Author's Response to Comments by Referees

We thank the reviewers for their thoughtful comments, which we think have led to substantial improvements in the manuscript. Below we provide a detailed list of responses.

Anonymous Referee #1
Received and published: 18 April 2016

1. Referee #1: This study presents a comparison of different proxies for assessing the nitrogen status of terrestrial ecosystems. Such proxies are essential if we are to assess the influence of nitrogen on these ecosystems, but various proxies exist and it is yet unclear which proxies are to preferred above others, and how the different proxies relate to one another. The current study addresses primarily the latter. Although I find this a useful effort, I think especially the analyses require revisions to improve the quality of this study.

General comments: As the study is now it is only indicative of the relationship between different proxies of nitrogen availability, and although differences between biomes are discussed, I think additional analyses are required to be able to draw firm conclusions regarding the difference among e.g. boreal, temperate and tropical systems. So, I suggest that the authors test statistically if for example the relationship between soil d15N soil and foliar d15N differs between temperate and tropical systems (as suggested on l. 167-168).

Response: We thank you for this suggestion and agree with the idea. Although we lack statistical power in boreal regions to test how these relationships change, we do discuss how they differ between temperate and tropical ecosystems in lines 177-183. We have also included the following text to address the way in which these relationships change:

Lines 179-185: "Some relationships between proxies differed with latitude. Soil and foliar $\delta^{15}N$ were more tightly correlated in the tropics (n=24, τ=0.68, p<0.0001) than in the temperate zone (n=49, τ=0.23, p=0.02). Soil $\delta^{15}N$ was correlated with net nitrification in tropical (n=17, τ=0.39, p=0.03), but not temperate regions. Conversely, soil $\delta^{15}N$ was correlated with net N mineralization (n=44, τ=0.34, p=0.001) in temperate but not tropical areas. Stream DIN:DON was correlated with net nitrification (n=10, τ=0.63, p=0.01) and N mineralization (n=10, τ=0.78, p=0.002) in the temperate zone, and not in the tropics (n=4, p>0.05)."

Lines 241-250: "While most observed correlations were consistent across latitudes, a few differed between the tropics and the temperate zone. The correlations of soil $\delta^{15}N$ with foliar $\delta^{15}N$, foliar $\delta^{15}N$ with net nitrification, and net nitrification with N mineralization were consistent across both tropical and temperate regions. Net nitrification and N mineralization were correlated with stream DIN:DON only in temperate regions. These data suggest that while terrestrial proxies may be a useful across biomes, stream DIN:DON requires further research to understand the extent of its applicability across space. The correlation between foliar and soil $\delta^{15}N$ also differs across latitudes, in that the correlation in the tropics was much tighter than in the temperate zone. Bias in the literature towards natural abundance isotopic data from the temperate zone may explain why previous research looking at this relationship has been noisy (Craine et al., 2009)."

Finally, we note that we did not test these relationships using regression, rather non-parametric correlation. Other than comparing the strength of the correlation, this makes it difficult to determine statistically whether the nature of the relationships vary with biome.

2. Referee #1: Although I understand that the potential proxies are numerous and choices need to be made, I wonder why authors did not include soil C:N ratio. This ratio is often considered a good indicator of soil nutrient status (see e.g. Alberti et al 2015, iForest 8, 195-206). It is also an easy measurement to make so I advocate to include this ratio in the study.

Response: We agree, soil C:N would be another useful proxy, since as the reviewer notes it is commonly measured, and is highly correlated with soil and plant $\delta^{15}N$ (Craine et al. 2015, Plant and Soil, (369): 1-26). However, at this stage including C:N would require us to withdraw the manuscript and revisit the literature, an exercise that might prove useful but our suspicion is would not change the conclusion of this manuscript. We have however updated the sentence in lines 90-94 to say: "We chose these metrics because 1) other authors have suggested that they are indicative of soil nutrient status (Martinelli et al., 1999, Amundson et al., 2001, Brookshire et al., 2012; Figure 1), and 2) they are thought to integrate N fluxes on different timescales (e.g. soil $\delta^{15}N$ integrates N losses over decades while net N mineralization rates integrate inorganic N production over days; Binkley and Hart, 1989, Hogburg 1997)."

3. Referee #1: I wonder if the authors could test for the influence of the distance between watershed and stream in the analysis of the correlation between soil proxies versus stream DIN:DON (perhaps with a subset for which this info is available).

Response: Unfortunately distance from the stream is rarely reported, especially when terrestrial metrics are gathered from different papers than that of water-based metrics (which was typical). We have updated the text to include this point in lines 123-124: "Terrestrial metrics were typically gathered from different papers than that of water-based metrics, requiring validation of congruent watershed location."

Another issue along the same lines it that other land-uses in the greater watershed are rarely reported either, which can potentially influence the relationship between soil and stream metrics. We revised the text to raise this point in lines 238-240: "varied land-use (e.g. pasture, N fixing plant species, etc.) upstream of undisturbed sites is typically not reported in the literature, but is another possible explanation for the break down between terrestrial and water-based proxies."

4. Referee #1: Finally, I suggest that the authors make their dataset available. At minimum, a list of sites and coordinates should be given, but I hope authors can also provide the associated data for the various proxies.

Response: Along with the manuscript, we submitted a supplementary zip file with the complete data set, including a list of sites and complete citations for papers we extracted data from. We apologize if this was not made available and will inquire with the editor about its status. We also plan to include an additional figure that outlines the approximate location of our sites (see Fig. 2b).

Figure 2. a) Distribution of grassland (grey) and forest (black) watershed mean annual temperature (MAT; °C) and mean annual precipitation (MAP; mm yr^{-1}) included in meta-analysis (left), and b) location of 154 sites (some black dots represent multiple watersheds; right).
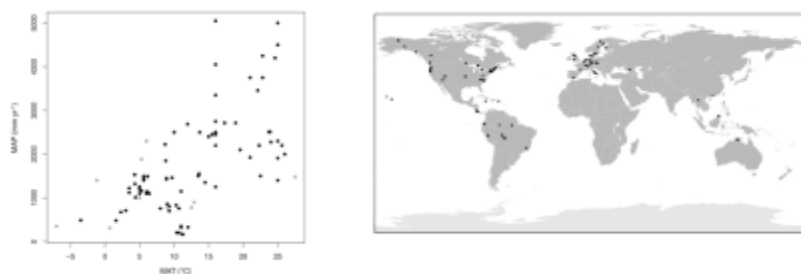
5. Referee #1: Specific comments:
l. 9: bracket after 'correlate' should come before 'correlate'
l. 46: remove 'in solution' (dissolved is obviously in solution)
l. 70: I suggest to rephrase 'we asked which were correlated'

Response: We corrected these. Thank you.

6. Referee #1:
l.181-182: I disagree with this statement that category 1 and 2 proxies showed a robust relationship. In a dataset with huge variation (like this one), a significant correlation between 2 metrics does not necessarily imply a robust relationship, and visual inspection of the relationships in Fig. 3 does not support the statement. There is some correlation, but there is a lot of unexplained variance.

Response: We agree this statement was misleading as stated. We have adjusted the text to point out the statistical significance and removed the word "robust". The sentence (lines 191-192) now reads: "Our data suggest that category 1 and 2 metrics are correlated".

7. Referee #1: l.194- 198: How is d15N of soil (and plants) influenced by N deposition? If N deposition influences soil and plant d15N, the use of these proxies globally may not be ok because similarly rich sites would differ in d15N depending on the N source.

Response: This is a good point. Our data show no evidence of N deposition on plant or soil $\delta^{15}N$, but many of our sites do not have N deposition data. We have revised the text to reflect this in lines 206-209: "In our dataset, N deposition was not correlated with stream DIN:DON ($\tau=0.03$, $p>0.05$), or any other metric. Thus our data do not support the idea that N deposition is responsible for the lack of correlation between these two long-term proxies."

8. Referee #1:
l.211: 'that' after 'seem' and 'to' after 'used

Response: We corrected this. Thank you.

Anonymous Referee #2
Received and published: 28 April 2016

1. Referee #2: BG review This paper poses an interesting and important question about whether different metrics used to characterize N availability (which represent different spatial and temporal scales) are correlated. This topic is of potential interest to a broad group of researchers who consider N availability in their studies. The paper attempts to evaluate some underlying assumptions that are included implicitly or explicitly in interpreting ecosystem N dynamics. The scope of the analysis is not really clear from the paper. It is a little surprising that the authors did not include several large syntheses of similar data (Aber at al. 2003, BioScience, Pardo et al. 2006 Biogeochemistry, the CANIF study in Europe, Schulze 2000 Springer).

Response: Thank you for this comment and for the citations. We did our best to find all the literature available, however we are sure to have missed some. We did not include Aber et al. (2003) because they do not report the variables we are focused on, at least not in a manner that was useable in this analysis (i.e. they report NO_3^- but not NH_4^+ or DON, and they report percent nitrification rather than a nitrification rate in ug N/g/d). We did use Pardo et al. 2006 (which included CANIF sites) to find original papers, from which we extracted data. Any sites that seem to be excluded may have been done because we were unable to find multiple proxies from that site. We thank you for the Schulze 2000 reference and have added these data to our analysis.

2. Referee #2: The scope of the analysis is important, because it can be difficult to make assertions about different climatic zones or life forms unless enough variation is included among the samples to represent that observed

Response: We agree and have included the following text to strengthen that point.

Line 76-79: "This review assesses the correlation between common foliar, surface soil (i.e. $\delta^{15}N$, nitrification and mineralization), and nutrient loss (i.e. soil solution and stream N concentrations) metrics of N availability from unmanaged ecosystems globally."

3. Referee #2: Several issues should be addressed: Nitrate leaching is referred to as if it were the driver of the fractionation that would lead to 15N-enrichment of material remaining in the ecosystem (soil, foliage). In fact the elevated nitrification which leads to an increase d15N of the plant available (including the nitrate that leaches to the stream) is the driver. The authors are, no doubt, well aware of this, but it is worth taking the trouble to be more precise for the reader less familiar with these dynamics. This should be addressed at several points in the paper.

Response: Thank you. We did not intend to give the impression that leaching is a key driver of isotopic enrichment in leaves and soil. To remedy this, we inserted: "primarily denitrification" in lines 61-62, and "during nitrification" in line 65.

4. Referee #2: The isotope literatures is not as current as it could be. I have given some examples of possible additional citations.

Response: Thank you for those. We have included the caveat that our literature search included only papers published prior to 2013 (line 84).

5. Referee #2: I assume that when the authors talk about long-term patterns and measures that are invariant temporally, that they mean in undisturbed systems. This should be stated explicitly, since over the long term, at many of these study sites, various disturbances have occurred which disrupt that N cycle and which would affect the values of these metrics.

Response: We added "in relatively undisturbed ecosystems" (line 32). However, one weakness of our approach for stream measurements is the nature of land use change upstream from a particular site described in the papers we searched. We have made that caveat clearer on lines 238-240, which now reads: "varied land-use (e.g. pasture, N fixing plant species, etc.) upstream of undisturbed sites is typically not reported in the literature, but is another possible explanation for the lack of correlation between terrestrial and water-based proxies."

6. Referee #2: Need to define what is meant by N status.

Response: Agreed. We rephrased this to include "relative abundance of plant available N" (line 31-32).

7. Referee #2: More explanation about the differences between observed correlations in tropical versus temperate systems would be useful (why were foliar and soil d15N correlated in tropical, but not temperate?)

Response: We added lines 241-250: "While most observed correlations were consistent across latitudes, a few differed between the tropics and the temperate zone. The correlations of soil $\delta^{15}N$ with foliar $\delta^{15}N$, foliar $\delta^{15}N$ with net nitrification, and net nitrification with N mineralization were consistent across both tropical and temperate regions. Net nitrification and N mineralization were correlated with stream DIN:DON only in temperate regions. These data suggest that while terrestrial proxies may be a useful across biomes, stream DIN:DON requires further research to understand the extent of its applicability across space. The correlation between foliar and soil $\delta^{15}N$ also differs across latitudes, in that the correlation in the tropics was much tighter than in the temperate zone. Bias in the literature towards natural abundance isotopic data from the temperate zone may explain why previous research looking at this relationship has been noisy (Craine et al., 2009)."

8. Referee #2: Abstract:
10 if space permit, include the region considered in this study
19 is there a 'that' missing? i.e., given that both. . .

Response: We corrected these. Thank you.

9. Referee #2: 27 why 'Nevertheless'? what follows doesn't not seem to contrast with what was said in the first sentence.
31 don't really need 'such' on this line

32 I would suggest adding 'rates' after mineralization and nitrification, to make the comparison to another flux clearer. Also, the verb needs to agree with the subject is→are

Response: We corrected these. Thank you.

10. Referee #2: 33-4 This is an important point (basing annual budgets on short-term measures) and one that is often ignored.

Response: Agreed.

11. Referee #2: 34-6 There seems to be a word missing or a punctuation problem. Is 'are relevant' associated with scales or N status?

Response: We changed this to "While N status measured over longer temporal and larger spatial scales is relevant to many ecosystem properties and their response to global change, it is more difficult to measure." (lines 35-37)

12. Referee #2: 87-90 Is this level of detail necessary?

Response: See our response to Reviewer 1 (and our response now in lines 90-94), who had a query about why we chose these metrics and not others. We thought it best to present our thinking as fully as possible.

13. Referee #2: 90 What is meant by 'intact'? does this mean 'not fragmented'? Or is it intended to include disturbance as well? And if so, only anthropogenic disturbance (e.g., harvesting) or also natural (fire, wind, ice or pest events, etc.)?

Response: We agree "intact" was unclear. We changed this to: "We limited our search criteria to studies that took place in forest or grassland ecosystems that had not incurred any large disturbances that might impair their function." (lines 98-100).

14. Referee #2: 92 Is there a list of the sites in supplemental information? (cite supplemental material here)

Response: The list is available in the supplemental. We have now noted this on line 102. Thank you.

15. Referee #2: 101 Is it appropriate to lump net nitrification potential measures with measures of nitrification? This should be justified.

Response: We agree that this is a point worth clarifying in the text. We limited our nitrification methods to intact soil core, buried bag, and lab incubations in order to avoid any methodological differences (as state in lines 110-112). In the literature net nitrification and nitrification potential are terms that are sometimes (but not always) used interchangeably (Ross et al. 2012, Journal of Geophysical Research: Biogeosciences, 117(G1); Bohlen et al. 2001, Ecology, 82(4), 965-978). Some authors define a buried bag incubation as "potential" because it is not what is actually

happening in intact soils. However, others define nitrification "potential" as how much nitrification happens when soils are amended to overcome potential substrate limitations to nitrification. We did not include any nitrification assays where the soils were amended and have thus revised the text to reflect this (lines 110-112): "In order to control for methodological differences, we limited our net nitrification and N mineralization methods to those which used intact soil core, buried bag, and laboratory incubations of unamended soils".

16. Referee #2: 105-6 This level of detail is unnecessary.

Response: We thought that including this level of detail might help field reader questions regarding the analyses we chose to run and have chosen to leave the text as is unless the editor prefers we remove it.

17. Referee #2: 107 Are these five watersheds identified somewhere? Supplemental material?

Response: Yes, the supplemental data lists full citations for each watershed, and there we state where we "collected soil".

18. Referee #2: 137 How is foliar d15N on the same timescale as bulk soil d15N? The plant available portion of the soil pool is very small and is not what is measured by bulk soil. Foliar %N and d15N can vary on very short time scales. Bulk soil d15N may vary in response to disturbance, but the soil N pool is many orders of magnitude larger than the foliar N pool.

Response: We agree that foliar $\delta^{15}N$ can differ among species, and that N in leaves turns over much more quickly than N in soil. However, our understanding is that average foliar $\delta^{15}N$ for a site is relatively stable in time, absent large changes in species composition. If the reviewer can point us to literature that suggests otherwise we would be happy to incorporate it in our discussion.

19. Referee #2: 141 What does 'that' refer to in this sentence (that of water-based proxies)

Response: We changed this to read "with water-based proxies" (line 166).

20. Referee #2: 144 Does the absence of a correlation between soil solution and stream DIN:DON suggest that stream DIN:DON does not reflect what is available in the terrestrial ecosystem?

Response: We think so, at least for the dataset here. We discuss this in lines 210-225, which read: "Another surprise from our dataset is that soil solution DIN:DON was not significantly correlated with any other metric, not even with stream DIN:DON, despite ~40% of papers in our dataset reporting both soil solution and stream DIN:DON in the same watershed. While the correlation between soil solution DIN:DON below the rooting zone and N availability has been documented across gradients in soil age and fertility (Hedin et al., 1995), this correlation was not found across the range of sites examined here. We found no relationship between soil solution DIN:DON and lysimeter depth, suggesting that the majority of N transformations responsible for the discontinuity between soil solution DIN:DON and that of terrestrial metrics are likely

occurring either within the rooting zone or in riparian zones. Neither soil solution or stream DIN:DON was sensitive to environmental variability (i.e. elevation, temperature, precipitation, N deposition), suggesting that processing along flow paths may be responsible for the disconnect between soil solution and stream N concentrations. From these data, at least, it does not seem that soil solution DIN:DON can be used to infer terrestrial N status across this suite of unmanaged sites. These data also do not support the idea that soil solution DIN:DON is representative of N forms that leach into streams (Binkley et al., 1992; Pregitzer et al., 2004; Fang et al., 2008)."

21. Referee #2: 155 Foliar 15N is not an integrator on the time scale of decades to centuries

Response: The fact that foliar N is derived from soil N and that foliar $\delta^{15}N$ correlates with soil $\delta^{15}N$ across broad spatial scales suggests that these two values are dependent on one another. While we agree that average foliar $\delta^{15}N$ may change faster than soil $\delta^{15}N$ in perturbed sites that have experienced a turnover in species composition or large scale disturbances, we argue that foliar $\delta^{15}N$ in relatively undisturbed ecosystems (such as the sites that we analyzed) change on a similar timescale as soil $\delta^{15}N$.

22. Referee #2: 160 It seems a fairly broad interpretation to say that these data suggest that correlations between categories 1 and 2 are robust some of them may be, but not all of them. To what extent is it reasonable to extrapolate this finding?

Response: We agree that it was not well written. We have changed this line to read "Our data suggest that category 1 and 2 metrics are correlated" (lines 191-192).

Since these data incorporate as much of the available data that we could find across a broad geographic and climatic range, we would imagine these findings can be extrapolated, but when we look at differences within biomes it becomes apparent that these relationships may vary geographically, and for that reason we call for more research examining these relationships at smaller spatial scales in lines 254-257: "Explicit comparisons of these proxies to each other, with a focus on how they are influenced by hot-spots, hot-moments, biological diversity, and N transformation between the soil-stream interface, will enhance their utility for understanding N availability at the ecosystem scale."

23. Referee #2: 171 I don't see why one would expect DIN:DON to be correlated with soil 15N, they are measuring very different things.

Response: We agree. However, in the literature both are used as an indication of N status within watersheds. We hope that these data highlight that they are measuring different things, and that interpretations of terrestrial N status based on these metrics is not straightforward. This is a key point we hope this paper makes.

24. Referee #2: 178 Is DIN:DON more sensitive to N deposition than DIN?

Response: We would presume that DIN is more sensitive than DIN:DON, because N can be deposited in both forms, but the majority is deposited as DIN. As we suggest in lines 205-206,

because most N deposition comes in the form of DIN, DIN:DON is lower in pristine settings than in polluted ones.

Lines 205-206: "We note that stream DIN:DON is sensitive to N deposition, and that relatively pristine settings have a lower DIN:DON than polluted ones (Perakis and Hedin, 2001)."

25. Referee #2: 183 What does it tell you if soil solution DIN:DON is not correlated with stream DIN:DON?

Response: We propose several explanations for this in the text (lines 226-240). One of which is that N is removed along hydrologic flow paths, and another is that stream N is potentially affected by upstream land-use/inputs that overshadow local inputs.

26. Referee #2: 198 Hydrologic flowpath and flowrate are also probably important. 2002-4 Work by K. Lohse et al. addresses these issues.

Response: Agreed. Thank you for the citation. We touch on this in lines 226-240: "While nitrate (NO_3^-) removal along flow paths can reduce stream NO_3^- (Vidon et al., 2010), with higher percent removal in forested watersheds (Sudduth et al., 2013), DON has been shown to be relatively resistant to removal by decomposition and biologic uptake along subsurface flow paths (Carreiro et al., 2000, Neff et al. 2003). We found no correlation between stream and soil solution DIN:DON, and suggest that variation in NO_3^- removal (relative to DON) along flow paths of undisturbed ecosystems may explain this lack of correlation. The extent to which riparian zones influence nutrients varies spatially with geomorphology, soil texture, vegetation, and riparian zone development (McDowell et al., 1992, Mayer et al., 2007); and soils with high rates of leaching to ground water may bypass riparian processing. As nutrients leach down the soil profile, denitrification, biologic uptake, and storage are all potential mechanisms that could alter soil solution and stream N species concentrations. Investigation of soil profile processes and riparian zone spatial variability may help determine where and when watershed-scale N status can be inferred from these proxies. Alternatively, varied land-use (e.g. pasture, N fixing plant species, etc.) upstream of undisturbed sites is typically not reported in the literature, but is another possible explanation for the break down between terrestrial and water-based proxies."

27. Referee #2: Figures and tables
Fig 2 there is a lot of useful information in Figure 2, but the graphs are too small and are illegible. The format, in the end, is more clever than useful. It would be better to enlarge the graphs a bit so that it is easier to resolve the patterns. (The quality of the figure in the paper I downloaded is fair, but I assume there is a high resolution version).
The size of the statistical info is fine and legible. It might be easier to follow if it were presented in the same triangle configuration as the figures (as opposed to flipped) or else in a table.

Response: Thanks. Since there are 16 graphs, we chose this format to conserve space. However, we have attempted to make the panels bigger so that they can be seen more easily. We hope that the editor will inform us of any further issues with legibility/resolution.

Measuring ecosystem nitrogen status: a comparison of proxies

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

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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 in 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 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 N status measured over longer temporal and larger spatial scales is relevant to many ecosystem properties and their response to global change, it is more difficult to measure.

Land-based investigations of N cycling commonly measure 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 that are thought to average over space and/or time. The first is the ratio of dissolved inorganic

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(DIN) to organic (DON) N concentration lost from ecosystems. Losses of DIN are considered

controllable by biota, and thus should be low if soil N is in short supply. In contrast, most DON

is not accessible to plants, and thus represents a loss beyond biotic control (Hedin et al., 1995;

Figure 1). Thus low DIN:DON in streams has been used to infer relative N-poverty in

watersheds (e.g. McDowell and Asbury, 1994; Perakis and Hedin, 2002; Brookshire et al., 2012).

The few sites where such measurements have been made over decades (e.g. the Luquillo

Mountains of Puerto Rico, Harvard Forest in Massachusetts, Hubbard Brook LTER in New

Hampshire; McDowell et al., 1992, McDowell et al., 2004, Bormann and Likens 2012) suggest

stream DIN:DON is not particularly variable over this timescale, and thus this metric may

integrate over time as well as space (W.C. McDowell, pers. comm.). It is common that

researchers using DIN:DON to infer ecosystem N status implicitly assume that a few

measurements are indicative of longer-term patterns (e.g. Perakis and Hedin, 2002; Brookshire et

al,. 2012).

In contrast to stream DIN:DON, soil $\delta^{15}N$ integrates solely over time, and at steady state

reflects the isotopic signature associated inputs (N fixation and/or deposition) and fractionation

associated with outputs (Handley and Raven, 1992). The major N loss pathways (primarily

denitrification, and to a lesser extent nitrate leaching) discriminate against ^{15}N, which thus

remains in relative abundance in N-rich soils (Hogburg 1997; Martinelli et al., 1999; Craine et

al., 2009; Houlton and Bai, 2009, Craine et al., 2015; Figure 1). To some degree foliar $\delta^{15}N$

reflects soil $\delta^{15}N$ (Amundson et al., 2003), but there can be fractionation during nitrification,

between bulk and soil solution N pools (Hogburg, 1997), as well as during N uptake by roots and

mychorrhizae (Hobbie et al., 2009). For this reason, foliar $\delta^{15}N$ may display greater variability

between species in a single site than the bulk soil δ^{15}N (Vitousek et al., 1989; Nadlehoffer et al., 1996).

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

2.0 Methods

2.1 Literature Review

We surveyed the literature (prior to 2013) and contacted individual investigators to gather data from forested and grassland watersheds where more than one proxy of long-term N availability had been measured. We focused on the most commonly-used proxies for N status: foliar (n=78) and surface soil δ^{15}N (n=104; <20 cm depth), net nitrification (n=86; <20 cm depth), net N mineralization (n=88; <20 cm depth), the ratio of dissolved inorganic to organic N forms (DIN:DON) in soil solution below the rooting zone (n=43; >20 cm depth), and stream DIN:DON (n=32). We chose these metrics because 1) other authors have suggested that they are

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

Commented [AM17]: R1.2, R2.12

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

Commented [AM18]: R2.13

We collected data from 154 watersheds across a broad climatic range (Figure 2), in which at least two of the six N proxies of interest had been measured (see *Supplemental Data*). We used

Commented [AM19]: R2.14

DataThief II software (version 1.2.1) to extract data from figures when data were not available in text or tables. When necessary, data were converted to standardize units.

From each paper we collected the following site description data: country, site, watershed, biome, ecosystem type, latitude, longitude, elevation (m), mean annual temperature (MAT; °C), mean annual precipitation (MAP; mm yr^{-1}), N deposition rate (kg N ha^{-1} yr^{-1}), soil depth (cm), soil solution (lysimeter) depth (cm), and N mineralization method. Site description

Commented [AM20]: R2.16

data were gathered from other sources when they were not in the original publication.

In order to control for methodological differences, we limited our net nitrification and N mineralization methods to those which used intact soil core, buried bag, and laboratory incubations of unamended soils (Boone, 1992; Piccolo et al., 1994), and eliminated methods

Commented [AM21]: R2.15

such as ion resin exchange beads or ^{15}N tracer techniques (Binkley et al., 1986; Hart and

Firestone, 1989; Davidson et al., 1991; Templer et al., 2008). We did not limit net nitrification and N

mineralization data based on the length of the incubation, as we see little change in rates between 1-7 days (Tietema et

al., 1998), however we recognize that longer incubations may result in lower net rates. Soil values were from the

mineral soil only, and were preferentially collected in the 0-10 cm range, however if soil samples

were not in 10 cm increments, we selected the increment that was most similar (e.g. A horizon,

0-5 cm, 0-15 cm), and no deeper than 20 cm.

When data were missing, or we were uncertain about location or collection method, we

contacted the authors to request unpublished data, elucidation of data collection, data reduction,

or soil samples. Terrestrial metrics were typically gathered from different papers than that of

water-based metrics, requiring validation of congruent watershed location. For five watersheds.

(Puerto Rico's Pared, Sonadora, Bisley, Tronoja watersheds and Hubbard Brook's watershed 6)

we collected soil that we analyzed for δ^{15}N. In Puerto Rico, we collected mineral soil samples (0-

10 cm) in replicates of five using an open side soil sampler from locations that were >3 m away

from the stream. Replicate samples were combined in a Ziploc bag, air-dried and shipped to the

Marine Biological Laboratory for analysis. Colleagues at Hubbard Brook collected three

replicate horizon B samples for us from several soil pits dug across an elevation gradient in

watershed 6 (Christopher Neill, pers. comm.), which were air-dried at the Marine Biological

Laboratory prior to analysis.

2.2 Soil Sample Analysis

The few soils we analyzed in house for δ^{15}N were homogenized, sieved (2 mm) and

ground using a mortar and pestle. We analyzed samples at the Marine Biological Laboratory

Ecosystem Center Stable Isotope Laboratory for δ^{15}N using a Europa 20-20 continuous-flow

Commented [AM22]: R1.3

isotope ratio mass spectrometer interfaced with a Europa ANCA-SL elemental analyzer. The analytical precision based on replicate analyses of δ^{15}N of isotopically homogeneous international standards was ± 0.1 ‰.

2.3 Statistics

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

3.0 Results

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

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

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

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

Some relationships between proxies differed with latitude. Soil and foliar $\delta^{15}N$ were more tightly correlated in the tropics (n=24, τ=0.68, p<0.0001) than in the temperate zone (n=49, τ=0.23, p=0.02). Soil $\delta^{15}N$ was correlated with net nitrification in tropical (n=17, τ=0.39, p=0.03), but not temperate regions. Conversely, soil $\delta^{15}N$ was correlated with net N mineralization (n=44, τ=0.34, p=0.001) in temperate but not tropical areas. Stream DIN:DON was correlated with net nitrification (n=10, τ=0.63, p=0.01) and N mineralization (n=10, τ=0.78, p=0.002) in the temperate zone, and not in the tropics (n=4, p>0.05).

4.0 Discussion

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

It is particularly interesting that stream DIN:DON was not correlated with soil δ^{15}N as both are proxies used to infer long-term N status. There is a wealth of literature that uses stream DIN:DON to infer large spatial and temporal scale patterns in N availability (Hedin et al., 1995; Perakis and Hedin, 2002; McDowell et al., 2004; Fang et al., 2008). Similarly, many studies interpret soil δ^{15}N as an integrator of N losses over time (Martinelli et al., 1999; Houlton et al., 2006; Houlton and Bai, 2009, Craine et al., 2015). These are the only two proxies for N status that integrate over relatively long timescales, and their lack of correlation warrants more careful consideration. We note that stream DIN:DON is sensitive to N deposition, and that relatively pristine settings have a lower DIN:DON than polluted ones (Perakis and Hedin, 2001). In our

Commented [AM24]: R1.6, R2.22

Commented [AM25]: R2.24

dataset, N deposition was not correlated with stream DIN:DON (τ=0.03, p>0.05), or any other metric. Thus our data do not support the idea that N deposition is responsible for the lack of correlation between these two long-term proxies.

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

While nitrate (NO_3^-) removal along flow paths can reduce stream NO_3^- (Vidon et al., 2010), with higher percent removal in forested watersheds (Sudduth et al., 2013), DON has been shown to be relatively resistant to removal by decomposition and biologic uptake along subsurface flow paths (Carreiro et al., 2000, Neff et al. 2003). We found no correlation between stream and soil

solution DIN:DON, and suggest that variation in NO_3^- removal (relative to DON) along flow paths of undisturbed ecosystems may explain this lack of correlation. The extent to which riparian zones influence nutrients varies spatially with geomorphology, soil texture, vegetation, and riparian zone development (McDowell et al., 1992, Mayer et al., 2007); and soils with high rates of leaching to ground water may bypass riparian processing. As nutrients leach down the soil profile, denitrification, biologic uptake, and storage are all potential mechanisms that could alter soil solution and stream N species concentrations. Investigation of soil profile processes and riparian zone spatial variability may help determine where and when watershed-scale N status can be inferred from these proxies. Alternatively, varied land-use (e.g. pasture, N fixing plant species, etc.) upstream of undisturbed sites is typically not reported in the literature, but is another possible explanation for the break down between terrestrial and water-based proxies.

Commented [AM29]: R1.3, R2.5
Commented [AM30]: R2.26

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

Commented [AM31]: R2.7

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

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

Commented [AM32]: R2.22

Commented [AM33]: R1.1

5.0 Conclusions

The labor and expense associated with fertilization studies to assess nutrient limitation requires that we develop proxies to infer soil nutrient status. While nitrification and mineralization most frequently correlate with other metrics, they are short-term proxies that vary over short spatial and temporal scales. Soil $\delta^{15}N$ and dissolved N losses from streams are long-term integrators of N loss that have been relied on to advance our understanding of N cycling at the global scale (Martinelli et al., 1999; Amundson et al., 2003; Hedin et al., 2003; Brookshire et al., 2012), however their lack of correlation brings to light a need to better understand how these terrestrial and stream-based metrics vary in relation to each other and with nutrient limitation.

Understanding ecosystem N status at the watershed and landscape scale is a first step towards projecting their response to climate change and environmental pollution (Aber et al., 1998; Oren et al., 2001; Reich et al., 2004). Soil N status can determine the rate at which detrimental N losses occur, such as NO_3^- (a drinking water contaminant) and nitrous oxide (a potent greenhouse gas). Furthermore, it is becoming more evident that projections regarding the potential for a terrestrial CO_2 sink, and concomitant feedbacks to the trajectory of climate change, are dependent on the nutrient status of soils (Thornton et al., 2007; Zaehle et al., 2010; Wieder et al., 2015). The health and environmental implications of soil N status heighten the need to develop

methodology to adequately assess long-term soil N availability.

6.0 Author contribution

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

7.0 Acknowledgments

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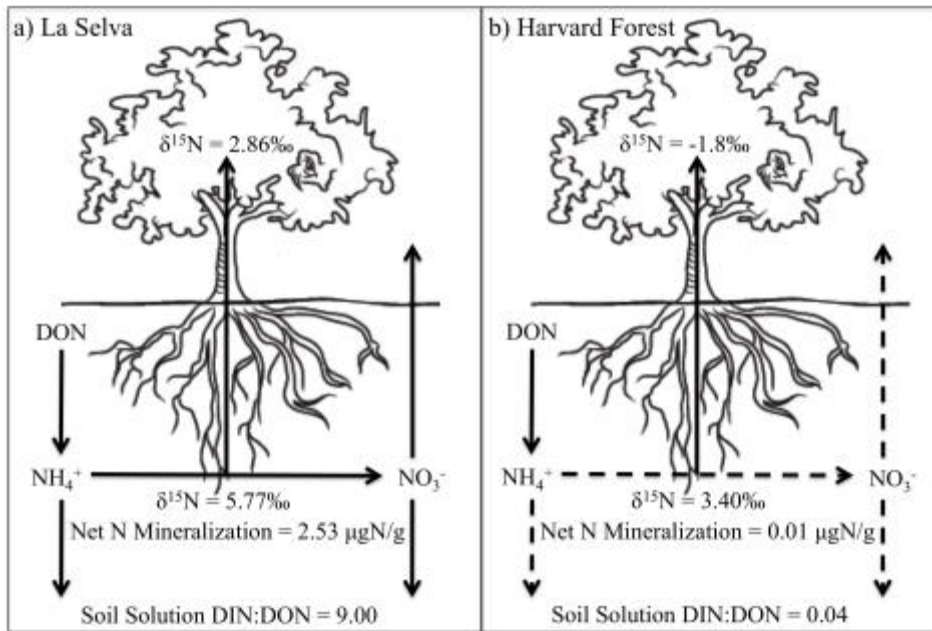
Figure 1. Nitrogen availability values for a) a nitrogen rich tropical forest at the La Selva field station in Costa Rica, and for b) a nitrogen limited temperate pine forest at Harvard Forest, Massachusetts. Solid and dotted lines represent the relative magnitude of fluxes (i.e. net N mineralization, denitrification to the atmosphere, dissolved organic and inorganic nitrogen leaching), which are contingent on ecosystem nitrogen status.
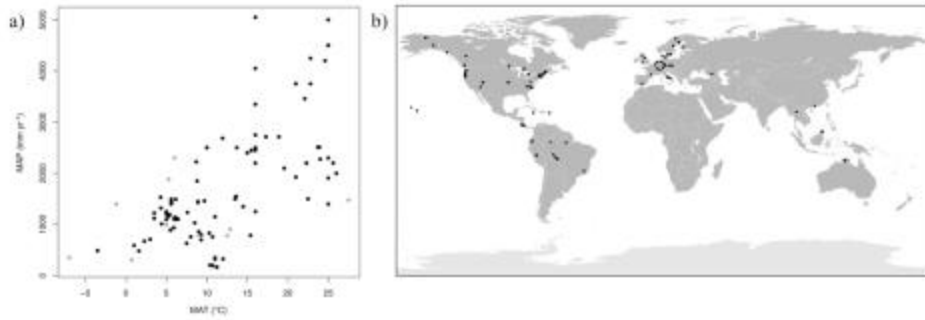
Figure 2. a) Distribution of grassland (grey) and forest (black) watershed mean annual temperature (MAT; °C) and mean annual precipitation (MAP; mm yr^{-1}) included in meta-analysis (left), and b) location of 154 sites (some black dots represent multiple watersheds; right).
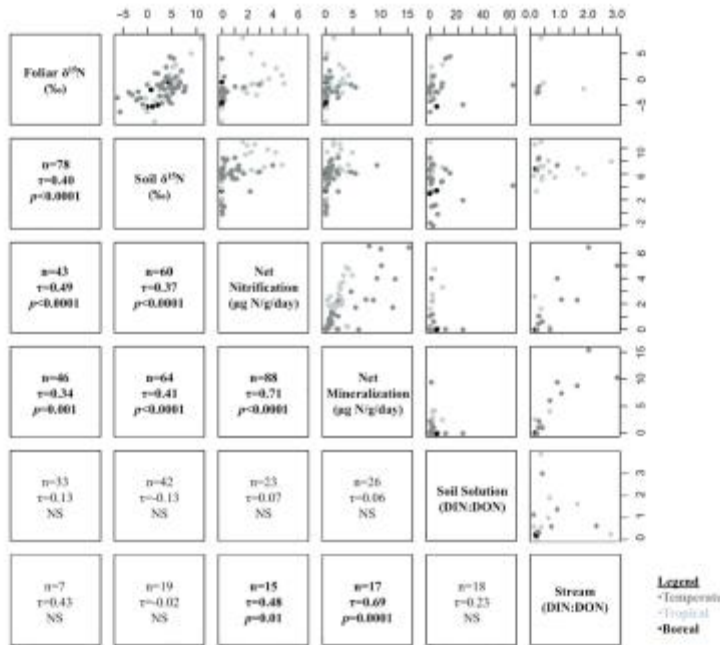
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Figure 3. Correlation matrix of N status proxies (foliar and soil δ^{15}N, net nitrification and N mineralization (<20 cm), the ratio of dissolved inorganic to organic N forms (DIN:DON) in soil solution below the rooting zone (>20 cm), and the DIN:DON in streams). Data are above the diagonal, summary statistics are below. NS signifies correlations that were not significant (p>0.05).

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