

# Chapter 12

## Nitrogen Isotopes in Tree Rings—Challenges and Prospects



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**Abstract** Nutritive, but detrimental if at high levels, several nitrogen (N) forms involved in air and soil biogeochemical reactions constitute the N load trees assimilate. Although a large body of literature describes series of tree-ring N isotopes ( $\delta^{15}\text{N}$ ) as archival systems for environmental changes, several questions relative to the isotopic integrity and reproducibility of trends still linger in the dendroisotopist community. This chapter reviews the fundamentals of forest N cycling and examines trees as N receptors in their very position, at the interface between the atmosphere and pedosphere. The related scrutiny of intrinsic and extrinsic mechanisms regulating isotopic changes also underlines flaws and forces of tree-ring  $\delta^{15}\text{N}$  series as environmental indicators.

### 12.1 Introduction

Key nutrient for trees, but forming reactive molecules potentially detrimental to forest ecosystems (e.g., Etzold et al. 2020), N constitutes a central object of research in terrestrial biogeochemistry. After several decades, the substantial body of literature on N in trees reflects the complexity of N cycling through trees, and how some intrinsic and extrinsic processes remain elusive. With anthropogenic emissions of reactive N ( $\text{N}_r$ ) rising globally and driving atmosphere-pedosphere exchanges that can perturb the external terrestrial N cycle, tree-ring  $\delta^{15}\text{N}$  series may record past changes in forest-N cycling.

Studies of long tree-ring  $\delta^{15}\text{N}$  series are rare, largely because ring wood includes very low amounts of N relative to carbon, evidently making tree rings difficult for

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isotopic determination. Additionally, N translocates between trunk rings, dampening environmental isotopic effects in time series. Nevertheless, several studies report  $\delta^{15}\text{N}$  trends interpreted in relations to changes in soil and air conditions.

How does N assimilation in non  $\text{N}_2$ -fixing trees operate? Do trees react to changes in air and soil  $\text{N}_r$  contents? Can tree-ring  $\delta^{15}\text{N}$  series help understand environmental changes? The purpose of this review primarily consists in scrutinizing the current understanding of mechanisms responsible for determining  $\delta^{15}\text{N}$  values in tree rings, appraising the type of information  $\delta^{15}\text{N}$  series can provide, and synthesizing the knowledge gaps of this research domain.

## 12.2 Sample Preparation and Analytical Procedures

The habitual mechanical separation of tree rings from stem samples using fine blades or microtome at the sought time resolution produces wood sub-samples for  $\delta^{15}\text{N}$  analysis. Treating these sub-samples prior to their isotopic analysis generates a debate regarding the utility of removing their mobile N (resins) to prevent producing false trends. But recent investigations suggest this type of pre-treatment does not modify significantly the final  $\delta^{15}\text{N}$  values (Elhani et al. 2005; Bukata and Kyser 2007; Couto-Vázquez and González-Prieto 2010; Caceres et al. 2011; Doucet et al. 2011; Tomlinson et al. 2014). Another observation arguing against pre-treatment is that samples from several species, for instance *Pinus ponderosa*, *Fagus grandifolia* and *Picea rubens*, show no change of concentrations after resin removal (Hart and Classen 2003; Doucet et al. 2011).

On another note, regardless of pre-treating wood samples or not, several studies have clearly shown trends of higher N concentrations in rings (and coniferous leaves) grown during sampling years, relative to concentrations in previous years. The general pattern forms an increasing trend from the heartwood-sapwood transition to the most recent ring; a physiological effect typical of N translocation. In addition, tree-ring N concentrations show poor inter-tree and inter-species coherence. These observations make N concentrations in tree rings (and dated coniferous leaves) useless in environmental research (Hart and Classen 2003; Saurer et al. 2004; Savard et al. 2009; Gerhart and McLaughlan 2014). However, this inter-ring N mobility does not seem to affect the final tree-ring  $\delta^{15}\text{N}$  values (Doucet et al. 2011).

For isotopic analysis, wood samples wrapped in tin capsules drop automatically from a carousel into an elemental analyser (EA) in continuous flow (CF) with an isotope ratio mass spectrometer (IRMS). The analytical procedure involves combustion in a reaction tube producing  $\text{N}_2\text{O}$ , followed by a reduction to  $\text{N}_2$ , which produces the analyses calibrated relative to air  $\text{N}_2$  (set at 0‰). Tree-ring wood harbors very low concentrations relative to roots or leaves (Scarascia-Mugnozza et al. 2000), and high C/N ratios, making its isotopic analysis difficult. For that reason, the EA-CF IRMS system for  $\delta^{15}\text{N}$  analysis needs close monitoring for performing complete combustion to prevent  $\text{CO}^+$  derived interferences at masses 28 and 29. A CO trap installed between ovens and GC columns helps for that step. The low N concentrations in wood

make internal standards a requirement to avoid poor analytical accuracy from low peak to background ratios (Couto-Vázquez and González-Prieto 2010). Inserting several internal wood standards in sample batches allows monitoring the external precision and accuracy of the complete laboratory procedure. Whole wood materials from three species of trees recently proposed as references may also support this essential task (Qi et al. 2016), although the  $\delta^{15}\text{N}$  range they cover is narrower ( $-2.4$  to  $+1.8\text{‰}$ ) than the natural extent in tree rings (generally between  $-10$  and  $+5\text{‰}$ ).

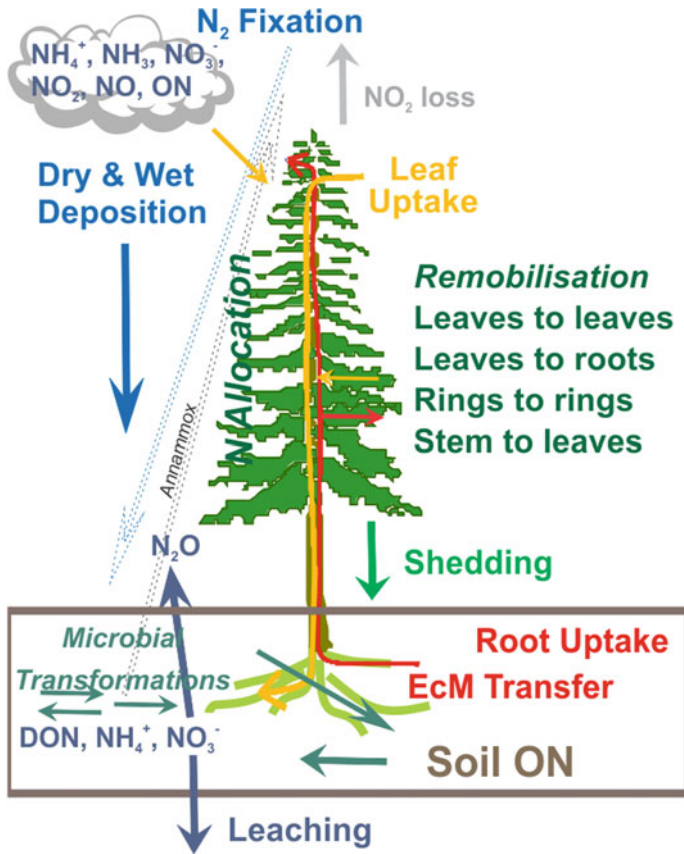
## 12.3 Assimilation, Storage and Fractionation of Nitrogen by Trees

Numerous tree-ring studies dealing with natural  $\delta^{15}\text{N}$  values or  $^{15}\text{N}$ -labelled N assume that uptake of soil inorganic N dominates the N assimilated in stems of non  $\text{N}_2$ -fixing trees. However, other means such as soil organic N assimilation and foliar uptake of various atmospheric N forms may significantly contribute to the N loads commonly characterized for  $\delta^{15}\text{N}$  values (Fig. 12.1). This section discusses the knowledge gains from controlled experiments, studies under natural conditions, recent developments in understanding the ultimate source and pathways of N to tree rings, and the role of N remobilization in determining the tree-ring  $\delta^{15}\text{N}$  values.

### 12.3.1 Nitrogen through Foliage

Many studies reveal that soil fertilization has direct impacts on foliar N characteristics, however, leaves also assimilate N (e.g., Gebauer and Schulze 1991; Arain et al. 2006; Pardo et al. 2007; Vizoso et al. 2008; Balster et al. 2009; Averill and Finzi 2011). Similarly, articles specifically addressing canopy functions report operational foliar uptake from air for all atmospheric N forms (Garten and Hanson 1990; Rennenberg and Gessler 1999; Krupa 2003; Sparks et al. 2003; Vallano and Sparks 2007; Chaparro-Suarez et al. 2011). In other words, it is widely accepted now that the foliar N loads come from soil as well as from air (e.g., Vallano and Sparks 2013). For its nutritive functions, leaf N plays a crucial role in enhancing activities of Rubisco, the proteins of photosynthesis (Warren et al. 2003; Wright et al. 2004). However, higher atmospheric N availability does not always translate into higher growth rates of stems. The crucial point for the present chapter lies with estimating the atmospheric foliar N contribution to the loads in stems of deciduous and coniferous trees, as atmospheric N transferred from leaves to stems may have a direct impact on the tree-ring  $\delta^{15}\text{N}$  series.

Atmospheric  $\text{N}_r$ -forms include N in ammonia gases ( $\text{NH}_4^+$ ,  $\text{NH}_3$ ), oxides ( $\text{NO}_3^-$ ,  $\text{NO}_2$ ,  $\text{NO}$ ), nitric acid ( $\text{HNO}_3$ ), and organic compounds (amino acids, peroxyacetyl nitrate PAN). These N-forms get to ground through wet scavenging or dry deposition



**Fig. 12.1** Representation of the forest nitrogen cycle. Processes influencing the bioavailability of N forms taken up by boreal and temperate trees are included;  $\text{NO}_2$  loss is significant mostly in wetlands and tropical settings; the tropical cycle would include  $\text{N}_2$  fixation by trees (not shown). EcM stand for ectomycorrhiza (Sect. 12.3.2)

upon contact with surfaces such as leaves. The N forms enter leaves either as wet or dry (gaseous) phases through stomata, although the liquid phases appear to pass in the foliar system more readily (Rennenberg and Gessler 1999; Harrison et al. 2000b; Krupa 2003; Choi et al. 2005; Gerhart and McLauchlan 2014). A series of enzymatic reactions transform  $\text{NH}_4^+$  and  $\text{NO}_3^-$  into amino acids, which generally enriches the reactants and depletes the products in  $^{15}\text{N}$  (e.g., Rennenberg and Gessler 1999). Once incorporated in organic compounds within leaves, N shortly resides in active and non-active parts (Millard and Grelet 2010). Experiments using  $^{15}\text{N}$ -labelled N show that the remobilized N can reach down to the root systems (Macklon et al. 1996; Rennenberg and Gessler 1999; Bazot et al. 2016).

Studies rarely quantify stem N originating from foliar uptake. In one known experimental example, the estimated proportions of N from previous-year needles

exported to support the growth of shoots vary between 10 and 37% in 5 year-old or younger coniferous trees (Millard and Grelet 2010). Otherwise, in 30-year-old spruce trees, between 8 and 22% of the annual N demands come from leaves, the range depending on the N forms selected for experiments (Harrison et al. 2000a, and references therein). Also, natural abundance of  $^{15}\text{N}$  has helped estimating foliar assimilation at 10% in 10- to 20 year-old Norway spruce trees, given that the signal of car exhaust, the single local source of anthropogenic  $\text{NO}_2$  emissions, was known to strongly deviate from the natural N sources (Ammann et al. 1999). However, in general, the precise quantification of anthropogenic N in the canopy constitutes a complex task because the isotopic signals of N in air can significantly change in space and time, and an array of emitters show overlapping  $\delta^{15}\text{N}$  ranges (e.g., Savard et al. 2017).

In deciduous specimens, the proportion of canopy N uptake used up for annual wood production appears to vary between <5 to >40% (Harrison et al. 2000b). In the case of young poplars exposed to  $\text{NO}_2$ -enriched air with low  $\delta^{15}\text{N}$  values, and grown on high and low N-supplied soils, the calculated foliar contributions were 14 and 18% of the total amount of plant N, respectively, based on  $\delta^{15}\text{N}$  measurements of plant material and the known isotopic signal of  $\text{NO}_2$  (Siegwolf et al. 2001). In another example, with labelled fertilizers applied at both the foliage and root levels of oak trees, soil N and internal storage contributed 60 and 40%, respectively, to the N of spring leaves (Bazot et al. 2016). Whereas the total autumn root N reserves included 73% from leaves and 27% from soils. At the broad scale, modeling studies reported the canopy to contribute between 3 and 16% of the total N demands for new growth in plants (Vallano and Sparks 2007, and references therein). Thus, on one hand, N in leaves comes partly from soils, and several studies clearly demonstrated partial remobilization of this N. On the other hand, the estimated contributions from leaves to the demands of trees may be more variable than the range covered in the literature because they largely depend on the atmospheric concentrations and the involved N-forms, the studied tree species, and the methodology selected for quantifying the foliar uptake/contribution.

Although trees acquire atmospheric N directly through leaves and without intermediate transformation steps as through soils before root uptake, the overall influence foliar uptake has on the tree-ring N loads is difficult to determine. To our knowledge, research efforts never estimated its contribution to stem N loads of mature trees. Accordingly, the remaining key questions regarding foliar uptake does not relate to its assessment but to the magnitude of its contribution in determining the final tree-ring  $\delta^{15}\text{N}$  values.

## 12.3.2 From Soils through Roots to the Stems

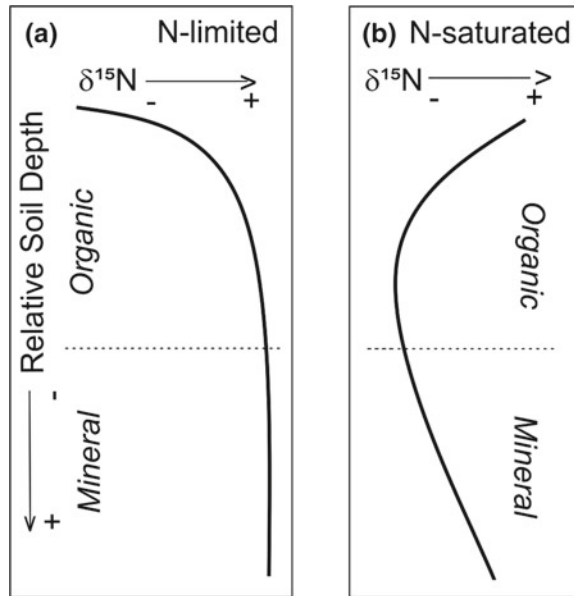
### 12.3.2.1 Soil Nitrogen Species and Processes

Dinitrogen-fixing microbes and forest organic matter represent the ultimate sources of N in soils of non-disturbed forests (Fig. 12.1). Geological N from rocks and minerals can provide a background influencing the forest N cycling and the overall  $\delta^{15}\text{N}$  values of organic soils, particularly if developed over clay-rich mineral horizons or sedimentary rocks, which generally have high  $\delta^{15}\text{N}$  values (Holloway and Dahlgren 2002; Craine et al. 2015; Houlton et al. 2018). Variability in the distribution of geological N contributes to the heterogeneity of soil N properties. Several studies also report evidence for microbial communities (fungi and bacteria) involved in mineral weathering (e.g., Courty et al. 2010).

Even though the absolute amount of N in soils is large, the dominant proportion of N is immobilized (carbon bound) in organic matter (Knicker 2004; Näsholm et al. 2009), with a small part of this matter available for nutrition (labile N or dissolved organic N—DON). The main N-rich constituents of DON, amino acids, derive from rapid hydrolysis of soil proteins (Näsholm et al. 2009). Furthermore, the soil inorganic ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) parts, largely derived from organic matter constitute only about 1% of the total soil N (Kendall et al. 2007). Forest N demands generally exceed the inputs in bioavailable N forms, limiting the net productivity in most of the boreal and temperate forests. As a consequence, trees compete for soil  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and DON. Anthropogenic N emissions can add to the regional N loads by wet or dry deposition, and enter the series of transformations leading to the bioavailable N pool mined by tree roots (Fig. 12.1).

The main transformation processes affecting the concentration and  $\delta^{15}\text{N}$  values of inorganic and organic bioavailable N in soils consist in fixation, immobilization, ammonification (mineralization), volatilization, nitrification and denitrification (Hopkins et al. 1998), and the rates of these N transformations vary seasonally and regionally (Handley et al. 1998). Whereas fixation and ammonification generally create minor isotopic N fractionation, volatilization of  $\text{NH}_4^+$ , nitrification and denitrification tend to significantly increase the  $^{15}\text{N}$  content in the reacting substrates and decrease it in the products (Högberg 1997; Pardo and Nadelhoffer 2010; Hobbie and Högberg 2012). Consequence to the interplaying N transformation processes, nitrate, ammonium and DON generally show  $\delta^{15}\text{N}$  values in increasing order. In addition, in N-limited forests,  $\delta^{15}\text{N}$  values of bulk N tend to increase with sample depth, and its concentration, to decrease (Fig. 12.2a). The general explanations for this pattern are that low nitrification rates and leaching from top horizons depletes the components of the organic horizons in  $^{15}\text{N}$ . Shedding of leaves depleted in  $^{15}\text{N}$  relative to soil and preferential uptake of  $^{15}\text{N}$  by fungi associated to roots (see Sect. 12.3.2.2) may accentuate this pattern (Hobbie and Colpaert 2002; Compton et al. 2007; Högberg et al. 2011). However, in less N-limited forests, the top horizons may show high  $\delta^{15}\text{N}$  values in soil N-species due to increased rates of nitrification in the N- and organic-rich horizons (Fig. 12.2b; Högberg 1997; Mayor et al. 2012; Shi et al. 2014).

**Fig. 12.2** The general  $\delta^{15}\text{N}$  trends of soil bulk N as a function of sample depth for N-limited **a** and N-saturated **b** forests (see text)



Hence, the overall N status of the forest, the proportions of the various soil N forms trees use up, and the depth of root N uptake from the soil all have direct influence on the final  $\delta^{15}\text{N}$  values of tree tissues. A key point to note here is that isotopic studies rarely characterize root (or tree-ring) samples along with individual bioavailable soil N forms, even though this combination would greatly help determining fractionation steps before N uptake by trees.

### 12.3.2.2 Direct and Ectomycorrhizal Root Uptake

Trees can use up inorganic N forms and DON directly through their roots (Näsholm et al. 2009; Averill and Finzi 2011). This direct uptake by physical transport shows no evidence of fractionation; N isotopic fractionation occurs during assimilation processes involving enzymatic functions (Handley et al. 1998; Pardo et al. 2013). Alternatively, trees can gain N (and other nutrients) while providing C, through symbiotic associations with fungi (ectomycorrhiza EcM; e.g., Näsholm et al. 2009; Courty et al. 2010; Lilleskov et al. 2019). It is well accepted that EcM generally show higher  $\delta^{15}\text{N}$  values relative to N sources in soils, and to roots and stems of trees. In the process of N uptake, they preferentially incorporate the heavy  $^{15}\text{N}$  during the production of their tissues, and provide light N to their hosts (Gebauer and Taylor 1999; Hobbie and Högberg 2012). The extent of this biogenic fractionation and thus the isotopic values of fungi vary widely (Trudell et al. 2004; Mayor et al. 2009; Hobbie and Högberg 2012), inasmuch as different EcM communities may efficiently assimilate specific soil N-compounds. Also, it is established that EcM communities



change in structure and abundance under varying soil chemistry (pH), N deposition, N transformation rates, and climatic conditions (Chalot et al. 1995; Wallander et al. 1997; Qian et al. 1998; Schulze et al. 2000; Lilleskov et al. 2002; Averill and Finzi 2011; Högberg et al. 2011; Kjoller et al. 2012; Kluber et al. 2012; van der Linde et al. 2018). The role of EcM in regulating  $\delta^{15}\text{N}$  values in plants during N assimilation in field conditions is illustrated by the measured  $\delta^{15}\text{N}$  patterns in Alaskan trees, EcM and soils (Hobbie et al. 1999). Modeling of these results indicates a net fractionation during the N transfer from EcM to trees.

Another example compares the foliar  $\delta^{15}\text{N}$  values of *Acer rubrum* seedlings from seven sites distributed along a gradient of atmospheric  $\text{NO}_2$ , with active EcM or manipulated absence of EcM in native soils of New York state (Vallano and Sparks 2013). The foliar  $\delta^{15}\text{N}$  results for seedlings devoid of EcM show no influence of increasing N, but for EcM seedlings, they correlate significantly with ambient  $\text{NO}_2$  levels, indicating the aid EcM provides to trees for N assimilation. These examples and the above observations make EcM causative agents for changes in tree-ring (and foliar)  $\delta^{15}\text{N}$  series, a key point for understanding the overall  $\delta^{15}\text{N}$  values of N transferred from soils to trees. However, the inventory of responses and functionalities of EcM communities under various environmental conditions, particularly the extent of their isotopic fractionation and implication during N uptake by roots, is not yet comprehensive. Research in that domain could help elucidate the causes of shifts in tree-ring  $\delta^{15}\text{N}$  series.

### 12.3.2.3 Preference of Trees for Soil N Species

Most trees absorb  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , but experiments conducted using fertilization with  $^{15}\text{N}$ -labelled N demonstrated that various species of trees show improved performances if grown with a specific soil N form (Kronzucker et al. 1997; Zhang et al. 2016; Miller and Hawkins 2007). The relative preferences for specific N forms mostly derive from the energy requirement for the production of proteins and the needed level of carboxylates (Arnold and van Diest 1991). Many species of deciduous trees take up  $\text{NO}_3^-$  preferentially (e.g., *Quercus alba*, *Fagus grandifolia*), whereas it is well established that most coniferous trees lacking the enzyme nitrate reductase assimilate  $\text{NH}_4^+$  more favorably, up to 20 times more than  $\text{NO}_3^-$  (Kronzucker et al. 1997; Templer and Dawson 2004; Islam and Macdonald 2009). Other studies have addressed the question of assimilation of DON, and found that coniferous trees such as *Chamaecyparis obtusa* do not use this form of N-compound (Takebayashi et al. 2010), while *Pinus sylvestris* and *Picea abies* assimilate as much DON as  $\text{NH}_4^+$  if the soil contains similar amounts of each N form (Ohlund and Näsholm 2002). To explain long-term deviations between tree-ring  $\delta^{15}\text{N}$  series of various deciduous species, McLauchlan and Craine (2012) linked differences to N-form preferences. Given that soil N compounds undergo different transformation paths and carry distinct  $\delta^{15}\text{N}$  signals, diverse N preferences by trees growing at the same site or under similar conditions ought to generate distinct tree-ring  $\delta^{15}\text{N}$  trends over time.



### ***12.3.3 N Remobilization, Inter-ring Translocation and Fractionation Within Stems***

Many studies explain well the fractionation along the length of trees, from root to stems and leaves (Yoneyama et al. 1998; Gebauer et al. 2000; Evans 2001). Briefly, after assimilation of N-species by trees, enzymatic functions transform  $\text{NH}_4^+$  and  $\text{NO}_3^-$  into amino acids (Handley et al. 1998). As mentioned previously, these steps generally enrich the reactants in  $^{15}\text{N}$  and deplete the products (Yoneyama et al. 1998; Gebauer et al. 2000). Research efforts also indicated that deciduous trees store N in their bark and wood, whereas coniferous trees predominantly store N as photosynthetic proteins in their youngest needles. The remobilization of these amino acids is seasonal. During spring, deciduous trees transfer non-structural N compounds (arginine and asparagine) from twigs and coarse roots (and stems) to forming leaves (Bazot et al. 2013; Brereton et al. 2014). For instance, N proportion in twigs of oak trees decreases by 55% during that period. During summer, leaves are the dominant storage of N (>50% in June, compared to only 10% in stems; see also Sect. 12.3.1). During autumn, while leaves are shedding, storage begins in stems, coarse roots and twigs. For willow trees, the stems become a major N reserve (Brereton et al. 2014), a pool that new leaves will solicit later on.

During spring, coniferous trees transfer N stored in their youngest needles to support new growth of leaves and stems (Millard and Proe 1993; Bauer et al. 2000; Krupa 2003; Millard and Grelet 2010; Couto-Vázquez and González-Prieto 2014). Translocation generates fractionation and systematically decreases  $\delta^{15}\text{N}$  values in old needles relative to young needles (Gebauer and Schulze 1991; Couto-Vázquez and González-Prieto 2010). In contrast, there is no systematic difference between recent and old tree rings as mentioned in Sect. 12.2. The remobilization steps described above may largely explain why foliage and tree-ring  $\delta^{15}\text{N}$  trends in coniferous trees are different from broad-leave trees (Pardo et al. 2006; Gerhart and McLauchlan 2014; Tomlinson et al. 2015).

For further assessing the impact of N mobility in stems on growth ring  $\delta^{15}\text{N}$  values, various research groups investigated the distribution of  $^{15}\text{N}$  after fertilization or misting labelled-N compounds (e.g., Elhani et al. 2005; Tomlinson et al. 2014). In such studies, labelled N detected in rings predating and postdating the marking events, clearly indicate that rings include both C-bound and mobile N (not removable by sample pretreatments). However, in most cases, the  $^{15}\text{N}$  maximal contents always peak in rings of the marking years (Schleppi et al. 1999; Elhani et al. 2003, 2005; Tomlinson et al. 2014). These experiments indicate that the inter-ring translocation of N does not erase the record (direction and year of changes) of environmental events, but may minimize the extent of its isotopic impact.

## 12.4 Tree-Ring $\delta^{15}\text{N}$ Responses to Changing Conditions

### 12.4.1 *Physiological Changes*

Some studies suggested that physiological functions, for instance lignification, may modify the  $\delta^{15}\text{N}$  values of rings with age of Spanish *Pinus radiata*, and proposed further experimentation in order to assess the validity of the hypothesis (Couto-Vázquez and González-Prieto 2010). *Acer saccharum* and *Fagus grandifolia* trees investigated for assessing the importance of potentially changing root depth with age on the evolution of  $\delta^{15}\text{N}$  values in leaves (and tree rings by extension) show no significant changes with age, but significant  $\delta^{15}\text{N}$  differences between root, stems and leaves, and averages between the two species (Pardo et al. 2013). These results suggest fractionation during transport and assimilation of N, and physiological differences between species. Such a finding agrees with former studies of temperate trees reporting a general increasing  $\delta^{15}\text{N}$  trend along the height of trees (Kolb and Evans 2002; Couto-Vázquez and González-Prieto 2010), with differences existing between species.

### 12.4.2 *Regional and Global Climate*

Based on the concepts explored in the former sections, in theory climatic conditions can imprint the  $\delta^{15}\text{N}$  values transferred to tree rings. Namely, temperature and precipitation variations may modify the soil bioavailable N pools through changes in organic matter degradation, ammonification and nitrification rates, functions of EcM communities, and depth of drawing available soil water and N species (Savard et al. 2009; Courty et al. 2010; Durán et al. 2016). Such changes modify the overall isotopic signal of bioavailable N, which will reverberate in the  $\delta^{15}\text{N}$  values of trees. Indeed, several studies have linked foliar  $\delta^{15}\text{N}$  results from various species of trees with precipitation, showing either direct or inverse correlations depending on the amounts of precipitation considered (Pardo et al. 2006; and references therein). Likewise, in rain exclusion experiments (simulated droughts) deciduous trees clearly increased their foliar  $\delta^{15}\text{N}$  values due to a relative decrease in soil N availability (Ogaya and Peñuelas 2008). At large scales, plant foliar  $\delta^{15}\text{N}$  trends correlate inversely with mean annual precipitation, but directly with mean annual temperature (MAT) possibly due to higher soil N availability under moist and warm conditions (Craine et al. 2009; Dawes et al. 2017). Instead, inverse correlation of temperature with foliar  $\delta^{15}\text{N}$  values of *Populus balsamifera* may reflect changing dominance in soil N transformation pathways, from DON leaching (low MAT) to denitrification (high MAT; Elmore et al. 2017). In general, we must keep in mind that soil N availability derives from microbial activity, and thus hinges on temperature and soil water content. Depending on the habitat, microbial activity reaches an optimum at a specific range of soil temperature and water content: too much or too little water reduces or inhibits microbial activity. The

same is true for temperature. As such, N availability depends on soil temperature and water content, which ultimately leave their fingerprints on the  $\delta^{15}\text{N}$  values of soil N compounds.

If leaf  $\delta^{15}\text{N}$  values of a given time contain climatic information, tree-ring  $\delta^{15}\text{N}$  series should also record this information. This suggestion is supported by a few studies reporting significant statistical correlations between precipitation or temperature with  $\delta^{15}\text{N}$  series from *Fagus grandifolia*, *Pinus strobus*, *Pinus massioniana*, *Fagus sylvatica* and *Pinus radiata* (Savard et al. 2009; Sun et al. 2010; Härdtle et al. 2013; Couto-Vázquez and González-Prieto 2014). Causes for the climate-induced  $\delta^{15}\text{N}$  variations include modified ratios of soil  $\text{NH}_4^+/\text{NO}_3^-$ , and change in soil depths of root uptake.

Despite these expressions of climatic triggers for changes in tree-ring  $\delta^{15}\text{N}$  series, one has to consider the potential limitations when evaluating potential climatic effects. High frequency changes in climatic parameters may be impractical to resolve using  $\delta^{15}\text{N}$  values of annually sampled tree rings, as remobilization and translocation of N tend to minimize the isotopic responses (Sect. 12.3.3). Such attempt for quantitative climatic–isotopic correspondence at this resolution may fail. However, tree-ring  $\delta^{15}\text{N}$  series may record low-frequency climate variability. This proposition is supported by a recent investigation of  $\delta^{15}\text{N}$  series in six and ten *Picea glauca* trees from two Canadian sites (Savard et al. 2020). The results indicate that short-term variations (<7 years) show no inter-tree coherence, whereas middle- (7–15 years) to long-term (>15 years) isotopic changes show strong coherence, encouraging their use as an environmental indicator. One option that may deserve further testing is to pass running averages through long tree-ring  $\delta^{15}\text{N}$  series, and evaluate their correlations with similarly treated climatic series (Savard et al. 2009; similarly treated for global climatic changes, see Sect. 12.4.3—cause number 4). As can be seen, climatic tree-ring  $\delta^{15}\text{N}$  studies require further exploration considering that climatic effects may interplay with anthropogenic impacts, and that improved knowledge on that front may help deciphering intricate  $\delta^{15}\text{N}$  responses of trees to intrinsic and extrinsic triggers.

### 12.4.3 Anthropogenic Impacts

There are four main reasons why anthropogenic N emissions are expected to affect the  $\delta^{15}\text{N}$  values in rings of specific species of trees. (1) Shifts in signals in N forms assimilated by trees through addition of large anthropogenic N loads with isotopic ratios markedly different from natural N isotopic abundance. (2) Change in N availability of the forest ecosystem due to high anthropogenic N supply relative to demands, modifying the overall soil N isotopic contents. (3) Modifications of the overall soil microbial structure and related N dynamics having an impact on the signal of N assimilated by stems under moderate anthropogenic N deposition. (4) Global change (climate and  $\text{pCO}_2$ ) interplaying with one or a combination of the former causes.

In the first case, much of the N in trees derives from the inorganic soil N pool ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), which forms only a small portion of the total soil N, but that has  $\delta^{15}\text{N}$  signals that may vary with changes in environmental conditions, particularly with enhanced N deposition from anthropogenic emissions. After transition of N contaminants in the soil compartments, trees assimilate anthropogenic N through roots, or root N possibly combines in stems with anthropogenic N transiting through leaves. Key studies have invoked changes in the isotopic signals of assimilated N to account for shifts in tree-ring  $\delta^{15}\text{N}$  values (Saurer et al. 2004; Savard et al. 2009). However, determining the cause of changes in tree-ring  $\delta^{15}\text{N}$  series is possible only when N deposits chronically and in abundance, from a dominant source with  $\delta^{15}\text{N}$  values deviate significantly from soil N, and if the other potential causes for change do not blur this effect.

In the second case, increased N deposition in temperate and boreal forest ecosystems may cause acidification of soils, and nutrient nitrogen imbalances in trees (Aber et al. 1998; Högberg et al. 2007). The soil N status may pass from semi-closed to open, if a high N supply exceeds demands. Such a forest soil would see a high rate of  $^{15}\text{N}$ -poor nitrate loss through leaching, increasing the overall  $\delta^{15}\text{N}$  values of the residual pool (Fig. 12.2). The chronic exposure of a forest to such a rate of N input would generate a long-term  $\delta^{15}\text{N}$  increase in tree rings. In contrast, a decrease of anthropogenic N supply would generate a decrease in long-term tree-ring  $\delta^{15}\text{N}$  series in the recovering forests. This interpretation explains the declining  $\delta^{15}\text{N}$  series over 75 years in *Picea rubens* trees of the central Appalachians (Mathias and Thomas 2018).

In the third case, a combination of modified biogeochemical processes under low to moderate long-term exposure to anthropogenic N inputs alters the overall signal of soil N species prior to their assimilation by trees. On a theoretical basis, one can conceive that microbial communities in forest soils with very low N availability ( $<1$  kg/ha/y) will quickly adapt to enhanced input and chronic exposure to anthropogenic N. As mentioned in Sect. 12.3.2, in such conditions, existing EcM communities may thrive or shift in terms of diversity, and rates of bacterial N transformation may change. A study of four different deciduous species of trees in Indiana (USA), found long-term increasing and decreasing  $\delta^{15}\text{N}$  trends explained by species-specific preferences for inorganic N forms while nitrification increases (McLauchlan and Craine 2012).

In the fourth potential cause for  $\delta^{15}\text{N}$  changes in plant tissue, climatic conditions or rising  $\text{pCO}_2$  generate long-term changes in soil N processes and N availability. At a continental scale, centennial, standardized, 10 year resolution tree-ring  $\delta^{15}\text{N}$  series of temperate forests seem to evolve independently from anthropogenic N deposition in the USA (McLauchlan et al. 2017). The series instead may reflect changes in N transformation rates and EcM assimilation, and the declining N availability under rising  $\text{pCO}_2$ . Further along this line, at the global scale, rising atmospheric  $\text{CO}_2$  may have generated decreasing foliage and tree-ring  $\delta^{15}\text{N}$  trends through the last 150 years due to prolonged growing seasons, increased photosynthesis and overall enhanced plant-N demands, ultimately lowering the terrestrial N availability (Craine et al. 2019).

To summarize, tree-ring series may record changes in the forest N cycle or reflect successive N-cycling patterns, but the potential causes for these changes are complex and rigorous interpretations require excellent knowledge of the setting in which trees are growing. Given the attenuated nature of the isotopic changes due to intra-tree N remobilization and intricate enzymatic fractionations along the assimilation path within trees, attempts to quantify anthropogenic impacts on the forest N cycle using tree rings or leaves could be scant. On a more positive note, recognizing and dating perturbations of the forest N cycle using tree-ring  $\delta^{15}\text{N}$  series appears achievable.

#### **12.4.4 Other Applications**

The literature also documents several applications other than the ones presented in Sects. 12.4.1, 12.4.2 and 12.4.3. For tropical and  $\text{N}_2$ -fixing trees, the reader can consult Craine et al. (2015). Boreal wetland and tropical trees emit  $\text{N}_2\text{O}$  (Rusch and Rennenberg 1998; Welch et al. 2019), a process significant for understanding the global N cycling, for which  $\delta^{15}\text{N}$  results in stems and emitted gasses may turn useful. Moreover, tree-ring  $\delta^{15}\text{N}$  applications exist on effects of wild fires (Cook 2002; Beghin et al. 2011; Hyodo et al. 2012), clear cutting (Pardo et al. 2002; Bukata and Kyser 2005), and bird nesting (Mizota 2009; Holdaway et al. 2010; Larry et al. 2010). Understandably, researchers should select sites devoid of these perturbations in order to achieve meaningful results and refine our understanding of climatic and anthropogenic influences on tree-ring  $\delta^{15}\text{N}$  series.

### **12.5 Knowledge Gaps and Future Directions**

Studies dealing with the assimilation of N through either leaves or roots have mostly operated independently, with root assimilation experiments disregarding the potential foliar assimilation, and vice versa. As a consequence, the proportions of N in stems contributed from the foliar and rooting systems still need resolving even if these proportions are highly pertinent for relating tree-ring  $\delta^{15}\text{N}$  values to mechanistic processes and environmental changes.

As with ice cores, lakes sediments and skeletal corals reflecting complex and irrefutable anthropogenic impacts on the atmospheric, aquatic, and marine N cycling, tree-ring series likely represent another natural archive offering potential for unravelling impacts on forest N cycling. Although each archival system potentially offers many applications, in all cases the interplaying mechanisms responsible for changes through time need to be further constrained. With trees, difficulties arise from the requirement to have an excellent understanding of soil conditions to interpret tree-ring trends adequately. Further research should address the knowledge gaps on the steps of fractionation of individual bioavailable N forms in the soil compartments. Similarly, the role of EcM should be explored as it might be effective or non-effective

during the transfer of N forms to roots under the broad ranges of existing soil conditions. Tree-ring studies seldom involve the investigation of the full spectrum of N transformations in the air–soil–tree continuum. However, such an interdisciplinary approach may solve several questions regarding the extrinsic controls on tree-ring  $\delta^{15}\text{N}$  changes, perhaps with the combination of running well-adapted models of soil N budgets.

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