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Abstract

Although recent anthropogenic effects on the global nitrogen (N) cycle have been significant, the consequences of increased anthropogenic N on terrestrial ecosystems are unclear. Studies of the impact of increased reactive N on forest ecosystems—impacts on hydrologic and gaseous loss pathways, retention capacity, and even net primary productivity— have been particularly limited by a lack of long-term baseline biogeochemical data. Stable nitrogen isotope analysis (ratio of ^{15}N to ^{14}N, termed $\delta^{15}N$) of wood chronologies offers the potential to address changes in ecosystem N cycling on millennial timescales and across broad geographic regions. Currently, nearly 50 studies have been published utilizing wood $\delta^{15}N$ records; however, there are significant differences in study design and data interpretation. Here, we identify four categories of wood $\delta^{15}N$ studies, summarize the common themes and primary findings of each category, identify gaps in the spatial and temporal scope of current wood $\delta^{15}N$ chronologies, and synthesize methodological frameworks for future research by presenting eight suggestions for common methodological approaches and enhanced integration across studies. Wood $\delta^{15}N$ records have the potential to provide valuable information for interpreting modern biogeochemical cycling. This review serves to advance the utility of this technique for long-term biogeochemical reconstructions.

Keywords

I. Introduction

Recent human alteration to the global N cycle has been immense, with supplies of reactive N (Nr including NH_3, NH_4, NO_x, and N_2O) from fossil fuel burning and fertilizer production now equal to natural N fixation rates on Earth (Galloway et al. 2008). However, the consequences of this unprecedented biogeochemical alteration on terrestrial ecosystems have been difficult to quantify. Despite the potential for a variety of harmful consequences from Nr deposition— including eutrophication, production of greenhouse gases, acid rain, and biodiversity loss (Houlton et al. 2013) — many terrestrial ecosystems have demonstrated stable or declining N availability during several decades of monitoring (Bernal et al. 2012). Thus, assessment of the consequences of increased Nr inputs on forests has been limited by the lack of long-term

"baseline" biogeochemical data. Nitrogen compounds preserved in wood have the potential to provide these data at decadal to millennial timescales, but significant variation in methods and intellectual viewpoints has prevented a unified approach.

The standardized ratio of ^{15}N to ^{14}N ($\delta^{15}N$) in wood of living trees can serve as a proxy record for the history of N cycling in terrestrial ecosystems. The benefits of using wood $\delta^{15}N$ for reconstructing N cycling are: (1) the potentially very long period of time covered, (2) good spatial coverage and associated geospatial information, and (3) an isotopic signal directly reflective of plant N uptake. Thus, wood $\delta^{15}N$ provides an integrated metric of N cycling that is clearly terrestrial. These characteristics, combined with improved analytical capability, have led to an increasing number of studies using this technique. The total number of published wood $\delta^{15}N$ studies has grown from a handful to more than 45 studies in the past 10 years (Online Resource 1). The existing literature is distributed very broadly among nearly 30 journals (including *Biogeochemistry*). Because specialized knowledge about the fields of dendrochronology, forest ecology, and stable N isotopes is required to perform these studies, there can be significant variation in research questions, study design, methods, and interpretation.

Interpretation of stable N isotopes in terrestrial ecosystems has advanced on both theoretical and empirical fronts in the past decade. Stable N isotopes are now commonly measured in terrestrial ecosystem compartments including foliar, soil, and wood. Recent syntheses of the $\delta^{15}N$ values of these compartments have yielded global-scale insights on N cycling controls, for foliar tissue (n> 11,000, Craine et al. 2009) and soil (n>6,000, Craine et al. In review, 2014). Ecosystem $\delta^{15}N$ values integrate a variety of fractionating processes in the terrestrial N cycle (Fig 1). The main fractionating pathway in the terrestrial N cycle is microbially-mediated gaseous N loss to the atmosphere, with multiple factors including climate and disturbance influencing that flux (Houlton et al. 2006). Second, nitrification in soil N pools produces differences in $\delta^{15}N$ values of soil N pools, namely higher $\delta^{15}N$ of NH_4^+ and lower $\delta^{15}N$ of NO_3^- (Högberg 1997). Hydrologic loss of soil NO_3^- through leaching and preferential uptake of NH_4^+ by trees amplifies the importance of fractionation between inorganic soil N pools. A third fractionation pathway involves plant associations with mycorrhizal fungi that give depleted N to plants (Hobbie and Högberg 2012). The most significant fractionation is observed in plants associated with ericoid

mycorrhizal fungus, with decreasing fractionation effects in plants associated with ectomycorrhizal, arbuscular mycorrhizal, and non-mycorrhizal fungus (Craine et al. 2009). Model results using natural variations in N isotopes, and mechanisms of fractionating loss pathways, have indicated spatial variation in global pathways of N loss from the land to the water and atmosphere (Bai et al. 2012). Finally, evidence is emerging that microbial processing of soil organic matter, independent of loss pathways, may imprint the soil $\delta^{15}N$ signature (Craine et al. In review, 2014).

Regardless of fractionation mechanisms, trees take up nitrate and ammonium with a $\delta^{15}N$ signature reflective of ecosystem processes, and when incorporated into proteins associated with structural compounds, this signal can persist for thousands of years, as has been shown with carbon (Gerhart et al. 2012). As with other stable light isotope approaches, wood $\delta^{15}N$ studies are geographically limited to ecosystems with woody plant cover (including forests and shrubland) and where seasonality allows for the formation of annual rings. This review focuses primarily on temperate and boreal forest ecosystems, though the few studies performed in the tropics and sub-tropics are also discussed. This review also focuses on studies published in English, although studies have been published in other languages (Guerrieri et al. 2006).

Here, we address some of the variation in published wood $\delta^{15}N$ studies and synthesize the technical and conceptual frameworks required to advance this potentially powerful biogeochemical approach. The objectives of this synthesis are: a) to describe the temporal and spatial scope of the existing studies of stable N isotopes in wood; b) to identify the main types of study design; c) to explore the consequences of methodological differences in extraction, pooling samples, and reporting data; and d) to synthesize what has been learned and what could be learned with retrospective studies of wood $\delta^{15}N$. With an increasing number of studies, sample sizes, and reported metadata, a true meta-analysis will likely be possible in the very near future.

II. Spatial and temporal coverage of wood $\delta^{15}N$ studies

The recent development of wood $\delta^{15}N$ studies means that sampling depth (number of studies on a given region or species) and breadth (number of regions or species studied) is generally low. Globally, only four regions have more than a single study: 1) the Pacific

Northwest United States, 2) The northeast United States and southern portions of Quebec and Ontario, Canada, 3) Western Europe, particularly Switzerland and Italy, and 4) Japan (Fig 2, an interactive version of this figure is also available through ArcGIS Explorer Online at *http://tinyurl.com/ksnhadj*). Outside of these areas, study locations are sparsely scattered with vast expanses of unstudied regions throughout China, Russia, Eastern Europe, the Middle East, Africa, Central America, South America, and central and northern regions of North America (Fig 2). Given the regional variation in wood $\delta^{15}N$ reported in the literature (eg. -3 to +7‰ in the northeastern U.S. and southeastern Canada, Bukata and Kyser 2005, 2007; McLauchlan et al. 2007; Poulson et al. 1995), increasing the breadth of regional studies is necessary before building a global (or even continental) synthesis of wood $\delta^{15}N$ patterns. Additionally, increased sampling density within regions may illuminate differences in wood $\delta^{15}N$ patterns between forest types and across biomes.

The power of dendrochronological analysis lies in the numerous growth rings of trees, allowing researchers to develop a record of past $\delta^{15}N$ patterns with potentially annual resolution. Isotope analysis can be performed on wood milled for construction or naturally-preserved tree remains, allowing researchers to stretch farther back in time than the life span of a single individual. Wood $\delta^{15}N$ studies have focused exclusively on living trees, limiting the depth of time analyzed (Fig 3). Although the oldest natural abundance wood N reported is from 1835 AD, nearly 60% of all wood $\delta^{15}N$ studies cover less than the last 60 years, and over 75% cover less than the last century (Fig 3). Yet, dendrochronological records of ring width extend more than 7000 years into the past (Eronen et al. 2002, Ferguson 1968) and preserved tree remains can date back to nearly 50,000 years ago (Gerhart et al. 2012). Analyses spanning a longer temporal duration would help determine more robust patterns of N cycling in ecosystems, and provide a baseline of N availability before the advent of anthropogenic disturbance on a global scale. The short-duration of the majority of wood $\delta^{15}N$ chronologies provides little data from times prior to large-scale fossil fuel combustion and fertilizer application. Consequently, no wood $\delta^{15}N$ data currently exist that can provide a pre-Industrial baseline level of N availability in any region for any species.

A taxonomically diverse array of tree species have been analyzed, with 21 genera sampled, but again sampling density is low in any particular taxon (Fig 3). The majority of genera (57%) have been studied only once, and an even higher proportion (62%) are represented

by only one species (Fig 3). The clear exception, however, is *Pinus*, which has been reported in the literature 22 times, incorporating 10 different species (Fig 3), representing the most breadth and depth of any genus. This cosmopolitan genus with good dendrochronological properties holds potential for cross-site comparisons at some point. Currently, however, small sample sizes within a genus make it difficult to identify differences or similarities between genera, or between broad classes (hardwoods/softwoods). Mixed species within a stand are frequently sampled, making this the appropriate spatial scale for analysis. Tree species do have individualistic effects on forest N cycling (Lovett et al. 2004), and when wood $\delta^{15}N$ records have been analyzed at the species level, there are some differences in temporal trajectory that indicate partitioning of available soil N (McLauchlan and Craine 2012). Increasing the diversity of taxonomic sampling for wood $\delta^{15}N$ analysis could further inform the potential consequences of species-specific nutrient use.

III. Four types of study design

1. *Natural abundance studies* account for a large proportion (n=15) of published wood $\delta^{15}N$ studies. In these studies, a researcher selects trees that have not been experimentally manipulated and analyses the naturally-occurring $\delta^{15}N$ trends within the tree rings. The key assumption is that $\delta^{15}N$ measured in wood reflects the terrestrial N cycle for that tree in that year. Studies addressing disturbance effects, such as fire (Beghin et al. 2011), tree-clearing events (Bukata and Kyser 2005, Hietz et al. 2010, Stock et al. 2012), bog drainage (Choi et al. 2007), and the cessation of anthropogenic disturbance (McLauchlan et al. 2007) were also included in this category, even though some of these studies incorporated experimental manipulation of the system to mimic disturbance events.

Numerous studies have documented decreasing wood $\delta^{15}N$ values toward present during the last century from a wide variety of locations including the United States (McLauchlan and Craine 2012, McLauchlan et al. 2007, Poulson et al. 1995), Spain (Cuoto-Vázquez and González-Prieto 2010, Peñuelas and Estiarte 1997), and Canada (Härdtle et al. 2013, Kranabetter et al. 2013). In some cases, decreasing trends were attributed to lower ecosystem N loss and increased reliance of plants on mineralized N or N from fixation by mycorrhizae (Cuoto-Vázquez and González-Prieto 2010, Peñuelas and Estiarte 1997), or a general decline in ecosystem N availability (Kranabetter et al. 2013, McLauchlan et al. 2007). The key to

interpreting temporal trends in these studies was relatively high sample size, because individual trees in the same location showed increasing, decreasing, or flat trends (Kranabetter et al. 2013, McLauchlan and Craine 2012).

2. *Fertilizer and tracer studies* were the second most common type of study, accounting for a quarter of wood δ^{15}N studies. These studies feature an experimental manipulation of the N cycling of the studied system by the addition of nitrogenous fertilizers. These studies sometimes do not reflect natural conditions, for example when several hundred kilograms of fertilizer per hectare were added (Balster et al. 2009). They can, however, provide a valuable insight into plant uptake of N and subsequent translocation within plant tissues, the fate of N within the ecosystem, and changes in ecosystem N cycling under higher N availability. The key assumption is that measurements of stable N isotopic signatures in plant tissues (such as wood) reflect both the amount of N retained in the system and its partitioning among plant and soil pools.

Fertilizer and tracer studies have indicated, through a meta-analysis of recovery rates of the enriched ^{15}N tracer, a relatively high capacity of temperate forests to retain added N (Templer et al. 2012). Some of these studies in no way reflect natural N availability, as they apply large amounts of N fertilizer; however, they do allow for direct quantification of N movement between various pools in the ecosystem and enhance our understanding of N cycling under high N availability. In the past, tracer studies utilized smaller amounts of fertilizer (~50 kg N ha^{-1}) with high δ^{15}N values (~300-43,000 ‰), though recently researchers have focused more on high N inputs (350 kg N ha^{-1}) with δ^{15}N near 0 ‰. This difference in structure represents a shift from tracing a unique ^{15}N signal through the ecosystem to observing changes in δ^{15}N values of ecosystem pools under non-limiting N supply. Mass balance techniques found that less than 1/3 of added N was retained in above-ground tree biomass, with woody tissue accounting for only 2-5% of N uptake (Koopmans et al. 1996, Nadelhoffer et al. 1995, Schleppi et al. 1999). In most cases, the majority of N was either retained in the soil, or lost through leaching (Nadelhoffer et al. 1999a). Such low retention in woody biomass may explain the lack of growth increases under increased N deposition (Nadelhoffer et al. 1999b).

Fertilizer and tracer studies have helped to interpret how inorganic N taken up by trees is deposited in wood tissue, particularly the potential for translocation of N-containing compounds across ring boundaries (Hart & Classen 2003). This is a key issue for all wood δ^{15}N studies (not just fertilizer and tracer studies), as significant translocation of N across rings could mask or

remove signals of environmental N availability. Under N additions with high δ^{15}N signatures, rings corresponding to the years of N addition often show a clear and dramatic peak (Elhani et al. 2003) although the peak may be delayed by 1-2 years after the fertilization treatment (Hart and Classen 2003). When N additions are highly enriched (high δ^{15}N), ring δ^{15}N shows a dramatic increase at the onset of N addition and a slow decline over several years following the initial peak (Elhani et al. 2003, Hart and Classen 2003). When N additions do not have uniquely high δ^{15}N signatures, the increase in tree ring δ^{15}N may be less dramatic (Balster et al. 2009, Elhani et al. 2005, Guerrieri et al. 2011). In these cases, the increase in ring δ^{15}N is not due to a direct manipulation of source δ^{15}N, but to indirect transformation of the soil N pool under high N availability due to pool mixing and isotope fractionation effects.

Slower reductions in δ^{15}N following fertilization may reflect translocation of N taken up immediately following fertilization, or continued uptake of ^{15}N-labeled compounds from the soil for several years after fertilization (Fig. 4). Mass balance studies tend to assume the former, for two reasons. First, rings formed prior to N fertilization also show increased δ^{15}N indicating translocation does occur (Elhani et al. 2003, Hart and Classen 2003). Second, mass-balance studies indicate the majority of labeled-N uptake occurs in the first year following application (Mead and Preston 1994). Since the majority of ^{15}N-labeled compounds remain in the soil profile (Koopmans et al. 1996, Nadelhoffer et al. 1999a, Schleppi et al. 1999), δ^{15}N of rings formed after fertilization may reflect the combination of continued, but minor, uptake of ^{15}N-labeled compounds from the soil as well as translocation of ^{15}N-labeled compounds from previously-formed rings. This combination would explain why the pre-treatment side of the δ^{15}N curve is much steeper than the post-treatment side, despite translocation occurring in both directions. Alternatively, if translocation occurs more strongly towards recent rings than older rings, the slower reduction in δ^{15}N following the fertilizer peak could be explained solely by translocation. At this point, the balance of these two processes (translocation and continued uptake) is as yet unclear. Continued analysis of trees several decades after treatment might help elucidate the long-term impacts of pulse N increases on wood δ^{15}N and help quantify wood translocation of N over decadal time scales.

3. The *pollution input studies* represent more than a quarter of the publications. These studies analyze trees growing near point sources of pollutant N (such as oil refineries, major

highways, or oil sands, ex. Saurer et al. 2004) or in highly populated and industrial areas experiencing high levels of N deposition from numerous sources (such as the Windsor-Québec City Corridor in Canada, ex. Savard et al. 2009). Tree ring chronologies represent a unique opportunity to develop an historical indicator of pollution impacts in a given region, particularly when multi-isotope approaches (δ^{15}N, δ^{13}C, and δ^{18}O) are utilized (Savard 2010).

Pollution input studies rely heavily on two key assumptions: 1) that anthropogenic inputs of N are large compared with internal cycling of natural N inputs, and 2) that pollutant N deposition has a unique δ^{15}N signature detectable against intrinsic δ^{15}N ecosystem values. A major underpinning of pollutant studies is the difference in δ^{15}N of pollution given its source with δ^{15}N values of vehicular exhaust ranging from -13 to -2 ‰ and coal-fired boiler emissions ranging from +6 to +13 ‰ (Heaton 1990) although subsequent atmospheric alterations may mask differences in point-source emission values under a broader deposition footprint (Freyer et al. 1993). Problems associated with these assumptions are discussed in detail in the discussion on atmospheric deposition of inorganic N (Section V).

Historical reconstructions of pollution impacts using tree ring δ^{15}N have been developed in China (Sun et al. 2010), South Korea (Choi et al. 2005, Kwak et al. 2009, 2010), Luxembourg (Härdtle et al. 2013), Switzerland (Saurer et al. 2004, Guerrieri et al. 2009), Italy (Guerrieri et al. 2009, Battipaglia et al. 2010, Guerrieri et al. 2010, Leonelli et al. 2012) and Canada (Bukata & Kyser 2007, Savard et al. 2009, Doucet et al. 2012, Jung et al. 2013). Wood δ^{15}N, δ^{13}C, and calcium/aluminum ratios (Ca:Al) have been used to reconstruct acid precipitation, though the signal was regionally specific, precluding large-scale extrapolation (Kwak et al. 2011, Kwak et al. 2009, Sun et al. 2010). Additionally, some tree ring δ^{15}N chronologies record a significant shift at the onset of increased pollution (Saurer et al. 2004) or long-term temporal trends correlated with a pollutant proxy, such as number of registered cars (Doucet et al. 2012) or regional NO_x emissions (Savard et al. 2009). In response to point-source pollution, trees exhibited increased (Guerrieri et al. 2009, Saurer et al. 2004) or decreased (Guerrieri et al. 2009, Leonelli et al. 2012, Sun et al. 2010) δ^{15}N values, which were attributed to the previously-discussed differences δ^{15}N signatures of emission sources (Guerrieri et al. 2009, Leonelli et al. 2012). In other cases, temporal trends in wood δ^{15}N were unaltered by pollution (Battipaglia et al. 2010, Jung et al. 2013), though interannual variation in δ^{15}N increased in some cases (Battipaglia et al. 2010). In regions experiencing high NO_x emissions from multiple sources,

short-term fluctuations in tree ring $\delta^{15}N$ correlated to climatic variables, while long-term trends reflected regional emissions from vehicles and coal plants (Doucet et al. 2012, Savard et al. 2009). These differences in N inputs resulted in a long-term decreasing wood $\delta^{15}N$ trend at sites driven by vehicle emissions (Doucet et al. 2012, Savard et al. 2009), increasing or flat trends at sites driven by coal combustion (Doucet et al. 2012), and increasing trends at sites experiencing both emission sources (Savard et al. 2009) due to the presumed high $\delta^{15}N$ values of power plant NO_x emissions (Savard et al. 2009).

4. The *marine input studies* are the least common type of wood $\delta^{15}N$ study with only 6 publications. These studies attempt to track exogenous N from marine animal populations into terrestrial ecosystems where it is taken up by trees. The key assumptions are that organic matter of marine origin is highly enriched in $\delta^{15}N$ relative to terrestrial pools and that the marine inputs are of sufficient magnitude to affect the native N cycle. Marine input studies have focused on two exogenous marine N sources: carcasses of spawning salmon in the Pacific Northwest of the United States and British Columbia, Canada, (Drake et al. 2011, Reimchen et al. 2002), and marine avian breeding populations roosting in coastal forests in Japan (Lopez C. et al. 2010, 2011, Mizota et al. 2011) and New Zealand (Holdaway et al. 2007). Reportedly, over 15 research groups have attempted to track salmon-derived N inputs in forest ecosystems; however, only two studies have been published (Drake et al. 2011, Reimchen et al. 2002) due to the limited success of the approach given current methodologies (Drake et al. 2011). Reimchen et al. (2002) identified a spatial trend whereby wood $\delta^{15}N$ was high next to the stream and decreased with increasing distance from the stream. Attempts to reconstruct historical salmon density using temporal trends in wood $\delta^{15}N$ were unsuccessful (Drake et al. 2011). Salmon-derived N impacts on wood $\delta^{15}N$ may be hindered by the low signal potential of salmon $\delta^{15}N$, defined as the difference between salmon and background $\delta^{15}N$ values and the mass of salmon-derived N taken up by the tree (Drake et al. 2011). Under this calculation, the potential salmon-derived N signal is within the range of natural wood $\delta^{15}N$ variation, and therefore difficult to detect (Drake et al. 2011).

Tracking of avian-derived N signals in wood $\delta^{15}N$ have met with more success, with enriched $\delta^{15}N$ trends found in wood and soil of forests in New Zealand (Holdaway et al. 2007) and Japan (Lopez et al. 2010; Mizota et al 2011). Long-term chronologies have been used to reconstruct colonization of coastal forests by breeding marine birds (Holdaway et al. 2007;

Mizota et al 2011). Tracking of animal-derived marine N inputs is still rather understudied, though it is unclear what more could be gained in this avenue, particularly in the tracking of salmon signals. Interpretation of these studies may be aided by additional research on spatial and temporal wood $\delta^{15}N$ comparisons under marine N, assuming a clear signal can be identified.

IV. Methodological considerations

N concentrations in ring wood are rather low (usually on the order of 0.05-0.2% of total dry weight) and so more tissue is needed for isotopic N analysis than for other common tree ring isotope analyses (such as carbon and oxygen). In decades past, N isotope analysis required as much as 100 mg of ring tissue to perform a single isotopic measurement. Technological advances in isotope mass spectrometry have reduced the necessary mass to ~20-25 μg of N. Given the range of [N] in wood, and the analytical capabilities of a given lab, this reduces total tissue weight to 10-30 mg. Still, narrow rings (particularly if obtained from small diameter increment borers) may not contain enough tissue for N analysis, requiring combining of annual tree rings to reach the necessary tissue weights. This poses challenges for reporting data and for obtaining annual resolution if it is a requirement of the study design.

The low N concentrations in wood also mean that excess carbon produced by sample combustion must be trapped and removed prior to analysis in the mass spectrometer. Additionally, $\delta^{13}C$ analysis is performed on alpha-cellulose (Leavitt and Danzer 1993), which contains no N. Thus, dual isotope analysis with $\delta^{13}C$ on the same sample is not possible at this time, although it would potentially be very interesting (see below). The large sample volume and variety of preparation methods also lead to the risk of incomplete combustion. Grinding or chopping the sample to homogenize it prior to analysis may aid combustion. Even so, low [N] in wood generally leads to lower analytical accuracy for wood $\delta^{15}N$ than for other solid-phase environmental samples such as foliar tissue or soil.

Replication of measurements is also difficult with tree-ring N studies. Ideally, the same tissue measured in different labs, or in the same lab at different times, should produce the same isotopic value. The high tissue weights required for N isotope analysis of ring wood preclude replication testing in most cases. Confounding this problem is the issue of translocation of N compounds throughout the ring chronology. Translocation of N means that the same ring, sampled in different time periods, would not have the same true $\delta^{15}N$ value, regardless of any

analytical inaccuracies in measurements. Replication testing, therefore, must rely on ring tissue obtained from one sampling time period, in order to avoid the confounding impacts of N translocation on wood δ^{15}N values.

An additional issue for wood δ^{15}N replication is the lack of a universally accepted wood standard for N analysis. The National Institute of Standards and Technology (NIST) identifies and maintains international standards for a number of tissues, yet offers no standard for wood N studies. In the absence of a NIST standard, it is necessary for an individual lab to determine an in-house high-volume wood standard (such as saw dust). While in-house standards supply some assurance of accuracy, and inter-lab tests indicate similar absolute values for these standards, a universal standard would enable a true comparison of absolute wood δ^{15}N values among studies.

Analytical accuracy is often not reported (Jardine and Cunjack 2005) but could be reported in the following manner without a universal standard. "Accuracy, as determined by comparing measured values to the known value of an internal laboratory standard, was better than 0.2‰ for δ^{15}N across all batches of samples. Precision within each batch of samples analyzed, as estimated by the standard deviation of repeated measurements of a working standard, was lower than 0.1‰ for δ^{15}N across all batches of samples." These types of estimates of error are likely the best that can be achieved at this time.

Other methodological issues include standardization of sampling strategies such as pooling wood samples from different trees, either to increase environmental representation, boost temporal resolution, or both. Although pooling wood samples from multiple years is a common practice due to the sample size requirements, a full study of the effects of pooling samples for analysis has not been undertaken for wood δ^{15}N as it has for wood δ^{13}C (Leavitt and Long 1986). Pooling of rings produces two inter-related issues in data analysis: 1) reduced temporal resolution, leading to 2) reduced ability to detect short-term fluctuations in wood δ^{15}N values. Annual resolution is not necessary for building long-term temporal trends in ecosystem N dynamics; however, short-term fluctuations in δ^{15}N may reflect climatic conditions (Savard et al. 2009; Doucet et al. 2012) or discrete perturbations to the N cycle *via* disturbance events (Beghin et al. 2011; Stock et al. 2012). Consequently, pooling rings for N analysis may obscure short-term fluctuations in ecosystem N dynamics. As analytical capabilities continue to improve, annual and even subannual resolution of wood δ^{15}N patterns through separating earlywood and

latewood could occur, perhaps by using very large samples and laser ablation techniques, as has been done for $\delta^{13}C$ (Schulze et al. 2004)

Finally, there is huge variation in reporting wood $\delta^{15}N$ data in published manuscripts that must be addressed and standardized (Wolkovich et al. 2012). The most important point is that reporting the exact chronology used for the wood $\delta^{15}N$ measurements is essential. The number of years contained in a sample, the date assigned to that sample, and the raw values of wood $\delta^{15}N$ would be a minimum dataset that would enable maximum utility of the study (Table 1). To focus on temporal trends, some authors report wood $\delta^{15}N$ values standardized to the same mean value for each site, centered around 0‰. In these cases, raw data should also be reported.

In summary, several methodological and analytical questions remain. Methodological questions include variability in the true wood $\delta^{15}N$ value due to temporal translocation, pooling of rings, and variation in wood $\delta^{15}N$ values around the tree circumference and with sampling height (as has ben shown in carbon; Leavitt and Long1986). Additionally, analytical questions include the development of a universal wood N standard, replicability of wood $\delta^{15}N$ measurements within a single lab, and replicability of wood $\delta^{15}N$ measurements between labs. Studies addressing these remaining questions will contribute additional value and utility to wood $\delta^{15}N$ studies, and allow for more robust meta-analysis of current published data.

The Role of [N]

Until the mid 1990s, N analysis of tree rings focused only on [N]. Proteins bound to carbon-based structural compounds in the cell wall contain N (Bao et al. 1992, Showalter 1993). Early studies identified a rather predictable trend in ring [N], whereby rings in the heartwood generally show constant [N] levels, with a sharp increase at the heartwood/sapwood boundary, followed by a steady increase through the sapwood, spiking to the highest [N] level in the most recent ring (Fig 5). In some cases, the sharp increase at the heartwood/sapwood boundary is lessened, and rings show a constant increase in [N] through both the heartwood and the sapwood (Poulson et al. 1995; McLauchlan and Craine 2012). When the pith is present, it may also exhibit a spike in [N] comparable to, and sometimes exceeding, the spike in the most recent ring (Merrill and Cowling 1966). These general trends have been reported across numerous taxa, disparate environmental growing conditions, different preparation techniques (see extraction section below), and different levels of ecosystem N availability.

Studies assessing wood [N] and δ^{15}N response to a known perturbation of the N cycle support the lack of an N-availability signal in [N]. Fertilizer and tracer studies often do not report increases in tree ring [N] after fertilization (Koopmans et al. 1996; Schleppi et al. 1999; Hart and Classen 2003; Balster et al. 2009). In cases where tree ring [N] appears to reflect pollution inputs (Kwak et al. 2009; Leonelli et al. 2012), the response is inconsistent (Saurer et al. 2004; Kwak et al. 2011) or also documented in trees not exposed to pollution (Guerrieri et al. 2009). Consequently, some researchers have concluded that tree ring [N] patterns reflect physiological drivers within the tree and cannot be used to reconstruct ecosystem N availability during growth (Poulson et al. 1995; Bukata and Kyser 2005; Doucet et al. 2010) and that tree ring δ^{15}N is a more useful measure of pollution impacts than [N] (Guerrieri et al. 2009; Doucet et al. 2010; Doucet et al. 2012). In fact, many studies no longer report [N] data, or do so only in the context of comparisons to other ecosystem pools like foliage and soil.

It has been suggested that differences in [N] and δ^{15}N between sites may be a more useful measure than absolute values (Jung et al. 2013). Termed Diff_N and Diff_ δ^{15}N respectively, these measures may help focus on the impacts of atmospheric N deposition between sites while avoiding confounding site-specific factors such as N availability and transformation processes (Jung et al. 2013). As yet, Diff_N and Diff_ δ^{15}N have not been widely used, but may indicate differences in pollution inputs between different sites or differences in tree preference for N forms (Jung et al. 2013). For example, Jung et al. (2013) compared wood δ^{15}N values in a two watersheds with similar climatic and environmental characteristics, located at differing distances from the Canadian oil sands. In these watersheds, increasing Diff_N and decreasing Diff_ δ^{15}N reflected greater temporal increases in ^{15}N-depleted inputs in the watershed closer to the oil sands than the further watershed (Jung et al. 2013). Trends in Diff_N and Diff_ δ^{15}N were significant, despite a lack of significant differences between watersheds or significant temporal trends in [N] and δ^{15}N (Jung et al. 2013).

For the studies that have reported both wood [N] and δ^{15}N, it seems that there is no consistent correlation between these variables, unlike the strong global correlation between foliar δ^{15}N and foliar [N] (Craine et al. 2009). All three relationships have been reported—positive correlations (Leonelli et al. 2012, Lopez et al. 2010), negative correlations (Choi et al. 2005), and lack of correlations (Guerrieri et al. 2009, Doucet et al. 2012). Concurrent increases in both [N] and δ^{15}N have been reported under marine bird colonies (Mizota et al. 2011), and in response to

fire disturbance (Beghin et al. 2011), and decreasing [N] concurrent to increasing δ^{15}N occurred under acid precipitation (Kwak et al. 2009).

Because [N] trends are at least partially (if not fully) influenced by physiological processes within the tree, it is inappropriate to attribute correlations between [N] and δ^{15}N or temporal changes in [N] to environmental changes in N availability. This is particularly true if increases in N availability in the ecosystem coincide temporally with physiological periods that exhibit high [N] such as the heartwood-sapwood boundary, or the most recent rings. Although wood δ^{15}N may correlate with environmental N availability, a correlation to wood [N] becomes meaningless if [N] is driven by non-environmental factors. It is likely for these reasons that the majority of wood N studies either report no [N] values at all, or do not attempt to correlate [N] to δ^{15}N.

To Extract or Not Extract

Wood extraction procedures have been strongly influenced by the development of cellulose purification techniques, which removes variation in δ^{13}C among wood components and isolates an annual signal in wood δ^{13}C values (Leavitt and Danzer 1993). As early as 1966, researchers had documented translocation of nitrogen across tree-rings (Nommik 1966). Fertilizer and tracer studies recovered labeled N in rings formed prior to N application, indicating that trees redistribute N from recently formed rings into older rings *via* rays (Nommik 1966; Mead and Preston 1994; Schleppi et al. 1999; Elhani et al. 2003). In fact, studies have shown N mobility throughout all plant tissues over the course of numerous growing seasons, indicating that N redistribution is a continual process (Mead and Preston 1994). Mobility of N in wood and other tissues has contributed to the view described above that wood N (both [N] and δ^{15}N) may not directly represent growing season N availability. It has been argued that structural N would represent N availability, but is confounded by labile, translocated N (Sheppard and Thompson 2000). Consequently, removal of labile N could reveal the pattern of past N availability as recorded by structural N. To date, such studies have focused exclusively on chemical extraction of labile N, described in detail below. It has been argued that the predictable

nature of N translocation could allow these patterns to be removed mathematically, although this approach has not been tested (Hart and Classen 2003).

A variety of different extraction techniques have been tested with widely varying results (Table 2). The first, and most influential of these was Sheppard and Thompson's protocol in 2000. The Sheppard/Thompson extraction method significantly reduced total N concentrations and variability in N concentrations compared to control rings, while the longer extraction method supported, but failed to improve upon the results obtained at shorter extraction lengths (Sheppard and Thompson 2000). The Sheppard/Thompson method eliminated the variation in N concentrations attributed to the sapwood-heartwood boundary and to the most recently formed ring, leading the authors to conclude that this extraction method successfully removed soluble N compounds in rings and retained structural N contained in cell walls (Sheppard and Thompson 2000).

Subsequent studies complicated this original result. In fertilizer and tracer studies, extraction decreased δ^{15}N values prior to the application of fertilizer, but increased δ^{15}N values during the treatment period, which amplified the δ^{15}N peak under fertilization and produced clearer divergences in δ^{15}N between treatment and control trees (Elhani et al. 2003, 2005). Sheppard/Thompson extraction did not always significantly reduce total [N] (Hietz et al. 2010). Even when significant reductions were achieved, extraction failed to physiologically driven pattern of [N] in rings (see section on The Role of [N]), suggesting not all mobile N was removed (Elhani et al. 2003; Kwak et al. 2009). Under high pollutant inputs, the amount of extracted N was higher in recent rings, suggesting that increases in [N] in recent rings reflect increases in mobile N as opposed to structural N components (Doucet et al. 2010). Conversely, in natural abundance studies, sapwood retained significantly higher [N] and δ^{15}N than heartwood indicating that more recent rings do not contain higher proportions of extractable N than heartwood (Hietz et al. 2010). These confounding results may be due to differences in N loads of the ecosystem, species-specific differences in N translocation, or an ineffective extraction protocol.

Recently, researchers have tested several alterations to the Sheppard/Thompson technique (Doucet et al. 2011; Lopez et al. 2011). A simplified Sheppard/Thompson extraction requiring fewer steps significantly reduced wood δ^{15}N in trees exposed to marine avian colonies, but produced no change in control trees (Lopez et al. 2011). In addition, temporal trends in δ^{15}N of

all trees were unaffected by extraction (Lopez et al. 2011). Consequently, the authors concluded that extraction is necessary in systems with unusually high N inputs and when absolute values of $\delta^{15}N$ (as opposed to temporal trends) were the main focus of the study. An altered Sheppard/Thompson method utilizing different chemicals did not significantly alter [N] and $\delta^{15}N$ patterns and did not reduce interannual variability in [N] or $\delta^{15}N$ leading to the conclusion that extraction was unnecessary in these systems (Doucet et al. 2011, 2012).

Several other extraction techniques, not based on the Sheppard/Thompson method have also been analyzed (Table 2). Holocellulose extraction produced a small but consistent reduction in $\delta^{15}N$ of control trees, and larger and more variable reductions in N-labeled trees (Hart and Classen 2003). These results support the findings of Lopez et al. (2011) that indicated extraction was necessary for trees occurring in high-N systems. Soaking samples in deionized water produced no consistent change in [N] of heartwood rings, but significant reductions in [N] and increases in $\delta^{15}N$ of sapwood (Bukata and Kyser 2005). These authors argued DI-water extraction only produces consistent and significant results in resinous species and is therefore unnecessary for non-resinous species. Analytically, extraction of resinous species may also reduce accumulation of secondary combustion compounds in the mass spectrometer which can hinder analytical precision. A sterilization extraction technique removed significant proportions of N from both heartwood and sapwood, resulting in increased wood $\delta^{15}N$ in one species, but decreased wood $\delta^{15}N$ in another (Drake et al. 2011). The high levels of N removed from rings, and the significance of the shift in $\delta^{15}N$ post-extraction led the authors to conclude that extraction was necessary for interpreting temporal $\delta^{15}N$ patterns in this system (Drake et al. 2011).

No wood N study has specifically tested which N compounds extraction may remove from or retain in ring wood. There is no N-containing compound that is easy to identify and purify, like cellulose for C. Presumably, N stored as proteins adhered to structural compounds (cellulose and lignin in cell walls) will be retained, while non-structural compounds (tannins, polyphenols, fats, waxes, resins, alkaloids) will be removed by extraction (Sheppard and Thompson 2000; Elhani et al. 2003). The fact that extraction will remove non-nitrogenous soluble compounds as well as nitrogenous ones may be beneficial as it can increase extracted wood [N], potentially reducing analytical error (Sheppard and Thompson 2000). Drake et al. (2011) are the only authors to analyze extracted compounds, and reported that extracted sap exhibited $\delta^{15}N$ values nearly 20 ‰ lower than extracted wood, although the mechanism for this

difference was not determined. Considering the fact that N forms experience different translocation trajectories within the tree (Mead and Preston 1994), that trees exhibit species-specific preferences for certain forms of N over others (ex. (Templer and Dawson 2004), and that extraction can increase or decrease wood δ^{15}N signatures (Drake et al. 2011), it is necessary to verify which N compounds are removed by extraction, and which are retained. Performing extraction without understanding the processes and compounds affected may alter wood [N] and δ^{15}N in some cases, but the significance (both quantitative and qualitative) of those changes is as yet unclear.

The variability in techniques, and the inconsistency of results have led many researchers to forego extraction altogether in favor of raw wood analysis. Since the publication of the Sheppard/Thompson method in 2000, approximately half of all wood N studies have analyzed raw ring wood. The majority of studies performing extraction are among the exogenous input categories, which is consistent with the findings that extraction is necessary only under high-N conditions (Hart and Classen 2003; Lopez et al. 2011). With only a few exceptions (ex. Heitz et al. 2010), natural abundance studies perform no extraction, and in recent years, even tracer studies have begun to favor analysis of raw ring wood.

A given extraction technique would need to meet three requirements before its use could be justified. First, extraction must be able to document which wood N compounds are removed and which are retained in the extracted wood. Drake et al (2011) provided an important first step, but much additional work is needed. Second, extraction must consistently remove physiologically-driven temporal patterns in [N], such as the increase at the heartwood/sapwood boundary. Third, and most relevant to fertilizer and tracer studies, extraction must reduce wood δ^{15}N values prior to the treatment period such that these values reflect background ecosystem N cycling and not fertilizer inputs. Until and unless an extraction technique is developed that meets these requirements, the inclusion of routine extraction in wood δ^{15}N protocols is unwarranted.

V. What have we learned from wood δ^{15}N studies?
Atmospheric deposition of inorganic N

Temporal trends in wood δ^{15}N during the 20th century have been frequently attributed to increased atmospheric deposition of N from anthropogenic sources (Poulson et al. 1995; Choi et al. 2005; Bukata and Kyser 2007; Hietz et al. 2011; Härdtle et al. 2013). The conclusions of

these studies, taken alone, would indicate that recent anthropogenic N deposition is affecting terrestrial N cycling in a variety of temperate forested ecosystems. These studies, however, rely heavily on two key assumptions: 1) that anthropogenic inputs of N are large compared with internal cycling of natural N inputs, and 2) that pollutant N deposition has a unique δ^{15}N signature detectable against intrinsic δ^{15}N ecosystem values. Unfortunately, there is not widespread evidence for either of these assumptions.

Globally, anthropogenic N deposition equals natural N fixation rates (Galloway et al. 2008); however, on a regional scale, total N deposition varies widely. For example, total N deposition ranges from near 0 to >8 kg ha^{-1} yr^{-1} in the contiguous United States (NAPD 2007) and from near 0 to >25 kg ha^{-1} yr^{-1} throughout Europe (Holland et al. 2005). Low levels of anthropogenic N deposition could still represent a significant N input if background N availability was very low; however, high N deposition loads relative to internal ecosystem N cycling cannot be assumed in all regions.

Second, the regional δ^{15}N value of pollution N varies considerably, with reported values spanning almost 20 ‰. Different point sources display different NO$_x$ δ^{15}N values, with vehicular exhaust emissions ranging from -13 to -2 ‰ and coal-fired boiler emissions ranging from +6 to +13 ‰ (Heaton 1990). Differences can even exist among power plants depending on the type of catalytic reduction employed (Felix et al. 2012). Pollutant δ^{15}N values may also vary between the point source emission value and the broader N deposition footprint after NO$_x$ experiences atmospheric alteration (Freyer et al. 1993) and transboundary pollution transport (Park et al. 2004, Schaub et al. 2005). For example, ^{15}NO$_x$ near a motorway can reach values up to +2 to +8 ‰ (Ammann et al. 1999), despite the low signature of emissions straight from the tailpipe. Consequently, the combination of different point source δ^{15}N values and different atmospheric alterations results in mean regional deposition δ^{15}N that is highly variable, ranging from -8.5 to +3.2 ‰ across the contiguous United States (Elliott et al. 2007, Hastings et al. 2013). Additionally, regional deposition δ^{15}N values consistently show strong seasonal variation (Elliott et al. 2009, Jai and Chen 2010, Kundu et al. 2010, Mara et al. 2009, Rolff et al. 2008) sometimes reaching values as high as 15 ‰ (Elliott et al. 2009). Consequently, the assumption that deposition δ^{15}N is sufficiently different from background ecosystem δ^{15}N is not consistently warranted. The assumption that low δ^{15}N values of atmospherically-deposited N drive temporal decreases in tree ring δ^{15}N is particularly speculative without data about the actual δ^{15}N values of

atmospherically-deposited forms of N in the region (Elliott et al. 2007, Elliott et al. 2009, Kundu et al. 2010, Hastings et al. 2013)

In some studies, increased N emissions from vehicular exhaust reportedly both increased wood $\delta^{15}N$ due to high exhaust $\delta^{15}N$ (Saurer et al. 2004, Guerrieri et al. 2009) and decreased wood $\delta^{15}N$ due to low exhaust $\delta^{15}N$ (Savard et al. 2009, Doucet et al. 2012). These findings are not necessarily mutually exclusive, as the previously mentioned regional variation in pollution $\delta^{15}N$ could result in high deposition $\delta^{15}N$ in some regions and low deposition $\delta^{15}N$ in others (ex. Hastings et al. 2013). Unfortunately, there is little direct data to support either interpretation (anthropogenic $\delta^{15}N$ values are higher or lower than natural $\delta^{15}N$ values). One study provided direct NO_x $\delta^{15}N$ values for the studied region during the analyzed time period (Saurer et al. 2004). Doucet et al. (2012) and Savard et al. (2009) both reported regional NO_x emissions, but not N deposition loads or deposition $\delta^{15}N$ values. Guerrieri et al. (2009) reported no regional NO_x emission or N deposition data. Additionally, studies assuming low exhaust $\delta^{15}N$ signatures, may be focusing on values straight from the tailpipe and not addressing subsequent atmospheric alteration which can increase vehicular NO_x $\delta^{15}N$ (Ammann et al. 1999). Consequently, although all of these studies could potentially accurately report pollution effects on wood $\delta^{15}N$, only Saurer et al. (2004) provided pollution $\delta^{15}N$ data definitively supporting their conclusions. To ascribe temporal wood $\delta^{15}N$ trends to impacts of a unique external pollutant signature, it is necessary to report data about the $\delta^{15}N$ values of local pollution sources. General analyses of emission sources or local atmospheric concentrations are not sufficient for concluding a direct impact of pollution deposition on temporal wood $\delta^{15}N$ trends.

It should be noted that potential relationships between temporal wood $\delta^{15}N$ and anthropogenic N deposition are only relevant on recent, short timescales. Even detailed measurements of deposition amount and $\delta^{15}N$ may not accurately reflect deposition trends throughout the length of the tree ring chronology.

Forest dynamics

Tree demography influences N cycling, and a small subset of studies focused on the impact of dominance and suppression in forest stands (Weber et al. 2008; Couto-Vázquez and González-Prieto 2010). Overall, wood $\delta^{15}N$ was not different between dominant and regenerating trees, and no significant spatial trends were identified in regenerating trees, which suggests that dominant

trees exhibit little influence over the N dynamics of nearby regenerating trees (Weber et al. 2008). Prior to the establishment of dominance, both dominant and suppressed trees displayed decreasing δ^{15}N through time, attributed to a more 'closed' N cycle (Couto-Vázquez and González-Prieto 2010). Following the establishment of dominance, however, suppressed trees exhibited an abrupt and significant increase in δ^{15}N, while dominant trees maintained their decreasing trajectory (Couto-Vázquez and González-Prieto 2010). Increased δ^{15}N of suppressed trees was attributed to a reduction in the amount of N taken up from the soil, with translocation of N from wood serving as the primary N source for needles (Couto-Vázquez and González-Prieto 2010). Successional changes over decadal timescales indicate a decline in wood δ^{15}N, supporting other lines of evidence of decreased N availability during ecosystem succession due to increased plant uptake of N and reduced N supply (Batterman et al. 2013, Vitousek et al. 1989).

Climatic variables

Climatic variables appear to play an inconsistent, or unclearly defined role in the spatial patterns of wood δ^{15}N values. Principal component analysis grouped wood δ^{15}N with growing season precipitation and opposite spring/summer mean temperatures (Couto-Vázquez and González-Prieto 2010). In other cases, wood δ^{15}N was negatively (Stock et al. 2012) or positively (Härdtle et al. 2013) correlated with growing season precipitation, and negatively correlated with growing season temperature (Härdtle et al. 2013). Interestingly, correlations were not found in all sample locations (Stock et al. 2012, Härdtle et al. 2013). Wood δ^{15}N in some locations was significantly correlated with site productivity (Kranabetter et al. 2013), suggesting that site characteristics may play a stronger role than climatic drivers. Globally, foliar δ^{15}N tends to decline then increase with increasing MAT and decline with increasing MAP, providing an important context for individual and site-based studies (Craine et al. 2009); however, it remains to be seen if climatic variables affect temporal trends in wood δ^{15}N or simply influence mean values due to influences on fractionating N cycling processes. Additional analyses are needed before it will be clear which climatic variables most impact wood δ^{15}N and what other factors may affect these relationships. This will enable investigations on the important topic of the role of past climate change on N cycling.

Disturbance

Several natural abundance wood δ^{15}N studies have focused on the effects of changing disturbance regimes (McLauchlan et al. 2007), or discrete disturbance events such as fire (Beghin et al. 2011, Stock et al. 2012), bog drainage (Choi et al. 2007), and stand thinning or logging (Bukata and Kyser 2005; Hietz et al. 2010; Stock et al. 2012) on wood δ^{15}N. These disturbance events occurred naturally, were the result of anthropogenic activity, or were produced experimentally. In many cases, disturbance increased wood δ^{15}N, due to increased ecosystem loss of isotopically light N through nitrification or soil leaching, and reduced competition for resources by the remaining vegetation (Bukata and Kyser 2005; Choi et al. 2007; Hietz et al. 2010; Stock et al. 2012). In other cases, no consistent change in wood δ^{15}N was observed, either due to the seasonal timing of the disturbance (Beghin et al. 2011), distance from the disturbance event (Bukata and Kyser 2005), or type of disturbance (Stock et al. 2012). The cessation of chronic anthropogenic disturbance resulted in declining wood δ^{15}N trends, which was attributed to declining ecosystem N availability as carbon stocks increased (McLauchlan et al. 2007). Growth trends were similarly variable with bog drainage and stand thinning substantially increasing growth (Choi et al. 2007; Stock et al. 2012), while fire decreased growth and increased the prevalence of locally absent rings (Beghin et al. 2011). Perturbations in annual growth and wood δ^{15}N persisted for 3-4 years after stand thinning (Stock et al. 2012), 12 years after clear-cutting (Bukata and Kyser 2005), 6-10 years after fire (Beghin et al. 2011), and at least 15 years following drainage (Choi et al. 2007). At the ecosystem scale, N availability required 75 years to return to levels consistent with non-disturbance trajectories (McLauchlan et al. 2007).

As with pollution input studies, the effect of the disturbance event on the magnitude of the δ^{15}N response was somewhat localized. Trees along the perimeter of a clearing showed increases in δ^{15}N consistent with other disturbance events, while center-stand trees showed no discernible change in δ^{15}N (Bukata and Kyser 2005). Interestingly, the side of the perimeter tree facing the rest of the stand showed a smaller magnitude increase in δ^{15}N than the sides parallel to the perimeter or the side facing the clearing, while no radial variation was documented in the center-stand tree (Bukata and Kyser 2005). These results indicate that proximity to the disturbance event is significant not only at the stand level, but even on the scale of a single individual.

VI. Suggestions for future research

Based on what is currently known from published wood δ^{15}N studies, we aim to provide several suggestions for guiding future studies utilizing such data. Research communities coalesced early in the development of wood δ^{13}C and δ^{18}O analyses to identify uniform protocols, sampling procedures, sample sizes, and standardization of interpretations, and these early papers helped to galvanize the field (Leavitt and Danzer 1993, Roden and Ehleringer 2000). However, due to the disparate nature of the current literature, no clear guidelines have emerged for wood δ^{15}N studies. Here, we identify some key points regarding study structure, data collection, interpretation, reporting, and most importantly, knowledge transfer between different research groups utilizing wood δ^{15}N datasets.

(1) More wood δ^{15}N records could be acquired to increase the temporal and spatial scope of existing datasets. There is a severe dearth of sampling across large portions of the world, including China, Russia, Eastern Europe, the Middle East, Africa, Central America, South America, and central and northern regions of North America (Fig. 2). These regions represent unique ecosystems and forest types that are not currently represented in published studies. Future wood δ^{15}N studies should focus on these regions in order to build a global synthesis of wood δ^{15}N trends. The importance of incorporating understudied regions cannot be underestimated. Much of the Earth's climate space, including regions that are located at biogeochemical extremes for gaseous N loss, reactive N deposition, or organic N uptake, has not been sampled for wood δ^{15}N, and thus we have little to no temporal dimension to our current understanding of N cycling in these interesting locations (Fig. 2).

Similarly, much older wood δ^{15}N records need to be generated. Currently, the oldest published natural abundance wood δ^{15}N dates to 1835 AD (McLauchlan et al. 2007) and the majority of chronologies cover less than the last 60 years. Thus, the full potential of wood δ^{15}N to provide baseline information about N cycling prior to anthropogenic increases in reactive N, and to illuminate controls on N cycling during previous periods of rapid climate change, has not yet been realized. Much of the strength of dendrochronology lies in the long-term historical reconstructions that can be made with long-lived trees and/or preserved tree remains. An important next step in wood δ^{15}N research will be to develop a long-term record of wood δ^{15}N using established chronologies in conjunction with glacial wood repositories.

(2) There is potential to acquire or integrate more detailed biogeochemical data to help interpret wood δ^{15}N measurements. In particular, alongside wood δ^{15}N records, an overview of the current site-specific N cycle, including pool sizes and fractionating flux pathways would 0 help. A suite of traditional N cycle measurements— nitrate and ammonium concentrations, net N mineralization, hydrologic losses in organic or inorganic forms, mycorrhizal type—could be generated at sites where wood δ^{15}N is measured. Alternatively, wood δ^{15}N can be measured in sites where there are good N budgets, especially long-term N monitoring such as stream nitrate export (Argerich et al. 2013). Additionally, correlation of temporal patterns of wood δ^{15}N with other ecosystem processes and δ^{15}N signatures of other compartments would improve interpretations of long-term nutrient cycling and ecosystem function.

(3) It is important that wood δ^{15}N data be accessible and available, as much as possible in the publication and certainly in online data repositories for future use in meta-analyses. In publications, it is unnervingly frequent that studies will report partial descriptions of data, with limited utility for other researchers. For instance, a study may report only a temporal trend (i.e. rate of decrease in δ^{15}N per year) without reporting actual wood δ^{15}N values either in text or figures. In other cases, the methods section may indicate that certain data were collected, but these data are not reported or analyzed in the results and discussion sections. To increase the utility of data for comparison among studies, we propose a two-fold solution to this problem: 1) report full data in publications. This can be done through tables, figures, and aggregate measures (mean and standard deviation, measures of variability, etc) in the publication, or in online supplemental material. We suggest a list of minimum data to be reported, as well as best practices for maximizing the utility of published data (Table 1). 2) contribute full data sets to online repositories from which other researchers can extract data for large-scale, multi-dataset studies. Recent studies have documented the importance of preserving data *via* publicly accessible archives (Vines et al. 2013). We recommend that researchers submit data to the National Climate Data Center (NCDC, http://www.ncdc.noaa.gov/). The NCDC focuses on climate-related data and has an explicit tree-ring data category under its paleoclimate portal, including a submission category for isotopic data. The NCDC makes it easy to access data and easy to contribute data, streamlining the data sharing process on both sides. Depending on the focus of the research, other databases may also be available. Regardless of which repository a

researcher may use, it is important that data are freely available, and the repository documented in the publication for future access.

(4) For questions about climate change and carbon status, more supporting datasets should be used alongside wood δ^{15}N. The concurrent use of δ^{13}C and δ^{18}O isotopic analyses on wood chronologies would be particularly helpful. Approximately 20 studies have incorporated one or both of these analyses in conjunction with wood δ^{15}N; however, in many cases, the data are not strongly integrated. To what degree C and O dynamics directly relate to, or are impacted by N dynamics is unclear. Additionally, δ^{13}C and δ^{18}O values are affected by changing environmental parameters other than N availability, such as increasing atmospheric [CO_2], increasing temperatures, and changing precipitation patterns. Separating the relative impacts of these variables on each isotope, and on the relationship between isotopes, can be a formidable task. Still, if isotopic analyses other than δ^{15}N are included, integration among the datasets is important. Even if integration is unclear or impossible in an individual study, contributing concurrent datasets of δ^{15}N and other isotopes to data repositories for use in meta-analyses could clarify relationships at continental or global scales. Additionally, few natural abundance studies report correlations or lack thereof between wood δ^{15}N and climatic variables. In some cases, other measures (such as ring width or δ^{13}C) are correlated to climate, so it is clear that detailed climate data were available, but correlations with δ^{15}N are not discussed. Limited discussion of the relationship between climate and wood δ^{15}N makes it difficult to assess if climate has little or no impact on wood δ^{15}N, if impacts are restricted to certain species or environments, or if large-scale climatic variables such as MAT are predictive of average wood δ^{15}N values or temporal trends in wood δ^{15}N. Finally, ring width determination is an important dendrochronological dataset measured on almost all samples processed for wood δ^{15}N, and could be analyzed to assess the impact of changing N cycling on tree and forest growth.

(5) It is clear from the numerous studies assessing wood [N] patterns that the primary drivers of ring [N] are physiological in nature and do not reflect ecosystem N availability. Only in isolated circumstances (such as the acid precipitation reconstructions) has wood [N] reflected environmental conditions, and even in these cases, the response was inconsistent and wood δ^{15}N exhibited clearer and more consistent trends. Consequently, if the primary goal of the study is to assess ecosystem N cycling then wood [N] trends are not a useful tool, and researchers should focus on wood δ^{15}N.

(6) An extraction protocol that consistently removes all non-structural N compounds does not currently exist. Numerous methodologies have been tested and provide at best inconsistent results. The problem may lie in our understanding, or lack thereof, of labile and structural N compounds, or with the behavior of N translocation between and among tissues. Performing extraction without understanding the processes and compounds affected may alter wood [N] and δ^{15}N in some cases, but the significance (both quantitative and qualitative) of those changes is as yet unclear. Consequently, until and unless we can identify which N compounds are affected by extraction, and a protocol is developed that consistently resolves wood [N] and δ^{15}N patterns, it is difficult to justify the use of extraction in wood N studies.

(7) A review of marine input papers suggests that it may not be possible to clearly reconstruct salmon populations using wood δ^{15}N. Marine avian colonies have been more successful locations for identification of a detectable impact of fecal N, but it is unclear what more can be done in this regard, unless the focus shifts to longer-term reconstructions of colony size and inhabitance. Researchers performing marine input work using other exogenous animal-derived N sources would benefit from first estimating the potential signal strength of their animal-derived N source, akin to the tracer analysis performed by Drake et al (2011). Too small of a signal potential may undermine a study design, and researchers may be better suited by investing their resources elsewhere.

(8) Attribution of temporal changes in wood δ^{15}N to pollution or anthropogenic sources of N would be strengthened by inclusion of both a local measure of N deposition amount and the δ^{15}N signature of the deposited N. Without these local measures, attributing changes in wood δ^{15}N to N deposition signatures is presumptive at best. Broad regional trends, or general analyses of emission sources are not detailed enough for such comparisons, and assessment of N loads in close proximity to wood sampling locations must be incorporated into analyses of pollution impacts. The incredibly close proximity required for point source pollutants to impact wood δ^{15}N trends (Saurer et al. 2004), and the high variability of the N isotopic values measured in precipitation [δ^{15}N-nitrate ranges from -8.1 to +3.2 ‰ in northeastern North America, (Elliott et al. 2007)] indicate that other hypotheses in addition to pollution should be considered to explain wood δ^{15}N trends.

Conclusion

There is enormous potential for the field of wood δ^{15}N analysis to provide retrospective records of terrestrial nitrogen cycling that contribute to an assessment of current global changes, specifically the impact of increased Nr on forested ecosystems. But first, individual wood δ^{15}N studies need to move towards a more cohesive structure, to more easily enable comparison of results across study types and eventually to enable meta-analyses of continental and global datasets. We hope that incorporating minor changes in study design, data interpretation, and data reporting will serve to advance the utility of wood δ^{15}N studies, and strengthen our knowledge of ecosystem N cycling in current and past ecosystems.

Acknowledgements

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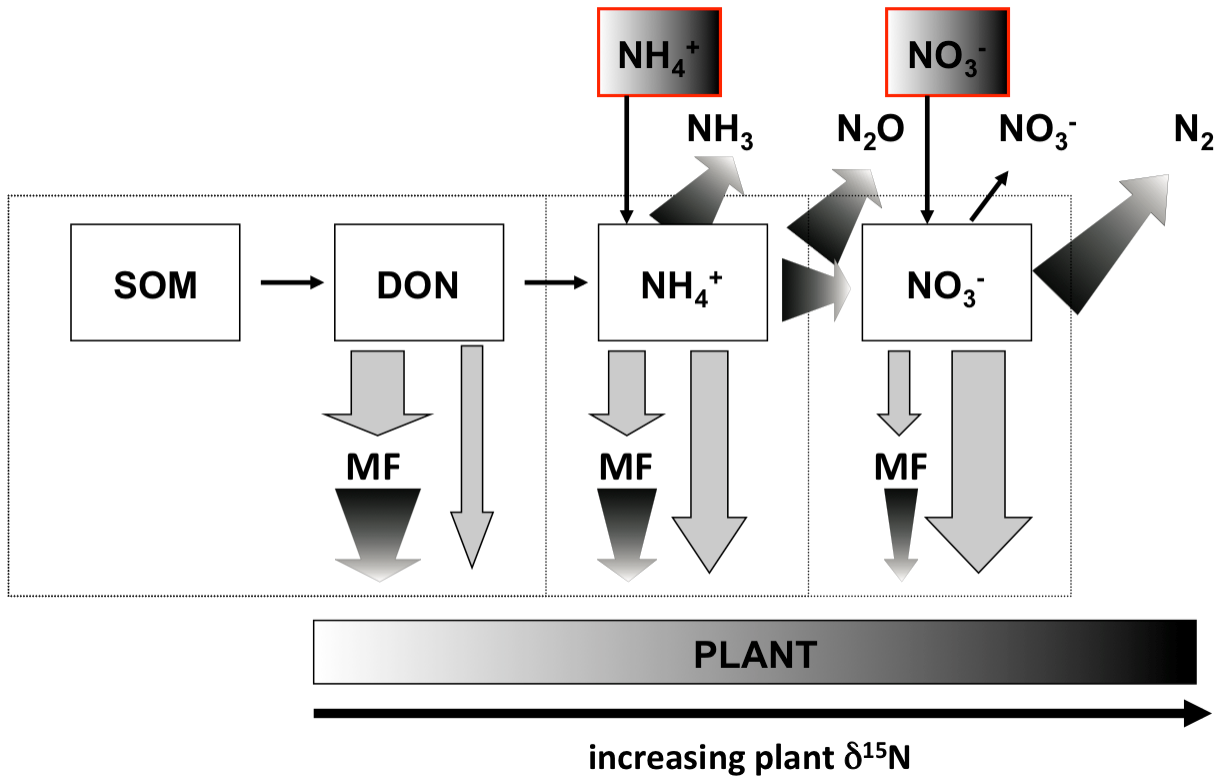science by individual researchers. Global Change Biology 18: 2102-2110.

Fig 1 The terrestrial N cycle is reflected in stable N isotopes of an ecosystem, with the integrated effects of isotopic fractionation affecting $\delta^{15}N$ values in wood. The major fractionating pathways are indicated by shading from dark (higher values or enrichment of ^{15}N) to light (lower values or depletion of ^{15}N). N cycles with high rates of gaseous loss lead to a progressive enrichment in $\delta^{15}N$ of soil available nitrogen; thus over time, plant $\delta^{15}N$ can be used as an indicator of nitrogen availability. The width of each arrow indicates the relative contribution of mycorrhizal transfers (MF), direct root uptake, and potentially atmospheric deposition to total plant N. SOM= soil organic matter, DON= dissolved organic nitrogen, NH_4^+= ammonium and NO_3^-= nitrate. Boxes outlined in red are deposited from the atmosphere, possibly from anthropogenic sources. Note that pool sizes and fluxes are likely to be highly variable among global ecosystems, and accurate N budgets at a site can help interpret wood $\delta^{15}N$ trajectories. Modified from (Chapin et al. 2003).
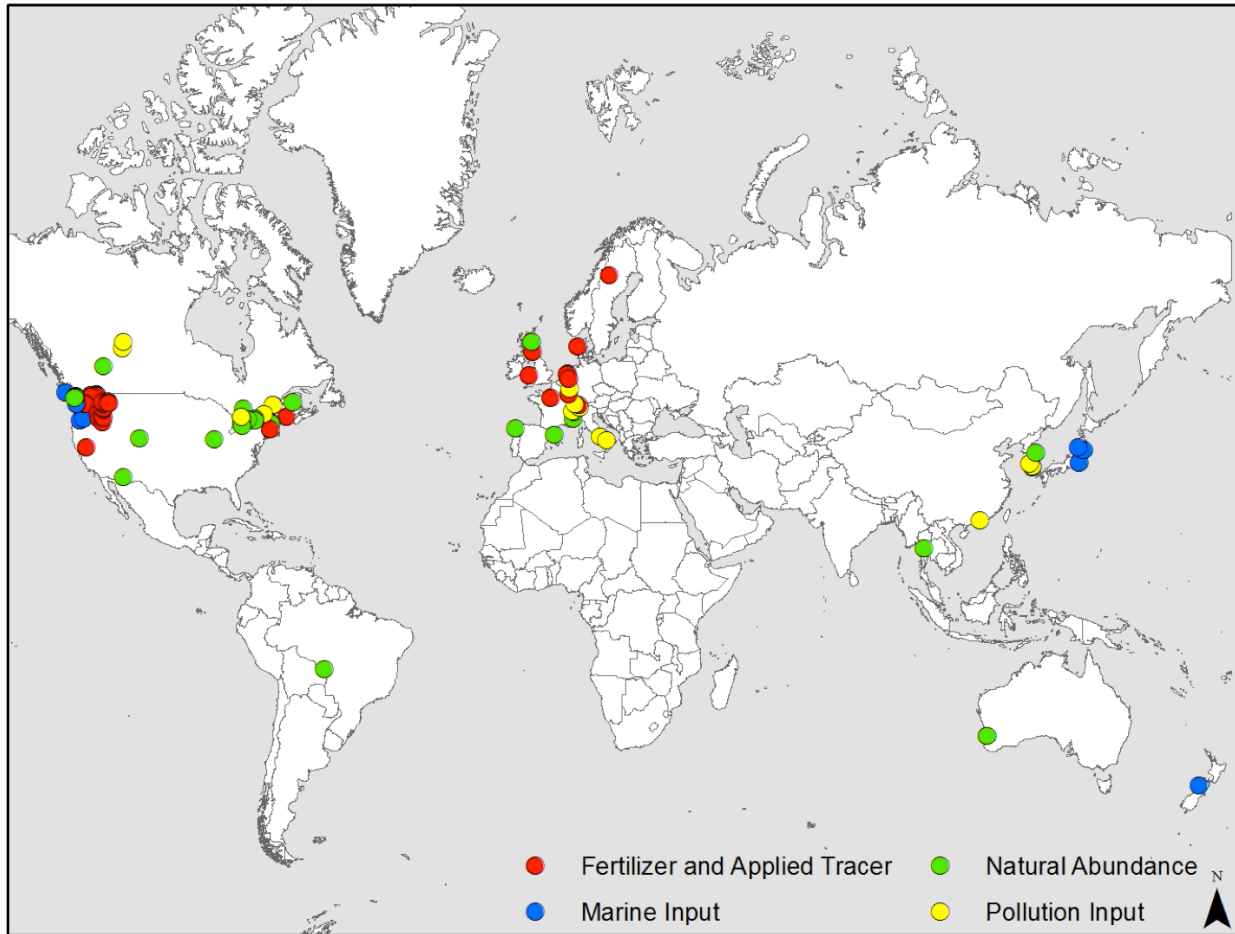
Fig 2 A global map of tree-ring nitrogen isotope studies published from 1995 to 2013. Green points indicate natural abundance studies (n=15), Red points indicate fertilizer and applied tracer studies (n=15), yellow points indicate pollution input studies (n=12) and blue points indicate marine input studies (n=6). An interactive version of this map is available through ArcGIS Explorer Online at *http://tinyurl.com/ksnhadj*
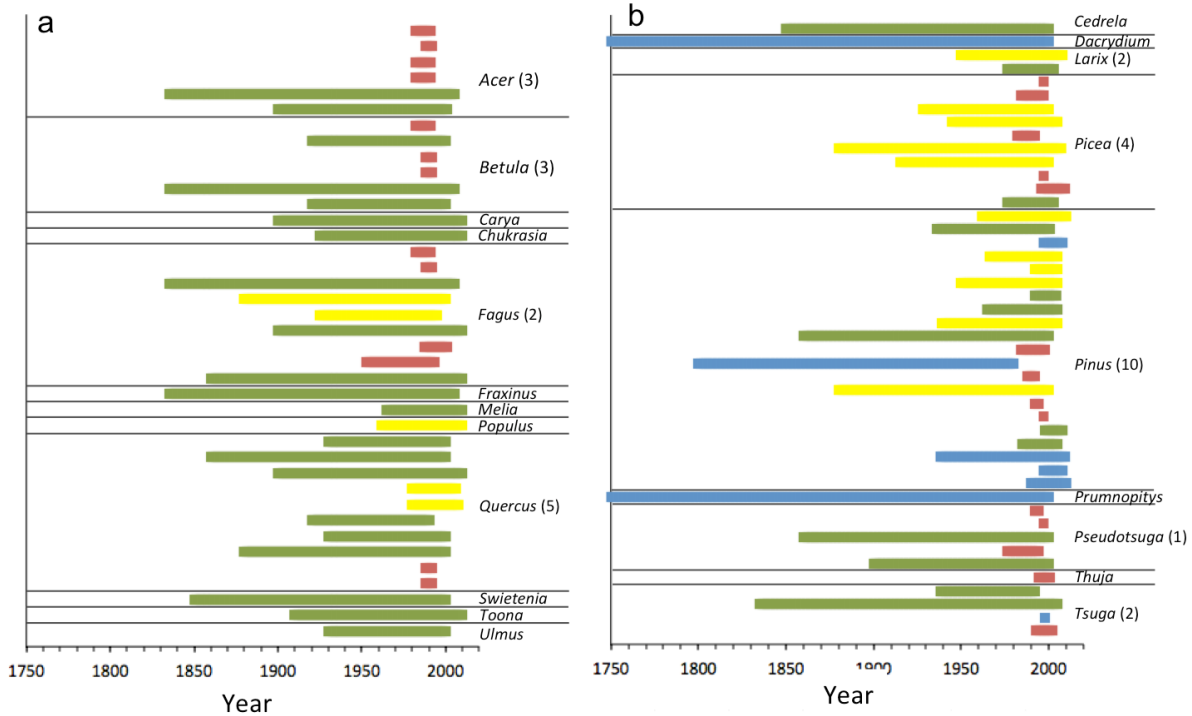
Fig 3 Chronology length of $\delta^{15}N$ measurements of tree genera studied for a) hardwoods and b) softwoods. Each line represents the time span covered by a single study. Numbers in parentheses after genus name indicate number of species studied within that genus. Green bars represent natural abundance studies, red bars represent fertilizer and applied tracer studies, yellow bars represent pollution input studies, and blue bars represent marine input studies.
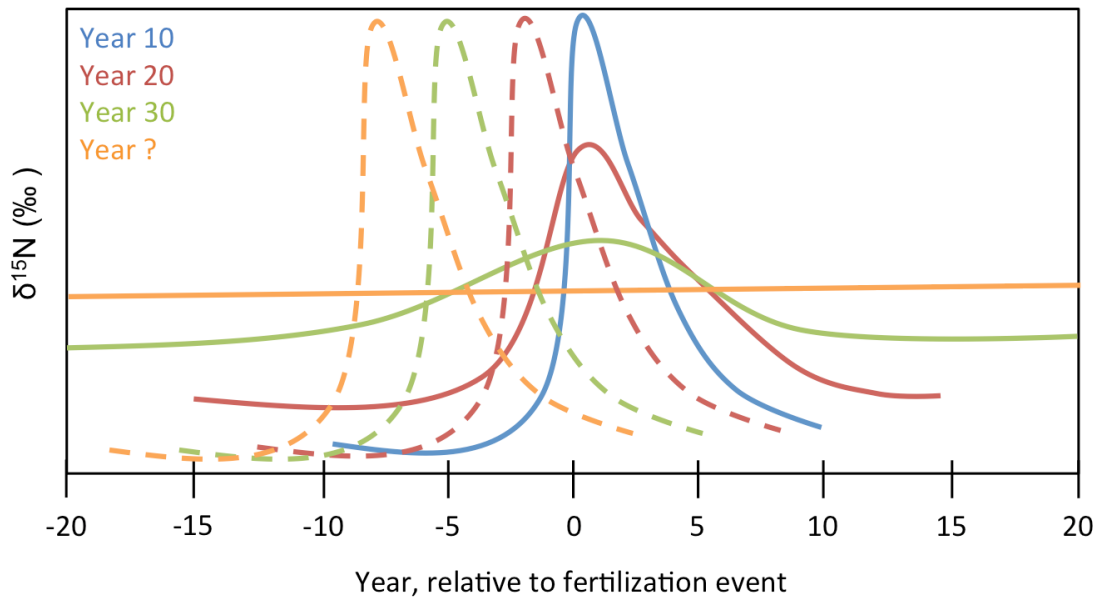
Fig 4 Conceptual diagram describing hypothetical changes in wood $\delta^{15}N$ through time due to translocation of applied tracer ^{15}N. As time passes, translocation of N might reduce the height of the initial peak such that sampling at longer time periods following the fertilization event might produce shorter and broader peaks and perhaps eventually a chronology of anomalously high $\delta^{15}N$ with no discernible peak (solid lines). Additionally, the peak may shift in location throughout the chronology (dashed lines), though still retaining the initial peak. Short-term peaks have been documented in Hart & Classen 2003 and Elhani *et al* 2003. It is as yet unclear how the peak might be altered under continued translocation throughout the life of the tree.
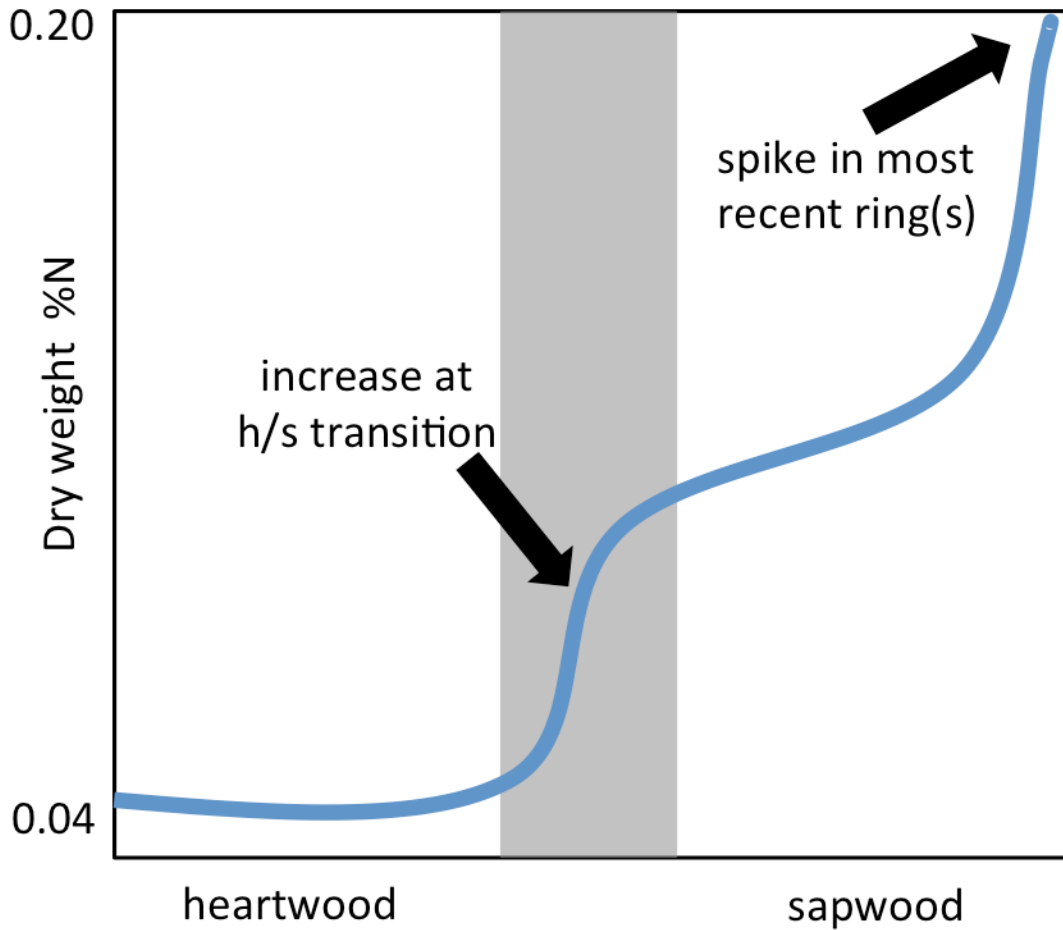
Fig 5 Summary diagram of empirical studies describing the general trend in [N] of tree rings from pith (left) to bark (right). This pattern seems to occur regardless of the nutrient history of the tree or current N status of the terrestrial ecosystem. Key changes are marked with arrows. Values on the y-axis represent the minimum and maximum [N] values reported in the literature (Poulson *et al* 1995, Choi *et al* 2007)

Table 1. Minimum acceptable and best practices for data reporting of wood $\delta^{15}N$ studies.

Data Type	Category	Minimum Acceptable	Best Practice	Includable in NCDC submission
Geospatial location of sampled trees	Metadata	X	X	X
Full species name of sampled trees	Data	X	X	X
Data manipulations used (detrending, standardization)	Metadata	X	X	X
Years included in sample	Data	X	X	X
Age assigned to sample	Data	X	X	X
Raw $\delta^{15}N$ values	Data	X	X	X
Raw [N] values	Data		X	X
Heartwood/Sapwood transition	Metadata		X	
Ring widths	Data		X	X

Table 2. Details of extraction methods used in wood $\delta^{15}N$ studies. For each method, a protocol, citations utilizing this protocol, and general findings of impacts are provided. References are Sheppard & Thompson 2000, Elhani et al. 2003, Hart & Classen 2003, Bukata & Kyser 2005, Elhani et al. 2005, Kwak et al. 2009, Hietz et al. 2010, Doucet et al. 2011, Drake et al. 2011, Lopez et al. 2011.

Method	Technique	Citations	Determination
Sheppard/Thompson	Step 1: Soxhlet extraction in 1:1 toluene/ethanol (short: 4 hours, long: 24 hours)	Sheppard & Thompson 2000	Reduced total [N] and variability in [N], long technique confirmed but did not improve upon short technique
	Step 2: Soxhlet extraction in 100% ethanol (short: 4 hours, long: 24 hours)	Elhani et al. 2003	Improved inter-annual resolution but did not remove all mobile N
	Step 3: Soxhlet extraction in distilled water (short: 1 hour, long: 24 hours)	Elhani et al. 2005	Enhanced differences in $\delta^{15}N$ between control and treated trees
		Kwak et al. 2009	More N extracted from sapwood, no change in

			temporal [N] trend
		Hietz et al. 2010	More N extracted from heartwood, no significant change in [N]
Holocellulose extraction	Step 1: Soxhlet etraction in 2:1 toluene/ethanol (16-18 hours) Step 2: Soxhlet extraction in 100% ethanol (16-18 hours) Step 3: Boil in deionized water (6 hours) Step 4: Soak in heated sodium chlorite and acetic acid (2 days)	Hart & Classen 2003	Stronger and more variable effect of extraction on N-labeled trees, no consistent effect on control trees
Water Bath	Soak in room temperature deionized water (3 days)	Bukata & Kyser 2005	No significant effect on $\delta^{15}N$ Extraction not necessary on non-resinous species
Altered Sheppard/Thompson	Step 1: Soxhlet extraction in 1:1 benzene/methanol (short: 12 hours, long: 48 hours) Step 2: Acetone (short: 1 hour ultrasonic bath, long: 48 hours Soxhlet extraction) Step 3: Boil in deionized water (1 hour)	Doucet et al. 2011	No significant change in [N] or $\delta^{15}N$ values or temporal trends
Sterilization	Step 1: Autoclaved samples in deionized water (1 hour) Step 2: Microwaved on high power (2 minutes) Step 3: Soaked in ~3% heated hydrogen peroxide (overnight) Step 4: Soaked in deionized water (10 hours)	Drake et al. 2011	Extraction increased $\delta^{15}N$ at one site, decreased $\delta^{15}N$ at other Extracted compounds exhibit $\delta^{15}N$ values ~18 per mil lower than remaining wood tissue
Simplified Sheppard/Thompson	Soak in 1:1 toluene/ethanol with sonic waves (overnight)	Lopez et al. 2011	Stronger impact of extraction under high-N conditions