

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

2 Maya Almaraz¹, Stephen Porder¹

3 ¹Department of Ecology and Evolutionary Biology, Brown University, Providence, 02912, USA

4 *Correspondence to:* Maya Almaraz (maya_almaraz@brown.edu)

5 **Keywords:** nitrogen availability, nutrient limitation, $\delta^{15}\text{N}$, nitrogen mineralization, dissolved
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}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}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⁻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 $\delta^{15}\text{N}$. 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 ± 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.

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 $\delta^{15}\text{N}$; 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.

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 $\delta^{15}\text{N}$ 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. Its 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),

Deleted:

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

286

287 **5.0 Conclusions**

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

297 Understanding ecosystem N status at the watershed and landscape scale is a first step towards
298 projecting ecosystem responses to climate change and environmental pollution (Aber et al.,
299 1998; Oren et al., 2001; Reich et al., 2004). Soil N status can determine the rate at which
300 detrimental N losses occur, such as NO_3^- (a drinking water contaminant) and nitrous oxide (a
301 potent greenhouse gas). Furthermore, it is becoming more evident that projections regarding the
302 potential for a terrestrial CO_2 sink, and concomitant feedbacks to the trajectory of climate
303 change, are dependent on the nutrient status of soils (Thornton et al., 2007; Zaehle et al., 2010;

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

306

307 **6.0 Author contribution**

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

310

311 **7.0 Acknowledgments**

312 We want to thank J. Campbell, C. Neill, and W. Wilcke for soil samples. M. Otter, C. Tamayo
313 and C. Silva for help with analyses. MA and SP received funding from NIH IMSD
314 R25GM083270, NSF DDIG GR5260021 and NSF EAR 1331841.

315

316 **References**

- 317
318 Abdi, H.: The Kendall rank correlation coefficient, in: Encyclopedia of Measurement and
319 Statistics, Sage, Thousand Oaks, CA, 508-510, 2007.
320
321 Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S.,
322 Currie, W., Rustad, L. and Fernandez, I.: Nitrogen saturation in temperate forest ecosystems.
323 BioScience, 48, 921-934, 1998.
324
325 Amundson, R., Austin, A.T., Schuur, E.A., Yoo, K., Matzek, V., Kendall, C., Uebersax, A.,
326 Brenner, D. and Baisden, W.T.: Global patterns of the isotopic composition of soil and plant
327 nitrogen, Global Biogeochem. Cy., 17, 1031, 2003.
328
329 Binkley, D., and Hart, S. C.: The components of nitrogen availability assessments in forest soils,
330 in: Advances in Soil Science, Springer, New York, 57-112, 1989.
331
332 Binkley, D., Aber, J., Pastor, J., and Nadelhoffer, K.: Nitrogen availability in some Wisconsin
333 forests: comparisons of resin bags and on-site incubations, Biol. Fert. Soils, 2, 77-82, 1986.
334
335 Binkley, D., Sollins, P., Bell, R., Sachs, D., and Myrold, D.: Biogeochemistry of adjacent conifer
336 and alder-conifer stands, Ecology, 73, 2022-2033, 1992.
337
338 Bland, J. M., and Altman, D. G.: Multiple significance tests: the Bonferroni method, BMJ, 310,
339 170, 1995.
340
341 Boone, R. D.: Influence of sampling date and substrate on nitrogen mineralization: comparison
342 of laboratory-incubation and buried-bag methods for two Massachusetts forest soils, Can. J.
343 Forest Res., 22, 1895-1900, 1992.
344
345 Bormann, F. H., and Likens, G.: Pattern and process in a forested ecosystem: disturbance,
346 development and the steady state based on the Hubbard Brook ecosystem study, Springer
347 Science and Business Media, 2012.
348
349 Bouwman, A.F., Beusen, A.H.W., Griffioen, J., Van Groenigen, J.W., Hefting, M.M., Oenema,
350 O., Van Puijenbroek, P.J.T.M., Seitzinger, S., Slomp, C.P. and Stehfest, E.: Global trends and
351 uncertainties in terrestrial denitrification and N₂O emissions, Phil. Trans. R. Soc. B., 368,
352 20130112, 2013.
353
354 Brookshire, E. J., Hedin, L. O., Newbold, J. D., Sigman, D. M., and Jackson, J. K.: Sustained
355 losses of bioavailable nitrogen from montane tropical forests, Nat. Geosci., 5, 123-126, 2012.
356
357 Carreiro, M. M., Sinsabaugh, R. L., Repert, D. A., and Parkhurst, D. F.: Microbial enzyme shifts
358 explain litter decay responses to simulated nitrogen deposition, Ecology, 81, 2359-2365, 2000.
359

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

406
407 Hart, S. C., and Firestone, M. K.: Evaluation of three in situ soil nitrogen availability assays,
408 Can. J. of Forest Res., 19, 185-191, 1989.
409
410 Hedin, L. O., Armesto, J. J., and Johnson, A. H.: Patterns of nutrient loss from unpolluted, old-
411 growth temperate forests: evaluation of biogeochemical theory, Ecology, 76, 493-509, 1995.
412
413 Hedin, L. O., Vitousek, P. M., and Matson, P. A.: Nutrient losses over four million years of
414 tropical forest development, Ecology, 84, 2231-2255, 2003.
415
416 Helsel, D. R., and Hirsch, R. M.: Statistical methods in water resources, Elsevier, 49, 1992.
417
418 Hobbie, J. E., Hobbie, E. A., Drossman, H., Conte, M., Weber, J. C., Shamhart, J., and
419 Weinrobe, M.: Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal
420 forests: ^{15}N is the key signal, Can. J. Microbiol., 55, 84-94, 2009.
421
422 Hogberg, P.: ^{15}N natural abundance in soil-plant systems, New Phytol., 137, 179-203, 1997.
423
424 Houlton, B.Z. and Bai, E.: Imprint of denitrifying bacteria on the global terrestrial biosphere, P.
425 Natl. Acad. Sci., 106, 21713-21716, 2009.
426
427 Houlton, B. Z., Sigman, D. M., and Hedin, L. O.: Isotopic evidence for large gaseous nitrogen
428 losses from tropical rainforests, P. Natl. Acad. Sci., 103, 8745-8750, 2006.
429
430 LeBauer, D. S., and Treseder, K. K.: Nitrogen limitation of net primary productivity in terrestrial
431 ecosystems is globally distributed, Ecology, 89, 371-379, 2008.
432
433 Lohse, K. A., and Matson, P.: Consequences of nitrogen additions for soil processes and solution
434 losses from wet tropical forests, Ecol. App., 15, 1629-1648, 2005.
435
436 Magill, A. H., Aber, J. D., Hendricks, J. J., Bowden, R. D., Melillo, J. M., and Steudler, P. A.:
437 Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition, Ecol.
438 App., 7, 402-415, 1997.
439
440 Martinelli, L.A., Piccolo, M.C., Townsend, A.R., Vitousek, P.M., Cuevas, E., McDowell, W.,
441 Robertson, G.P., Santos, O.C. and Treseder, K.: Nitrogen stable isotopic composition of leaves
442 and soil: tropical versus temperate forests, in: New Perspectives on Nitrogen Cycling in the
443 Temperate and Tropical Americas, Springer, Netherlands, 45-65, 1999.
444
445 Mayer, P. M., Reynolds, S. K., McCutchen, M. D., and Canfield, T. J.: Meta-analysis of nitrogen
446 removal in riparian buffers, J. Environ. Qual., 36, 1172-1180, 2007.
447
448 McDowell, W. H., and Asbury, C. E.: Export of carbon, nitrogen, and major ions from three
449 tropical montane watersheds, Limnology and Oceanography, 39, 111-125, 1994.
450

451 McDowell, W. H., Bowden, W. B., and Asbury, C. E.: Riparian nitrogen dynamics in two
452 geomorphologically distinct tropical rain forest watersheds: subsurface solute patterns,
453 *Biogeochemistry*, 18, 53-75, 1992.

454
455 McDowell, W. H., Magill, A. H., Aitkenhead-Peterson, J. A., Aber, J. D., Merriam, J. L., and
456 Kaushal, S. S.: Effects of chronic nitrogen amendment on dissolved organic matter and inorganic
457 nitrogen in soil solution, *Forest Ecol. Manag.*, 196, 29-41, 2004.

458
459 Nadelhoffer, K., Shaver, G., Fry, B., Giblin, A., Johnson, L., and McKane, R.: 15N natural
460 abundances and N use by tundra plants, *Oecologia*, 107, 386-394, 1996.

461
462 Neff, J. C., Chapin III, F. S., and Vitousek, P. M.: Breaks in the cycle: dissolved organic nitrogen
463 in terrestrial ecosystems, *Front. Ecol. Environ.*, 1, 205-211, 2003.

464
465 Oren, R., Ellsworth, D. S., Johnsen, K. H., Phillips, N., Ewers, B. E., Maier, C., Schäfer, K.V.,
466 McCarthy, H., Hendrey, G., McNulty, S.G. and Katul, G.G.: Soil fertility limits carbon
467 sequestration by forest ecosystems in a CO₂-enriched atmosphere, *Nature*, 411, 469-472, 2001.

468
469 Perakis, S. S., & Hedin, L. O.: Fluxes and fates of nitrogen in soil of an unpolluted old-growth
470 temperate forest, southern Chile, *Ecology*, 82, 2245-2260, 2001.

471
472 Perakis, S. S., and Hedin, L. O.: Nitrogen loss from unpolluted South American forests mainly
473 via dissolved organic compounds, *Nature*, 415, 416-419, 2002.

474
475 Piccolo, M. C., Neill, C., and Cerri, C. C.: Net nitrogen mineralization and net nitrification along
476 a tropical forest-to-pasture chronosequence, *Plant Soil*, 162, 61-70, 1994.

477
478 Pregitzer, K. S., Zak, D. R., Burton, A. J., Ashby, J. A., and MacDonald, N. W.: Chronic nitrate
479 additions dramatically increase the export of carbon and nitrogen from northern hardwood
480 ecosystems, *Biogeochemistry*, 68, 179-197, 2004.

481
482 Räike, A., Pietiläinen, O. P., Rekolainen, S., Kauppila, P., Pitkänen, H., Niemi, J., Raateland, A.,
483 and Vuorenmaa, J.: Trends of phosphorus, nitrogen and chlorophyll a concentrations in Finnish
484 rivers and lakes in 1975–2000, *Sci. Total Environ.*, 310, 47-59, 2003.

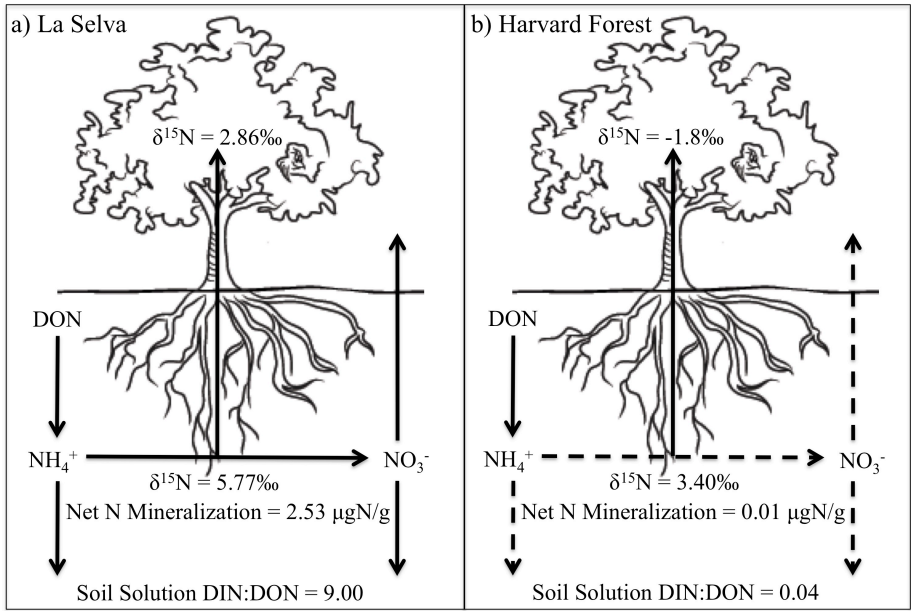
485
486 Reich, P. B., Hobbie, S. E., Lee, T., Ellsworth, D. S., West, J. B., Tilman, D., Knops, J. M. H.,
487 Naeem, S., and Trost, J.: Nitrogen limitation constrains sustainability of ecosystem response to
488 CO₂, *Nature*, 440, 922-925, 2006.

489
490 Robertson, G.P., Wedin, D., Groffman, P.M., Blair, J.M., Holland, E.A., Nadelhoffer, K.J.,
491 Harris, D., Coleman, D.C., Bledsoe, C.S. and Sollins, P.: Soil carbon and nitrogen availability.
492 Nitrogen mineralization, nitrification and soil respiration potentials, in: *Standard soil methods for*
493 *long-term ecological research*, 258-271, 1999.

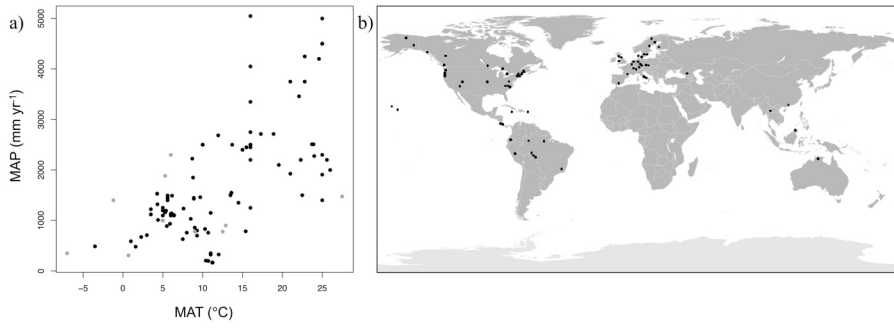
494

495 Schimel, J. P., Kielland, K., and Chapin III, F. S.: Nutrient availability and uptake by tundra
496 plants, in: *Landscape function and disturbance in arctic tundra*, Springer, Berlin, Heidelberg,
497 203-221, 1996.
498
499 Schmidt, I. K., Jonasson, S., and Michelsen, A.: Mineralization and microbial immobilization of
500 N and P in arctic soils in relation to season, temperature and nutrient amendment, *Appl. Soil*
501 *Ecol.*, 11, 147-160, 1999.
502
503 Smith, B., Warlind, D., Armeth, A., Hickler, T., Leadley, P., Siltberg, J., & Zaehle, S. (2014).
504 Implications of incorporating N cycling and N limitations on primary production in an
505 individual-based dynamic vegetation model. *Biogeosciences*, 11, 2027-2054.
506
507 Soper, F.M., Boutton, T.W. and Sparks, J.P.: Investigating patterns of symbiotic nitrogen
508 fixation during vegetation change from grassland to woodland using fine scale $\delta^{15}\text{N}$
509 measurements, *Plant Cell Environ.*, 38, 89-100, 2015.
510
511 Sparks, D.L., Page, A.L., Helmke, P.A., Loepfert, R.H., Soltanpour, P.N., Tabatabai, M.A.,
512 Johnston, C.T. and Sumner, M.E.: *Methods of soil analysis, Part 3-Chemical methods*, Soil
513 Science Society of America Inc., 1996.
514
515 Sudduth, E. B., Perakis, S. S., and Bernhardt, E. S.: Nitrate in watersheds: Straight from soils to
516 streams? *J. Geophys. Res. Biogeo.*, 118, 291-302, 2013.
517
518 Templer, P. H., Silver, W. L., Pett-Ridge, J., M. DeAngelis, K., and Firestone, M. K.: Plant and
519 microbial controls on nitrogen retention and loss in a humid tropical forest, *Ecology*, 89, 3030-
520 3040, 2008.
521
522 Thornton, P. E., Lamarque, J. F., Rosenbloom, N. A., and Mahowald, N. M.: Influence of
523 carbon-nitrogen cycle coupling on land model response to CO₂ fertilization and climate
524 variability, *Global Biogeochem. Cy.*, 21, GB4018, 2007.
525
526 Vidon, P., Allan, C., Burns, D., Duval, T. P., Gurwick, N., Inamdar, S., Lowrance, R., Okay, J.,
527 Scott, D. and Sebestyen, S.: Hot spots and hot moments in riparian zones: Potential for improved
528 water quality management, *J. Am. Water Resour. Assoc.*, 46, 278-298, 2010.
529
530 Vitousek, P. M., and Howarth, R. W.: Nitrogen limitation on land and in the sea: how can it
531 occur? *Biogeochemistry*, 13, 87-115, 1991.
532
533 Vitousek, P. M., Gosz, J. R., Grier, C. C., Melillo, J. M., and Reiners, W. A.: A comparative
534 analysis of potential nitrification and nitrate mobility in forest ecosystems, *Ecol. Monogr.*, 52,
535 155-177, 1982.
536
537 Vitousek, P. M., Shearer, G., and Kohl, D. H.: Foliar ^{15}N natural abundance in Hawaiian
538 rainforest: patterns and possible mechanisms, *Oecologia*, 78, 383-388, 1989.
539

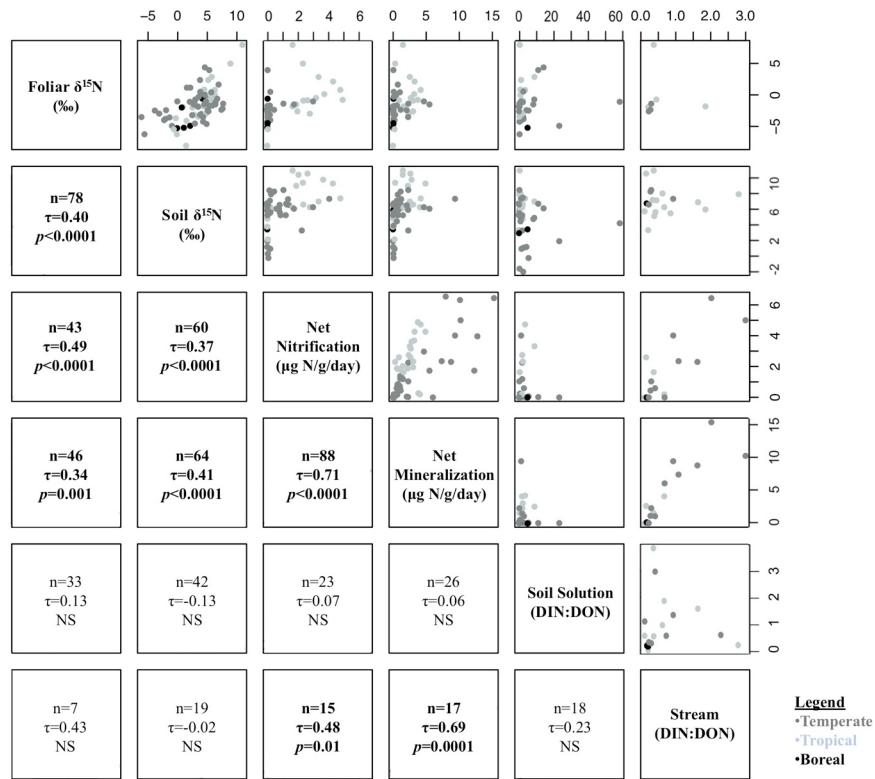
540 Wieder, W. R., Cleveland, C. C., Smith, W. K., and Todd-Brown, K.: Future productivity and
541 carbon storage limited by terrestrial nutrient availability, *Nat. Geosci.*, 8, 441-444, 2015.
542
543 Zaehle, S., Friedlingstein, P., and Friend, A. D.: Terrestrial nitrogen feedbacks may accelerate
544 future climate change, *Geophys. Res. Lett.*, 37, LD01401, 2010.
545
546



547
 548 **Figure 1.** Nitrogen availability values for a) a nitrogen rich tropical forest at the La Selva field
 549 station in Costa Rica, and for b) a nitrogen limited temperate pine forest at Harvard Forest,
 550 Massachusetts. Solid and dotted lines represent the relative magnitude of fluxes (i.e. net N
 551 mineralization, denitrification to the atmosphere, dissolved organic and inorganic nitrogen
 552 leaching), which are contingent on ecosystem nitrogen status.
 553



554
555 **Figure 2.** a) Distribution of grassland (grey) and forest (black) watershed mean annual
556 temperature (MAT; °C) and mean annual precipitation (MAP; mm yr⁻¹) included in meta-
557 analysis (left), and b) location of 154 sites (some black dots represent multiple watersheds;
558 right).
559



560
 561
 562
 563
 564
 565
 566
 567

Figure 3. Correlation matrix of N status proxies (foliar and soil $\delta^{15}\text{N}$, 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 ($p>0.05$).

568 **Supplemental Table 1.** Site description data, full citations, foliar and surface soil $\delta^{15}\text{N}$ (<20 cm
569 depth), net nitrification (<20 cm depth), net N mineralization (<20 cm depth), the ratio of
570 dissolved inorganic to organic N forms (DIN:DON) in soil solution below the rooting zone (>20
571 cm depth), and stream DIN:DON.
572

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

574

575 **Editor:** p.13, line 271, It's → Its

576

577 **Response:** We have changed this in the text. Thank you for catching this error.

578