trajectory of climate  
302 change, are dependent on the nutrient status of soils (Thornton et al., 2007; Zaehle et al., 2010;

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

305

306 **6.0 Author contribution**

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

309

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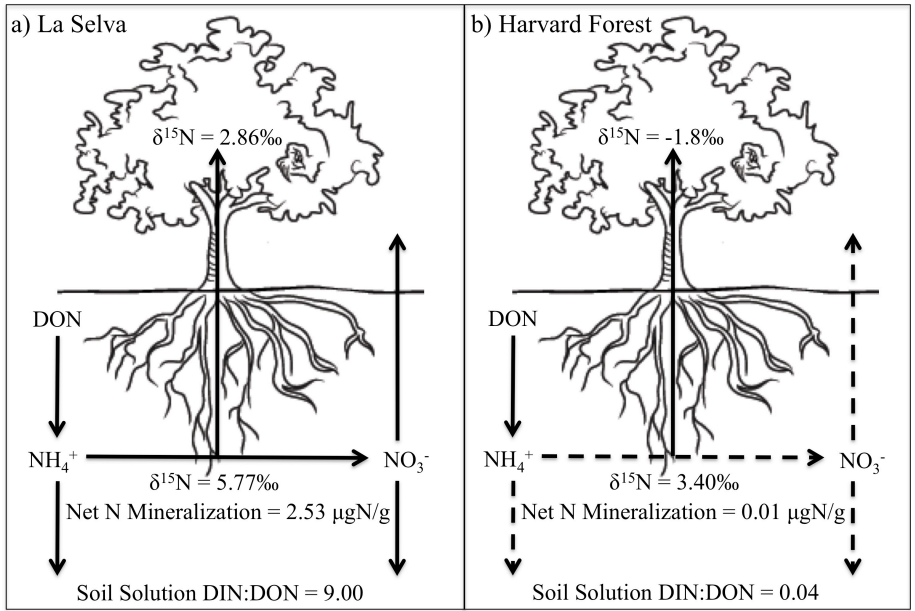
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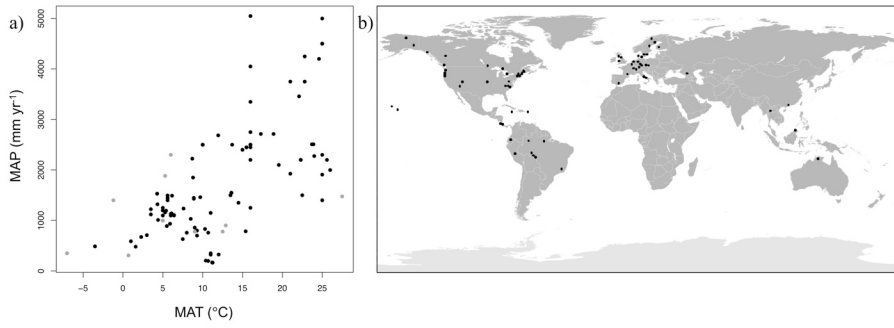
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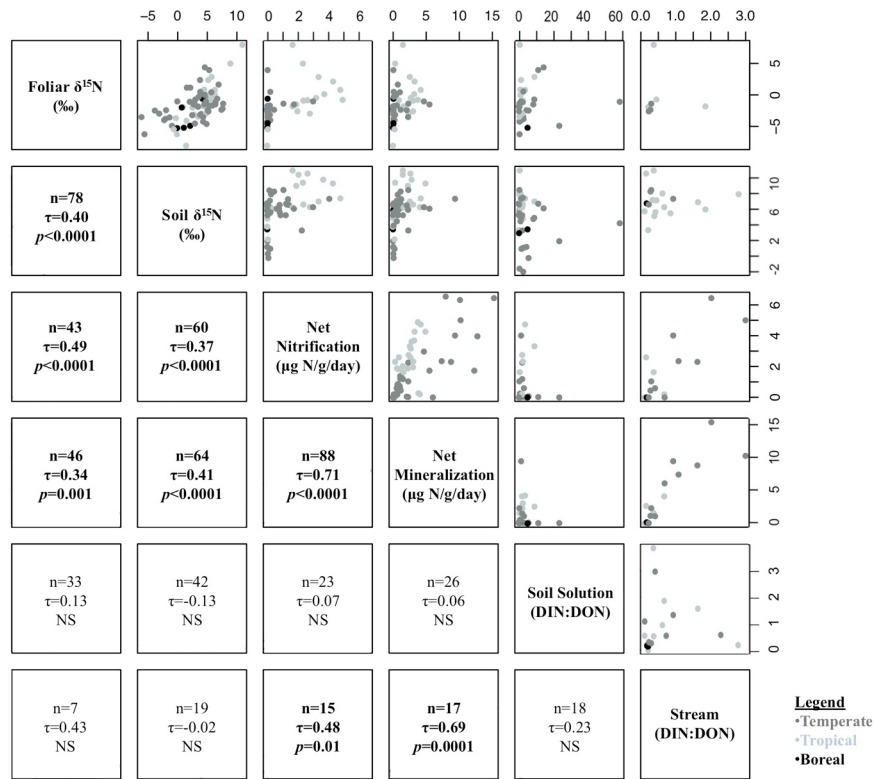
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 547 **Figure 1.** Nitrogen availability values for a) a nitrogen rich tropical forest at the La Selva field  
 548 station in Costa Rica, and for b) a nitrogen limited temperate pine forest at Harvard Forest,  
 549 Massachusetts. Solid and dotted lines represent the relative magnitude of fluxes (i.e. net N  
 550 mineralization, denitrification to the atmosphere, dissolved organic and inorganic nitrogen  
 551 leaching), which are contingent on ecosystem nitrogen status.  
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**Figure 2.** a) Distribution of grassland (grey) and forest (black) watershed mean annual temperature (MAT; °C) and mean annual precipitation (MAP; mm yr<sup>-1</sup>) included in meta-analysis (left), and b) location of 154 sites (some black dots represent multiple watersheds; right).

**Comment [AM4]:** Revision #5



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**Figure 3.** Correlation matrix of N status proxies (foliar and soil δ<sup>15</sup>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 ( $p>0.05$ ).

Comment [AM5]: Revision #4



567 **Supplemental Table 1.** Site description data, full citations, foliar and surface soil  $\delta^{15}\text{N}$  (<20 cm  
568 depth), net nitrification (<20 cm depth), net N mineralization (<20 cm depth), the ratio of  
569 dissolved inorganic to organic N forms (DIN:DON) in soil solution below the rooting zone (>20  
570 cm depth), and stream DIN:DON.  
571

572 **Reply to the Associate Editor's Comments**

573

574 Thanks very much for your continued handling of the manuscript and your constructive  
575 comments. Please find attached our responses point by point. We hope you find them  
576 satisfactory.

577

578 **1) Editor:** While I can see that you do not want to invest into gathering C:N ratio, I agree with  
579 reviewer #1 that this would have improved the impact of your manuscript substantially. This is  
580 of course your decision. However, either in the introduction or discussion you need to explain  
581 why you have omitted one of this easily measurable and relevant measure of nitrogen status, and  
582 why you think that this would not have affected your results.

583

584 **Response:** We agree that the inclusion of C:N data would have been an excellent addition to the  
585 manuscript, and we regret not having included it in the original analysis. Unfortunately, to redo  
586 the analysis using our original method would require performing a new search for every paper  
587 that reports C:N, following up on new watersheds included in the data set to find additional  
588 metrics measured there, and ultimately retracting the current version of the manuscript from  
589 Biogeosciences, as we estimate this amount of work taking months, if not longer. A meta-  
590 analysis of C:N has never been done and could potentially hold up as a paper on its own. Soil  
591 C:N is not usually used as a long term proxy for N availability in the same way as  $\delta^{15}\text{N}$ , because  
592 C:N is not solely sensitive to fractionating N losses. Further research looking at soil C:N  
593 dynamics at the global scale will shed light on the potential utility of this metric in assessing long  
594 term N availability. We want to acknowledge the importance of this metric in our discussion and  
595 have thus added the following paragraph to our discussion section:

596

597 Lines 262-277: "One commonly reported metric that was not included in our analysis is the bulk  
598 soil carbon to nitrogen ratio (C:N). The conception for this manuscript focused on the  
599 relationship between soil  $\delta^{15}\text{N}$  and stream DIN:DON, because these are most commonly used as  
600 long term proxies of N availability (Martinelli et al., 1999; Amundson et al., 2000; Perakis and  
601 Hedin et al. 2001; Brookshire et al. 2012). Specifically, theory regarding spatial differences in N  
602 availability, especially between the tropics and temperate zone, focus on the metrics we report  
603 here. Conclusions about N richness at the global scale have yet to use C:N data to support the  
604 theory for latitudinal gradation in N availability (Brookshire et al. 2011; Smith et al.; 2014). Soil  
605 C:N has already been shown to be tightly correlated with soil  $\delta^{15}\text{N}$  at the global scale (Craine et  
606 al., 2015), but has yet to be compared to the other metrics we present here. It's relationship with  
607 soil  $\delta^{15}\text{N}$  leads us to believe that soil C:N will likely reflect the same trends as that of soil  $\delta^{15}\text{N}$ .  
608 The measurement of soil C:N is perhaps reported more so than any other biogeochemical metric,  
609 and certainly more so than those included in this meta-analysis. We suggest that future research  
610 utilize meta-analysis techniques to look at how soil C:N changes across ecosystem gradients, and  
611 whether or not it agrees with latitudinal patterns observed for soil  $\delta^{15}\text{N}$  and stream DIN:DON  
612 (Martinelli et al., 1999; Brookshire et al. 2011).

613

614 **2) Editor:** L177ff: Please acknowledge that lacking data availability prevented you from  
615 identifying differences between temperate and boreal ecosystems (rather than not being there at  
616 all).

617

618 **Response:** We have added the following text to our results in lines 190-192: “Because we only  
619 found multiple proxies measured at eleven boreal sites, this limited our ability to compare  
620 correlated data in boreal regions with correlations in temperate or tropical areas.”

621  
622 **3) Editor:** L205ff: This discussion fails to acknowledge that N deposition data was lacking for  
623 an important fraction of the data set.

624  
625 **Response:** This is a good point. We have added the following text to the discussion in lines 217-  
626 219: “Although 48% of our sites lacked N deposition data, our data do not support the idea that  
627 N deposition is responsible for the lack of correlation between these two long-term proxies.”

628  
629 **4) Editor:** Figure 2: increase font size of axis labels

630  
631 **Response:** Change made.

632  
633 **5) Editor:** Figure 3: Improve resolution of figure. Use entire column width to maximise  
634 readability.

635  
636 **Response:** Change made. All of our figures are now 600dpi, please let us know if that resolution  
637 does not suffice.