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

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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 δ^{15}N,

12   soil δ^{15}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 (τ=0.71, p<0.0001). Net nitrification and N mineralization

17   were each correlated with foliar and soil δ^{15}N (p<0.05). Foliar and soil δ^{15}N were more tightly

18   correlated in tropical sites (τ=0.68, p<0.0001), than in temperate sites (τ=0.23, p=0.02). The only

19   significant correlations between terrestrial- and water-based metrics were those of net

20   nitrification (τ=0.48, p=0.01) and N mineralization (τ=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 δ^{15}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 δ^{15}N and

25   stream DIN:DON are used to infer long-term N status, their lack of correlation in watersheds

26   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

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 δ^{15}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}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 δ^{15}N reflects soil δ^{15}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 mychorrhizae 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 δ^{15}N may display greater

73   variability between species in a single site than the bulk soil $\delta^{15}N$ (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 $\delta^{15}N$) 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 relatinships

82   between common foliar, surface soil (i.e. $\delta^{15}N$, 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 $\delta^{15}N$ (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 δ^{15}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", "15N", "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^{-1}), N deposition rate (kg N ha^{-1} yr^{-1}), 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}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 δ^{15}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 δ^{15}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 δ^{15}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 δ^{15}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; Raike

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 δ^{15}N was positively correlated with both net

165 nitrification (n=60, τ=0.37, p<0.0001) and N mineralization (n=64, τ=0.41, p<0.0001). Foliar

166 δ^{15}N was also positively correlated with net nitrification (n=43, τ=0.49, p<0.0001), and N

167 mineralization (n=46, τ=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 δ^{15}N was positively correlated with

170 soil δ^{15}N (n=78, τ=0.40, p<0.0001). There was also a positive correlation between net

171 nitrification and N mineralization (n=88, τ=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, τ=0.48, p=0.01) and N mineralization (n=17, τ=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 δ^{15}N were more

185 tightly correlated in the tropics (n=24, τ=0.68, p<0.0001) than in the temperate zone (n=49,

186 τ=0.23, p=0.02). Soil δ^{15}N was correlated with net nitrification in tropical (n=17, τ=0.39,

187 p=0.03), but not temperate regions. Conversely, soil δ^{15}N was correlated with net N

188 mineralization (n=44, τ=0.34, p=0.001) in temperate but not tropical areas. Stream DIN:DON

189 was correlated with net nitrification (n=10, τ=0.63, p=0.01) and N mineralization (n=10, τ=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.

193

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 δ^{15}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 δ^{15}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 δ^{15}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 $\delta^{15}N$ 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.

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 (NO_3^-) removal along flow paths can reduce stream 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 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 $\delta^{15}N$ with foliar $\delta^{15}N$, foliar

253    $\delta^{15}N$ 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 δ^{15}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 δ^{15}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 δ^{15}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 δ^{15}N leads us to believe that soil C:N will likely reflect the same trends as that of soil δ^{15}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 δ^{15}N and stream DIN:DON

277 (Martinelli et al., 1999; Brookshire et al. 2011).

278   Although we found that temporal (soil δ^{15}N) and spatial (stream DIN:DON) integrators of

279 watershed N were correlated with short-term proxies (net nitrification and net N mineralization),

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 δ^{15}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 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 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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315 **References**
316
317 Abdi, H.: The Kendall rank correlation coefficient, in: Encyclopedia of Measurement and
318 Statistics, Sage, Thousand Oaks, CA, 508-510, 2007.
319
320 Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S.,
321 Currie, W., Rustad, L. and Fernandez, I.: Nitrogen saturation in temperate forest ecosystems.
322 BioScience, 48, 921-934, 1998.
323
324 Amundson, R., Austin, A.T., Schuur, E.A., Yoo, K., Matzek, V., Kendall, C., Uebersax, A.,
325 Brenner, D. and Baisden, W.T.: Global patterns of the isotopic composition of soil and plant
326 nitrogen, Global Biogeochem. Cy., 17, 1031, 2003.
327
328 Binkley, D., and Hart, S. C.: The components of nitrogen availability assessments in forest soils,
329 in: Advances in Soil Science, Springer, New York, 57-112, 1989.
330
331 Binkley, D., Aber, J., Pastor, J., and Nadelhoffer, K.: Nitrogen availability in some Wisconsin
332 forests: comparisons of resin bags and on-site incubations, Biol. Fert. Soils, 2, 77-82, 1986.
333
334 Binkley, D., Sollins, P., Bell, R., Sachs, D., and Myrold, D.: Biogeochemistry of adjacent conifer
335 and alder-conifer stands, Ecology, 73, 2022-2033, 1992.
336
337 Bland, J. M., and Altman, D. G.: Multiple significance tests: the Bonferroni method, BMJ, 310,
338 170, 1995.
339
340 Boone, R. D.: Influence of sampling date and substrate on nitrogen mineralization: comparison
341 of laboratory-incubation and buried-bag methods for two Massachusetts forest soils, Can. J.
342 Forest Res., 22, 1895-1900, 1992.
343
344 Bormann, F. H., and Likens, G.: Pattern and process in a forested ecosystem: disturbance,
345 development and the steady state based on the Hubbard Brook ecosystem study, Springer
346 Science and Business Media, 2012.
347
348 Bouwman, A.F., Beusen, A.H.W., Griffioen, J., Van Groenigen, J.W., Hefting, M.M., Oenema,
349 O., Van Puijenbroek, P.J.T.M., Seitzinger, S., Slomp, C.P. and Stehfest, E.: Global trends and
350 uncertainties in terrestrial denitrification and N2O emissions, Phil. Trans. R. Soc. B., 368,
351 20130112, 2013.
352
353 Brookshire, E. J., Hedin, L. O., Newbold, J. D., Sigman, D. M., and Jackson, J. K.: Sustained
354 losses of bioavailable nitrogen from montane tropical forests, Nat. Geosci., 5, 123-126, 2012.
355
356 Carreiro, M. M., Sinsabaugh, R. L., Repert, D. A., and Parkhurst, D. F.: Microbial enzyme shifts
357 explain litter decay responses to simulated nitrogen deposition, Ecology, 81, 2359-2365, 2000.
358

359 Compton, J. E., Church, M. R., Larned, S. T., and Hogsett, W. E.: Nitrogen export from forested
360 watersheds in the Oregon Coast Range: the role of N2-fixing red alder, Ecosystems, 6, 773-785,
361 2003.
362
363 Craine, J.M., Elmore, A.J., Aidar, M.P., Bustamante, M., Dawson, T.E., Hobbie, E.A., Kahmen,
364 A., Mack, M.C., McLauchlan, K.K., Michelsen, A. and Nardoto, G.B.,: Global patterns of foliar
365 nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient
366 concentrations, and nitrogen availability, New Phytol., 183, 980-992, 2009.
367
368 Craine, J. M., Brookshire, E. N. J., Cramer, M. D., Hasselquist, N. J., Koba, K., Marin-Spiotta,
369 E., and Wang, L. J. Marty Kranabetter, Michelle C. Mack, Marin-Spiotta, E., Mayor, J. R.,
370 McLauchlan, K. K., Michelsen, A., Nardoto, G. B., Oliveira, R. S., Perakis, S. S., Peri, P. L.,
371 Quesada, C. A., Richter, A., Schipper, L. A., Stevenson, B. A., Turner, B. L., Viani, R. A. G.,
372 Wanek, W., and Zeller, B: Ecological interpretations of nitrogen isotope ratios of terrestrial
373 plants and soils, Plant Soil, 396, 1-26, 2015.
374
375 Craine, J. M., Elmore, A. J., Wang, L., Augusto, L., Baisden, W. T., Brookshire, E. N. J., E.N.J.,
376 Cramer, M.D., Hasselquist, N.J., Hobbie, E.A., Kahmen, A. and Koba, K., ... & Koba, K.:
377 Convergence of soil nitrogen isotopes across global climate gradients, Sci. Rep., 5, 8280, 2015
378
379 Davidson, E. A., Hart, S. C., Shanks, C. A., and Firestone, M. K.: Measuring gross nitrogen
380 mineralization, and nitrification by 15N isotopic pool dilution in intact soil cores, J. Soil Sci., 42,
381 335-349, 1991.
382
383 Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H. and Tu, K.P.: Stable isotopes in
384 plant ecology, Annu. Rev. Ecol. Syst., 33, 507-559, 2002.
385
386 Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T.,
387 Seabloom, E.W., Shurin, J.B. and Smith, J.E.: Global analysis of nitrogen and phosphorus
388 limitation of primary producers in freshwater, marine and terrestrial ecosystems, Ecol. Lett., 10,
389 1135-1142, 2007.
390
391 Emmett, B.A., Kjønaas, O.J., Gundersen, P., Koopmans, C., Tietema, A. and Sleep, D.: Natural
392 abundance of 15 N in forests across a nitrogen deposition gradient, Forest Ecol. Manag., 101, 9-
393 18, 1998.
394
395 Fang, Y. T., Gundersen, P., Mo, J. M., and Zhu, W. X.: Input and output of dissolved organic
396 and inorganic nitrogen in subtropical forests of South China under high air pollution,
397 Biogeosciences, 5, 339-352, 2008.
398
399 Gundersen, P., Emmett, B.A., Kjønaas, O.J., Koopmans, C.J. and Tietema, A.: Impact of
400 nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data, Forest Ecol.
401 Manag., 101, 37-55, 1998.
402
403 Handley, L. L., and Raven, J. A.: The use of natural abundance of nitrogen isotopes in plant
404 physiology and ecology, Plant Cell Environ., 15, 965-985, 1992.

405

406   Hart, S. C., and Firestone, M. K.: Evaluation of three in situ soil nitrogen availability assays,
407   Can. J. of Forest Res., 19, 185-191, 1989.

408

409   Hedin, L. O., Armesto, J. J., and Johnson, A. H.: Patterns of nutrient loss from unpolluted, old-
410   growth temperate forests: evaluation of biogeochemical theory, Ecology, 76, 493-509, 1995.

411

412   Hedin, L. O., Vitousek, P. M., and Matson, P. A.: Nutrient losses over four million years of
413   tropical forest development, Ecology, 84, 2231-2255, 2003.

414

415   Helsel, D. R., and Hirsch, R. M.: Statistical methods in water resources, Elsevier, 49, 1992.

416

417   Hobbie, J. E., Hobbie, E. A., Drossman, H., Conte, M., Weber, J. C., Shamhart, J., and
418   Weinrobe, M.: Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal
419   forests: 15N is the key signal, Can. J. Microbiol., 55, 84-94, 2009.

420

421   Hogberg, P.: 15N natural abundance in soil–plant systems, New Phytol., 137, 179-203, 1997.

422

423   Houlton, B.Z. and Bai, E.: Imprint of denitrifying bacteria on the global terrestrial biosphere, P.
424   Natl, Acad. Sci., 106, 21713-21716, 2009.

425

426   Houlton, B. Z., Sigman, D. M., and Hedin, L. O.: Isotopic evidence for large gaseous nitrogen
427   losses from tropical rainforests, P. Natl, Acad. Sci., 103, 8745-8750, 2006.

428

429   LeBauer, D. S., and Treseder, K. K.: Nitrogen limitation of net primary productivity in terrestrial
430   ecosystems is globally distributed, Ecology, 89, 371-379, 2008.

431

432   Lohse, K. A., and Matson, P.: Consequences of nitrogen additions for soil processes and solution
433   losses from wet tropical forests, Ecol. App., 15, 1629-1648, 2005.

434

435   Magill, A. H., Aber, J. D., Hendricks, J. J., Bowden, R. D., Melillo, J. M., and Steudler, P. A.:
436   Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition, Ecol.
437   App., 7, 402-415, 1997.

438

439   Martinelli, L.A., Piccolo, M.C., Townsend, A.R., Vitousek, P.M., Cuevas, E., McDowell, W.,
440   Robertson, G.P., Santos, O.C. and Treseder, K.: Nitrogen stable isotopic composition of leaves
441   and soil: tropical versus temperate forests, in: New Perspectives on Nitrogen Cycling in the
442   Temperate and Tropical Americas, Springer, Netherlands, 45-65, 1999.

443

444   Mayer, P. M., Reynolds, S. K., McCutchen, M. D., and Canfield, T. J.: Meta-analysis of nitrogen
445   removal in riparian buffers, J. Environ. Qual., 36, 1172-1180, 2007.

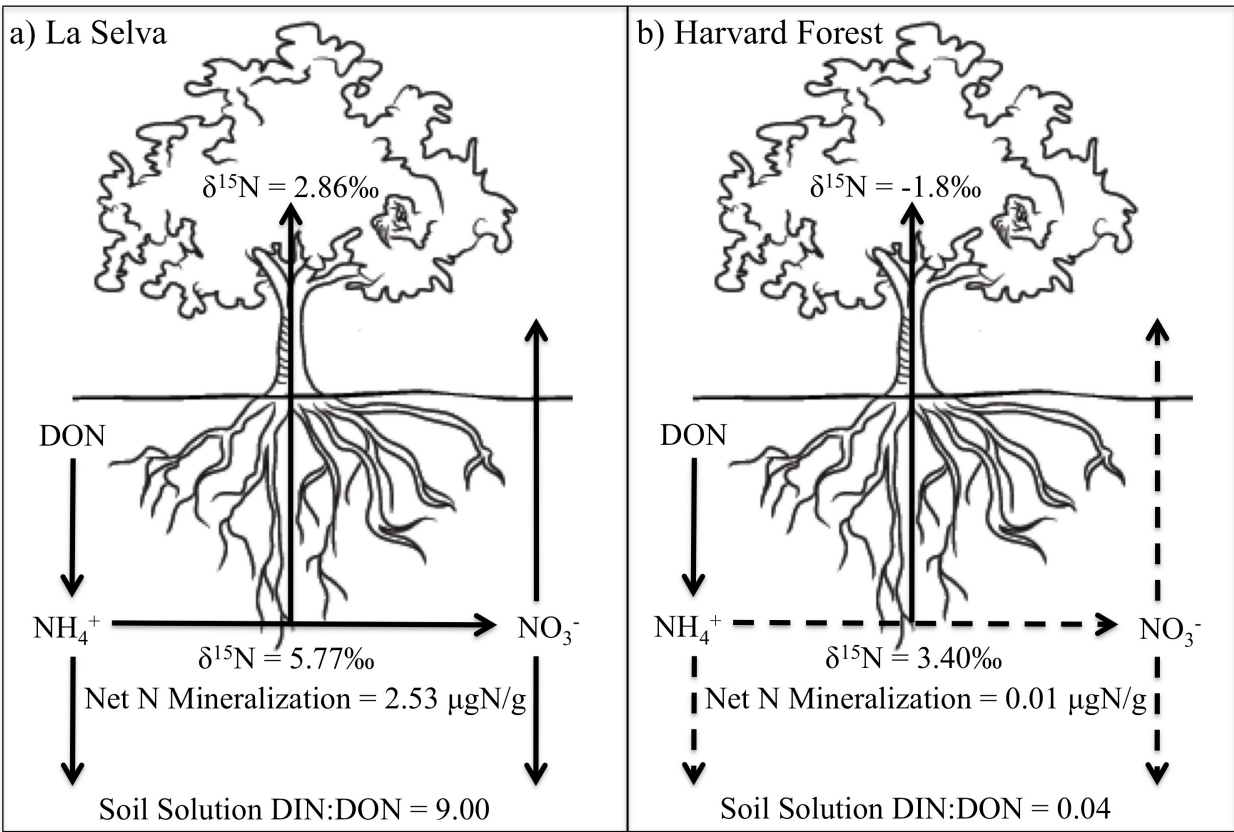
446

447   McDowell, W. H., and Asbury, C. E.: Export of carbon, nitrogen, and major ions from three
448   tropical montane watersheds, Limnology and Oceanography, 39, 111-125, 1994.

449

450 McDowell, W. H., Bowden, W. B., and Asbury, C. E.: Riparian nitrogen dynamics in two
451 geomorphologically distinct tropical rain forest watersheds: subsurface solute patterns,
452 Biogeochemistry, 18, 53-75, 1992.
453
454 McDowell, W. H., Magill, A. H., Aitkenhead-Peterson, J. A., Aber, J. D., Merriam, J. L., and
455 Kaushal, S. S.: Effects of chronic nitrogen amendment on dissolved organic matter and inorganic
456 nitrogen in soil solution, Forest Ecol. Manag., 196, 29-41, 2004.
457
458 Nadelhoffer, K., Shaver, G., Fry, B., Giblin, A., Johnson, L., and McKane, R.:15N natural
459 abundances and N use by tundra plants, Oecologia, 107, 386-394, 1996.
460
461 Neff, J. C., Chapin III, F. S., and Vitousek, P. M.: Breaks in the cycle: dissolved organic nitrogen
462 in terrestrial ecosystems, Front. Ecol. Environ., 1, 205-211, 2003.
463
464 Oren, R., Ellsworth, D. S., Johnsen, K. H., Phillips, N., Ewers, B. E., Maier, C., Schäfer, K.V.,
465 McCarthy, H., Hendrey, G., McNulty, S.G. and Katul, G.G.: Soil fertility limits carbon
466 sequestration by forest ecosystems in a CO2-enriched atmosphere, Nature, 411, 469-472, 2001.
467
468 Perakis, S. S., & Hedin, L. O.: Fluxes and fates of nitrogen in soil of an unpolluted old-growth
469 temperate forest, southern Chile, Ecology, 82, 2245-2260, 2001.
470
471 Perakis, S. S., and Hedin, L. O.: Nitrogen loss from unpolluted South American forests mainly
472 via dissolved organic compounds, Nature, 415, 416-419, 2002.
473
474 Piccolo, M. C., Neill, C., and Cerri, C. C.: Net nitrogen mineralization and net nitrification along
475 a tropical forest-to-pasture chronosequence, Plant Soil, 162, 61-70, 1994.
476
477 Pregitzer, K. S., Zak, D. R., Burton, A. J., Ashby, J. A., and MacDonald, N. W.: Chronic nitrate
478 additions dramatically increase the export of carbon and nitrogen from northern hardwood
479 ecosystems, Biogeochemistry, 68, 179-197, 2004.
480
481 Räike, A., Pietiläinen, O. P., Rekolainen, S., Kauppila, P., Pitkänen, H., Niemi, J., Raateland, A.,
482 and Vuorenmaa, J.: Trends of phosphorus, nitrogen and chlorophyll a concentrations in Finnish
483 rivers and lakes in 1975–2000, Sci. Total Environ., 310, 47-59, 2003.
484
485 Reich, P. B., Hobbie, S. E., Lee, T., Ellsworth, D. S., West, J. B., Tilman, D., Knops, J. M. H.,
486 Naeem, S., and Trost, J.: Nitrogen limitation constrains sustainability of ecosystem response to
487 CO2, Nature, 440, 922-925, 2006.
488
489 Robertson, G.P., Wedin, D., Groffman, P.M., Blair, J.M., Holland, E.A., Nedelhoffer, K.J.,
490 Harris, D., Coleman, D.C., Bledsoe, C.S. and Sollins, P.: Soil carbon and nitrogen availability.
491 Nitrogen mineralization, nitrification and soil respiration potentials, in: Standard soil methods for
492 long-term ecological research, 258-271, 1999.
493

494 Schimel, J. P., Kielland, K., and Chapin III, F. S.: Nutrient availability and uptake by tundra
495 plants, in: Landscape function and disturbance in arctic tundra, Springer, Berlin, Heidelberg,
496 203-221, 1996.
497
498 Schmidt, I. K., Jonasson, S., and Michelsen, A.: Mineralization and microbial immobilization of
499 N and P in arctic soils in relation to season, temperature and nutrient amendment, Appl. Soil
500 Ecol., 11, 147-160, 1999.
501
502 Smith, B., Warlind, D., Arneth, A., Hickler, T., Leadley, P., Siltberg, J., & Zaehle, S. (2014).
503 Implications of incorporating N cycling and N limitations on primary production in an
504 individual-based dynamic vegetation model. Biogeosciences, 11, 2027-2054.
505
506 Soper, F.M., Boutton, T.W. and Sparks, J.P.: Investigating patterns of symbiotic nitrogen
507 fixation during vegetation change from grassland to woodland using fine scale $\delta15N$
508 measurements, Plant Cell Environ., 38, 89-100, 2015.
509
510 Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A.,
511 Johnston, C.T. and Sumner, M.E.: Methods of soil analysis, Part 3-Chemical methods, Soil
512 Science Society of America Inc., 1996.
513
514 Sudduth, E. B., Perakis, S. S., and Bernhardt, E. S.: Nitrate in watersheds: Straight from soils to
515 streams? J. Geophys. Res. Biogeo., 118, 291-302, 2013.
516
517 Templer, P. H., Silver, W. L., Pett-Ridge, J., M. DeAngelis, K., and Firestone, M. K.: Plant and
518 microbial controls on nitrogen retention and loss in a humid tropical forest, Ecology, 89, 3030-
519 3040, 2008.
520
521 Thornton, P. E., Lamarque, J. F., Rosenbloom, N. A., and Mahowald, N. M.: Influence of
522 carbon‐nitrogen cycle coupling on land model response to CO2 fertilization and climate
523 variability, Global Biogeochem. Cy., 21, GB4018, 2007.
524
525 Vidon, P., Allan, C., Burns, D., Duval, T. P., Gurwick, N., Inamdar, S., Lowrance, R., Okay, J.,
526 Scott, D. and Sebestyen, S.: Hot spots and hot moments in riparian zones: Potential for improved
527 water quality management, J. Am. Water Resourc. Assoc., 46, 278-298, 2010.
528
529 Vitousek, P. M., and Howarth, R. W.: Nitrogen limitation on land and in the sea: how can it
530 occur? Biogeochemistry, 13, 87-115, 1991.
531
532 Vitousek, P. M., Gosz, J. R., Grier, C. C., Melillo, J. M., and Reiners, W. A.: A comparative
533 analysis of potential nitrification and nitrate mobility in forest ecosystems, Ecol. Monogr., 52,
534 155-177, 1982.
535
536 Vitousek, P. M., Shearer, G., and Kohl, D. H.: Foliar 15N natural abundance in Hawaiian
537 rainforest: patterns and possible mechanisms, Oecologia, 78, 383-388, 1989.
538

539   Wieder, W. R., Cleveland, C. C., Smith, W. K., and Todd-Brown, K.: Future productivity and
540   carbon storage limited by terrestrial nutrient availability, Nat. Geosci., 8, 441-444, 2015.
541
542   Zaehle, S., Friedlingstein, P., and Friend, A. D.: Terrestrial nitrogen feedbacks may accelerate
543   future climate change, Geophys. Res. Lett., 37, LD01401, 2010.
544
545

546
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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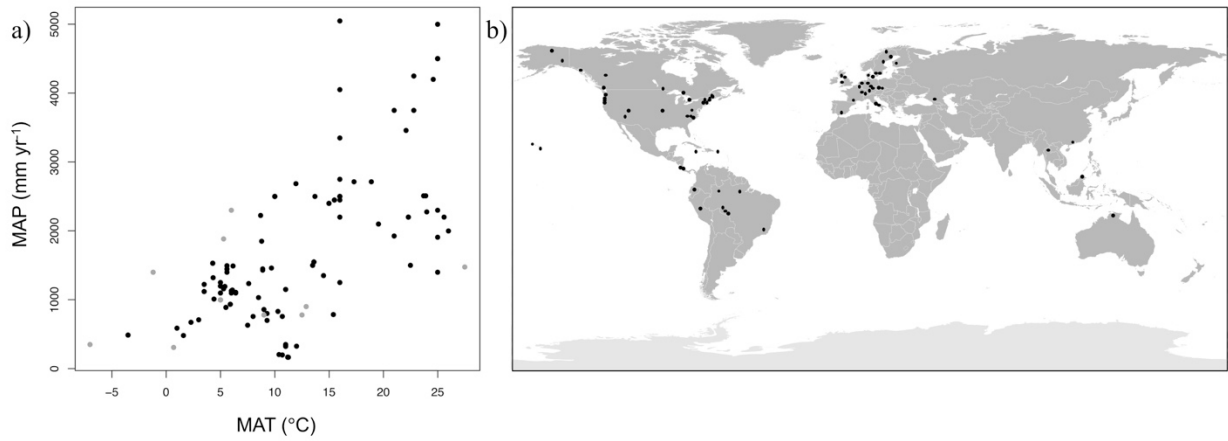
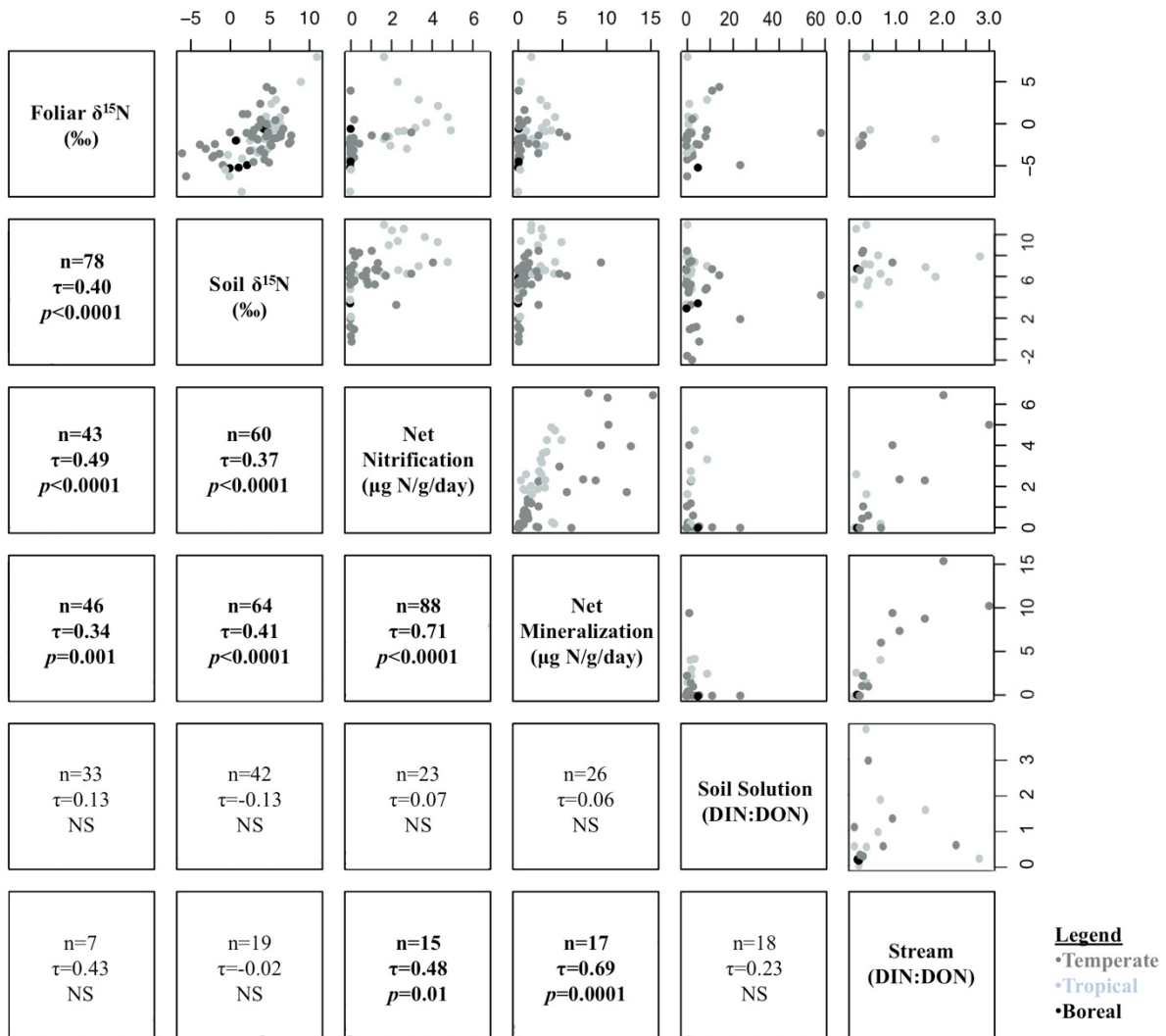

553
554 **Figure 2**. a) Distribution of grassland (grey) and forest (black) watershed mean annual
555 temperature (MAT; °C) and mean annual precipitation (MAP; mm yr^{-1}) included in meta-
556 analysis (left), and b) location of 154 sites (some black dots represent multiple watersheds;
557 right).
558

559
560 **Figure 3**. Correlation matrix of N status proxies (foliar and soil δ^{15}N, net nitrification and N
561 mineralization (<20 cm), the ratio of dissolved inorganic to organic N forms (DIN:DON) in soil
562 solution below the rooting zone (>20 cm), and the DIN:DON in streams). Data are above the
563 diagonal, summary statistics are below. NS signifies correlations that were not significant
564 (*p*>0.05).
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566

567   **Supplemental Table 1.** Site description data, full citations, foliar and surface soil $\delta^{15}N$ (<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.
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