

Can tree-ring $\delta^{15}\text{N}$ be used as a proxy for foliar $\delta^{15}\text{N}$ in European beech and Norway spruce?

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Abstract

Key message For long-term environmental investigations, tree-ring $\delta^{15}\text{N}$ values are inappropriate proxies for foliar $\delta^{15}\text{N}$ for both *Fagus sylvatica* and *Picea abies* under moderate N loads.

Abstract Currently it is unclear whether stable nitrogen isotope signals of tree-rings are related to those in foliage, and whether they can be used to infer tree responses to environmental changes. We studied foliar and tree-ring nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope ratios in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L.) from six long-term forest monitoring sites in Switzerland together with data on N deposition and soil N availability, as well as a drought response index over the last two decades. For both species, tree-ring $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were less negative compared to foliar $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, most likely due to recycling and reallocation of N within the tree and fractionation processes associated with the transport of sucrose and the formation of tree-rings, respectively. Temporal trends recorded in foliar $\delta^{15}\text{N}$ were not reflected in tree-ring $\delta^{15}\text{N}$, with much higher variations in tree-rings compared to foliage. Soil N availability and N deposition were partially able to explain changes in foliar

$\delta^{13}\text{C}$, while there were no significant correlations between environmental variables and either tree-ring or foliar $\delta^{15}\text{N}$. Our results suggest an uncoupling between the N isotopic composition of tree-rings and foliage. Consequently, tree-ring $\delta^{15}\text{N}$ values are inappropriate proxies of foliar $\delta^{15}\text{N}$ values under low-to-moderate N deposition loads. Furthermore, at such low levels of deposition, tree-ring $\delta^{15}\text{N}$ values are not recommended as archives of tree responses to soil C/N or bulk N deposition.

Keywords Tree-ring · Foliage · Nitrogen · Carbon · Isotope · Archive

Introduction

Forest ecosystems are experiencing changes in climate and other environmental conditions on both regional and global scales (de Vries et al. 2014; Settele et al. 2014). Atmospheric CO_2 concentrations, air temperature and precipitation are highly influential in controlling forest productivity, whilst forest stands themselves influence climate through the fixation and release of CO_2 or through climate cooling via evapotranspiration (Settele et al. 2014). At the same time, the increasing use of nitrogen (N) fertilizers, coupled with increased emissions of nitrogen oxides (NO_x) from fossil fuel combustion are causing large-scale changes in the global N cycle (Galloway et al. 2008). In N-limited forest ecosystems, increased N inputs can cause a short-term increase in tree growth (Solberg et al. 2009). In N-saturated ecosystems, however, further mineral N inputs (as ammonium or nitrate) will lead to soil acidification (de Vries et al. 2014), resulting in reduced tree productivity and even tree mortality (Aber et al. 1998; Wallace et al. 2007). Further effects of N saturation include

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decreased vegetation biodiversity and increased susceptibility to disease (Erisman et al. 2011; Gilliam 2006). While it is clear that forest ecosystems will undergo significant changes throughout the twenty-first century, it is difficult to quantify forest responses to predicted environmental changes.

The assessment of stable isotopic compositions in tree-rings and foliage is a valuable tool for assessing the influence of environmental changes on forest ecosystems (Dawson et al. 2002). Carbon and nitrogen stable isotopic compositions are determined by (1) isotopic composition of the respective source (2) climatic and environmental conditions, and (3) tree physiological and biochemical processes. As a result, they are often used within these three areas of research. There are a number of studies that have investigated the relationship between climate and C isotopic composition of both tree-rings and foliage (e.g. Di Matteo et al. 2014; Eilmann et al. 2010). A number of studies have also used a dual-isotope approach based on both carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope ratios of tree-rings and foliage to better understand the physiological processes that alter isotopic compositions (Saurer and Siegwolf 2007; Scheidegger et al. 2000). In addition, there have been a number of investigations that described the relationships between the isotopic composition in tree-rings and foliage for both C (Marshall et al. 2008) and O (Jäggi et al. 2003). The mechanistic processes that determine intra-tree variation of $\delta^{13}\text{C}$ have been particularly well documented and investigated (Cernusak et al. 2009). Foliar $\delta^{15}\text{N}$ values have also been used to assess site-specific environmental conditions, such as N emissions (Ammann et al. 1999), soil N (Garten 1993) and changes in the regional N cycle (Pardo et al. 2006). However, there are few studies that investigate tree-ring $\delta^{15}\text{N}$. This may be because N concentrations in tree-rings are low (relative to C in tree-rings and N in foliage) and related methodological difficulties (Savard 2010). Low tree-ring N concentrations also result in the movement of mobile N compounds between tree-rings (Tomlinson et al. 2014). These N recycling processes within the tree play an important role in meeting the N demand from sinks within, potentially masking N deposition signals and the impact of added N on tree-ring $\delta^{15}\text{N}$ values (Evans 2001; Kolb and Evans 2002). While some studies point out the potential of tree-ring $\delta^{15}\text{N}$ as a proxy for various forest N inputs (Savard 2010), the relationship between tree-ring and foliar $\delta^{15}\text{N}$ is still poorly understood. It is also not clear whether this relationship is species specific, and whether changes in climate and/or site-specific N conditions affect tree-ring and foliar N (and C) isotopic compositions in the same fashion. Furthermore, it is unclear whether the isotope ratios of tree-rings or of foliage better reflect site-specific environmental conditions such as water or N availability.

The assessment of the relationship between tree-ring and foliar $\delta^{15}\text{N}$ values would determine the potential use of the much longer chronologies of tree-ring $\delta^{15}\text{N}$ values as proxies for foliar $\delta^{15}\text{N}$ values in environmental investigations.

Long-term monitoring programmes are essential in both understanding and testing how forest ecosystems respond to environmental changes, particularly of climate and N availability. In this dual-isotope investigation, we combined chronologies of both tree-ring and foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L.) with environmental data collected from six long-term monitoring sites within Switzerland. Our objectives were to: (1) determine whether intra-tree $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ variations differ between *Fagus sylvatica* and *Picea abies*, (2) investigate differences in the inter-annual variability of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between foliage and tree-rings, and the influence of selected environmental variables on $\delta^{15}\text{N}$ tree isotopic composition, and (3) determine the potential of tree-ring $\delta^{15}\text{N}$ as a proxy for foliar $\delta^{15}\text{N}$.

Methods

Site description

Switzerland is one of approximately 30 European countries currently participating in the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (www.icp-forests.net). The 19 Swiss sites included in the programme are also part of the Swiss Long-Term Forest Ecosystem Research Programme (LWF) and the European Long-Term Ecosystem Research network (LTER-Europe) (Cherubini and Innes 2000; Schaub et al. 2011). These 19 sites are distributed across Switzerland, spanning a wide range of geographical/altitudinal gradients and cover various forest ecosystem types. Based on species distribution, the availability of archived foliar samples for both *Fagus sylvatica* and *Picea abies*, as well as the environmental data available (Table 1), six LWF sites (Alptal, Beatenberg, Bettlachstock, Lausanne, Othmarsingen, and Vordemwald) were selected for this study. All measurements were conducted in accordance with the ICP Forests Programme (ICP-Forests 2010).

Climate, nitrogen deposition and soil data

At each LWF site, there is one meteorological station within the forested site and another one in an open field nearby, within a radius of 2 km. In Alptal, meteorological data from the station Erlenhöhe, located in the hydrological research catchment Erlenbach (operated by WSL), were

Table 1 Main site characteristics, climate, deposition and soil (Walther et al. 2003) data from the six long-term forest monitoring sites of the LWF

Sites						
Site	Alptal	Beatenberg	Bettlachstock	Lausanne	Othmarsingen	Vordemwald
Abbreviation	ALP	BEA	BET	LAU	OTH	VOR
Main tree species	<i>Picea abies</i>	<i>Picea abies</i>	<i>Fagus sylvatica</i>	<i>Fagus sylvatica</i>	<i>Fagus sylvatica</i>	<i>Abies alba</i>
Forest type	Coniferous	Coniferous	Mixed broadleaved	Broad-leaved	Broad-leaved	Mixed coniferous
Region	Prealps	Prealps	Jura	Central Plateau	Central Plateau	Central plateau
Altitude a.s.l. (m)	1160	1511	1149	807	484	480
Orientation	NW	SW	S	NE	S	NW
Mean slope (%)	23	33	66	7	27	14
Mean annual temperature (°C)	5.3	4.7	6.0	8.6	8.6	8.4
Mean annual precipitation [mm]	2129	1725	1454	1062	1045	1106
Mean annual bulk N deposition (kg/ha/year)	12.0	7.4	7.0	9.7	8.0	9.7
Mean growing season (Apr–Oct) drought response index (mm)	−1044.8	−366.1	−179.0	81.5	−25.2	81.5
Soil type	Mollic Gleysol	Podzol	Rendzic Leptosol	Dystric Cambisol	Haplic Acrisol	Dystric Planosol
Soil C/N ratio	17	28	18	15	17	21
Rooting depth (cm)	40	55	>120	240	>190	60

used. Variables measured at these meteorological stations include precipitation (measured at 1.5 m height), air temperature (2 m height) and relative humidity (2 m height), global short-wave solar radiation (3 m height), and wind speed (4.6 m height). The values from the open field station were verified by comparing against corresponding values from the forest meteorological station and from the closest MeteoSwiss meteorological station (www.meteoswiss.ch). Non-plausible and missing values were replaced by values either from the forest meteorological LWF station or the MeteoSwiss station after applying regression analysis that was established from periods with plausible values. For precipitation, the values of the open field station were also checked against the values from deposition volume measurements and subsequently replaced or scaled as appropriate.

Bulk precipitation, from which bulk N deposition was determined, was continuously sampled using three replicated, custom-made funnel-type polyethylene collectors (replaced by bucket-type collectors during the winter). These collectors were positioned 1.5 m above ground in the open fields where the meteorological stations were situated. In Alptal, bulk precipitation measurements were carried out with only one funnel-type polyethylene collector, connected with a tube to a sample bottle placed beneath the soil surface. The content of each of these collectors was collected biweekly and brought to the lab where it was stored at 2 °C prior to chemical analyses. Bulk N deposition values were used in this investigation, as

total N deposition values were not available for all sites. However, similar temporal trends between bulk N deposition and total N deposition (where available) meant that we were able to make conclusions based on bulk N deposition measurements during our analyses. A detailed description of the sampling, N analysis, quality assurance and control procedures of atmospheric deposition calculations can be found in Thimonier et al. (2005) for LWF and Kloeti et al. (1989) for Alptal.

Soil matric potentials were measured using tensiometers at five different depths: 15, 30, 50, 80, and 130 cm, with eight replicates per depth and site, every 2 weeks at all sites (with the exception of Alptal) (Graf Pannatier et al. 2012). At Alptal, the ground water level was measured instead of soil matric potential because of high levels of precipitation, shallow soils and an impermeable underlayer. The C/N ratios of the forest floor (Oe and Oa layers) or, when these organic horizons were missing of the upper 5 cm of mineral soil, were determined once during the period 1994–1999 at each of the six sites. A detailed description of the sampling and analysis of soil samples can be found in Walther et al. (2003).

Drought response index

A drought response index (DRI) was calculated for each site and day as the difference between potential evapotranspiration (PET) and measured precipitation (P), with:

$$\text{DRI} = \text{PET} - \text{P} \quad (1)$$

PET was calculated with the Penman–Monteith approach (Monteith 1965) as implemented in the CoupModel (Jansson and Karlberg 2004), using the parameter settings of Gustafsson et al. (2004) for forests. Soil matric potential measurements were used to verify the PET model. Daily DRI values were totalled over the entire growing season (1st April–31st October) to obtain annual growing-season values between 1997 and 2011 (or 1998–2011, depending on data availability) that were subsequently used in the analyses.

Tree sampling

Foliage has been sampled biennially since 1997 at each of the LWF sites, with the exception of Vordemwald, for which samples were only taken in 2009. Samples were collected from the upper third of the tree crown from five dominant trees in the surrounding buffer zone of the LWF site. Sampling was undertaken during summer for *F. sylvatica* before the onset of autumnal leaf yellowing or senescence, and during autumn for *P. abies* after the onset of the dormancy period of coniferous species (Thimonier et al. 2010).

For tree-ring samples, ten dominant trees were selected adjacent to each LWF site, as close as possible to the trees from which foliage samples had been taken, in terms of distance and site topography. Trees were selected adjacent to the LWF site due to the fact that the coring of trees from within the site is prohibited under the guidelines set out by the ICP-Forests program. This is with the exception of *P. abies* at Vordemwald, from which it was possible to extract cores from the same trees from which foliage samples had been taken in 2009 as well as from an additional five trees adjacent to the LWF site. Two cores were extracted from each tree at a height of approximately 1.5 m in 2012 using a 0.5 cm diameter increment borer. Cores were air-dried and planed using a custom-made core microtome (WSL, Switzerland) to improve the visibility of individual tree-rings without contamination of wood material between tree-ring years.

Dendrochronological analyses

Annual tree-rings were identified and measured from bark to pith, with a resolution of 0.01 mm using the TSAPWIN tree-ring software (Version 3.5) connected to a LINTAB 3 measuring system (F. Rinn S.A., Heidelberg, Germany). The tree-rings were subsequently cross-dated, both visually by comparing individual chronologies to site mean chronologies and using standard methods (Cook and Kairiukstis 1990; Fritts 1976). The tree-ring chronologies were statistically verified using the TSAP and COFECHA

routines (Holmes 1983), before being detrended with a 30-year smoothing spline using the ARSTAN program (Version 44h2, Cook 1985) to remove tree-ring biases caused by tree age. Although chronologies were found to date back to 1725, we considered 1997–2011 only, as this was the time period for which archived foliage samples were available.

Sample preparation and isotope analyses

Biennial foliar samples (leaves and current-year needles) were dried at 65 °C, after which the weight of 100 leaves or 1000 needles was determined. The samples taken since 1997 have been stored in a micro-climate controlled archive at the WSL. In 2012, the archived foliage samples of each of the five trees were pooled to produce one composite sample per site and sampling year, and subsequently milled and homogenised using a MM200 centrifugal ball mill (Retsch, Haan, Germany). This was with the exception of needle samples taken from *P. abies* at Vordemwald in 2009 that were prepared and analysed at an individual tree level. The milled material was weighed into tin capsules (1.8–2.0 mg for *F. sylvatica* leaves and 3.5–3.8 mg for *P. abies* needles, according to species differences in foliage N concentration) for stable isotope analysis. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured simultaneously through combustion under excess oxygen in an elemental analyser (EA3000, EuroVector, Milano, Italy) interfaced to a continuous flow stable isotope mass spectrometer (Delta V, Thermo Fisher, Bremen, Germany), with a precision of 0.2 and 0.3 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Five trees (ten cores) from each site were selected for C and N tree-ring isotope analyses. Based on the findings of Tomlinson et al. (2014), where no significant changes in isotopic composition of either C or N were found after the extraction of mobile compounds, we decided to use whole wood material. Single tree-rings were separated with a razor blade under a microscope, and then pooled for each individual tree-ring year per site. These samples were milled and homogenised using the MM200 centrifugal ball mill. The resulting wood material was weighed into tin capsules (0.5–0.55 mg) for $\delta^{13}\text{C}$ analysis (site $n = 15$, total $n = 105$). For the analysis of wood $\delta^{15}\text{N}$, we applied the ‘10 year split pool approach’, whereby wood samples are pooled for three consecutive tree-ring years, with an individual year sample every 10th year (e.g. Kress et al. (2010); site $n = 6$, total $n = 35$). This ensures sufficient wood material for the $\delta^{15}\text{N}$ analysis, which requires much more material due to very low N concentrations. This approach also allows the assessment of annual inter-site variance every 10th year. Samples were pooled by combining 10 mg of material from each tree-ring year (e.g. 1998–2000, 2002–2004, etc.), and homogenized using a

vortexer (Select Bioproducts, Edison, USA). For *P. abies* from Vordemwald, the tree-ring samples were pooled differently between 2008 and 2011, as wood material was sampled from the same trees from which foliar samples were taken in 2009. The wood material from each tree at this site was measured individually (rather than all five trees pooled), whilst the tree-rings were pooled every 2 years between 2008 and 2011 (i.e. 2008/2009 and 2010/2011). All wood materials prepared for $\delta^{15}\text{N}$ analysis were then weighed into tin capsules (~ 15 mg). Both the C and N samples were analysed by combustion under excess oxygen in an elemental analyser (EA 1110, Carlo Erba, Milano, Italy) connected in continuous-flow mode to an isotope ratio mass spectrometer (Delta-S, Finnigan, Bremen, Germany), with a precision of 0.1 and 0.3 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. A blank (empty tin capsule) was also measured after each N sample due to the high C/N ratios in wood material (approx. 500:1).

Isotope values are expressed in the δ -notation as relative deviations from the international standards; Vienna Pee Dee Belemnite for carbon ($\delta^{13}\text{C} = ((^{13}\text{C}/^{12}\text{C}_{\text{sample}})/(^{13}\text{C}/^{12}\text{C}_{\text{standard}}) - 1) \cdot 1000$), and atmospheric N_2 for nitrogen ($\delta^{15}\text{N} = ((^{15}\text{N}/^{14}\text{N}_{\text{sample}})/(^{15}\text{N}/^{14}\text{N}_{\text{standard}}) - 1) \cdot 1000$). The tree-ring $\delta^{13}\text{C}$ was corrected for the Suess effect, caused by the production of ^{13}C -depleted CO_2 emissions during fossil fuel combustion. This was achieved using records of past atmospheric $\delta^{13}\text{C}$ obtained from ice cores and from historical $\delta^{13}\text{C}$ recorded at Mauna Loa (Francey et al. 1999; Leuenberger 2007). For both tree-ring and foliar $\delta^{13}\text{C}$ values, we used these Suess-corrected values for all further analyses.

Data analyses

Intra-tree and inter-species variation

All statistical analyses were performed using the statistical software program *R* (version 3.1.3). We applied paired *t*-tests to investigate whether there were significant differences in the isotopic composition of C and N between tree-rings and foliage within each tree species. We investigated the relationship between tree-ring and foliage isotope ratios of both C and N with linear regression. For C, both tree-ring and foliar $\delta^{13}\text{C}$ isotope data used in these models matched the time resolution of the foliage data (i.e. 1997, 1999, 2001, etc.; $n = 8/\text{site}$), with the exception of Vordemwald. Here, no foliage data were available for *F. sylvatica*, but individual tree-ring and foliar results for the five *P. abies* trees measured in 2009 were used. For all sites, as tree-ring $\delta^{15}\text{N}$ samples had been pooled prior to analyses, the foliage samples were averaged accordingly to match the tree-ring samples (site $n = 6$, with the exception of Vordemwald). Un-paired *t*-tests were applied to determine

species differences in tree-ring $\delta^{13}\text{C}$, tree-ring $\delta^{15}\text{N}$, foliar $\delta^{13}\text{C}$ and foliar $\delta^{15}\text{N}$ isotopic composition.

Environmental effects

Linear regression models were used to test for temporal trends in environmental variables between 1997 and 2011. Linear mixed-effects models were used to investigate the effects of environmental variables (explanatory variables) on both tree-ring and foliage isotopic composition and tree growth. Initial analyses on annual tree-ring $\delta^{13}\text{C}$ values demonstrated a lack of independency between tree-ring years, and subsequently it was decided to average the results of the tree-ring $\delta^{13}\text{C}$ analyses to match the pooling of the tree-ring $\delta^{15}\text{N}$ (i.e. 1998–2000, 2001, 2002–2004, 2005–2007, 2008–2010, 2011). Data for the environmental variables were averaged to match the tree-ring data (i.e. 10-year split pool approach) or biannually selected to match the foliar data (every second year between 1997 and 2011). Tree-ring width (TRW), tree-ring $\delta^{15}\text{N}$, foliar $\delta^{15}\text{N}$, tree-ring $\delta^{13}\text{C}$, and foliar $\delta^{13}\text{C}$ were assessed using individual linear mixed-effects models. In these models, species (*F. sylvatica* or *P. abies*), soil C/N, annual growing-season DRI ($\text{DRI}_{\text{Apr–Oct}}$), annual bulk deposition of mineral N (N deposition), year and site (as a random factor; $n = 6$) were included as explanatory variables in a complete model.

The significance of each explanatory variable was tested by removing each one in turn from the complete model. An analysis of variance (ANOVA) was subsequently used to determine whether there was a significant difference between the original model and the model lacking the particular explanatory parameter, i.e. to determine whether this variable significantly contributed to explaining the remaining variability.

Results

Intra-tree relationships and inter-species differences

Tree-ring $\delta^{15}\text{N}$ values were significantly less negative than foliar $\delta^{15}\text{N}$ values for both *F. sylvatica* and *P. abies* ($p \leq 0.001$; Table 2). Moreover, $\delta^{15}\text{N}$ of tree-rings were not related to that of foliage for either species ($p = 0.80$ and 0.55 for *F. sylvatica* and *P. abies*, respectively) (Fig. 1b). Tree-ring $\delta^{13}\text{C}$ was also significantly less negative than foliar $\delta^{13}\text{C}$ for both tree species ($p \leq 0.001$; Table 2). $\delta^{13}\text{C}$ of tree-rings and foliage of both species were positively related to each other, with highly significant relationships for *P. abies* ($p \leq 0.001$), but less so for *F. sylvatica* ($p = 0.05$) (Fig. 1a).

Overall, $\delta^{15}\text{N}$ of tree-rings and foliage of *F. sylvatica* were significantly ($p \leq 0.001$) less negative compared to those of *P. abies* (by 1.58 and 1.85 ‰, respectively;

Table 2 Average tree-ring and foliar $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (‰; \pm standard deviation (n)) of *F. sylvatica* and *P. abies* as well as the intra-tree isotopic differences and differences between species (‰; \pm standard deviation)

		<i>F. sylvatica</i>	<i>P. abies</i>	Species difference
$\delta^{15}\text{N}$	Tree-ring	-5.98 ± 1.44 (24)	-7.56 ± 1.36 (18)	$1.58 \pm 1.98^{***}$
	Foliage	-6.85 ± 0.34 (24)	-8.70 ± 0.47 (17)	$1.85 \pm 0.58^{***}$
	Intra-tree difference	$0.87 \pm 1.28^{***}$	$1.14 \pm 1.43^{***}$	
$\delta^{13}\text{C}$	Tree-ring	-25.69 ± 0.70 (60)	-23.71 ± 0.37 (45)	$-1.98 \pm 0.79^{***}$
	Foliage	-26.38 ± 0.50 (24)	-24.84 ± 0.88 (17)	$-1.54 \pm 1.01^{***}$
	Intra-tree difference	$0.69 \pm 0.58^{***}$	$1.13 \pm 0.86^{***}$	

*, **, *** for $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively

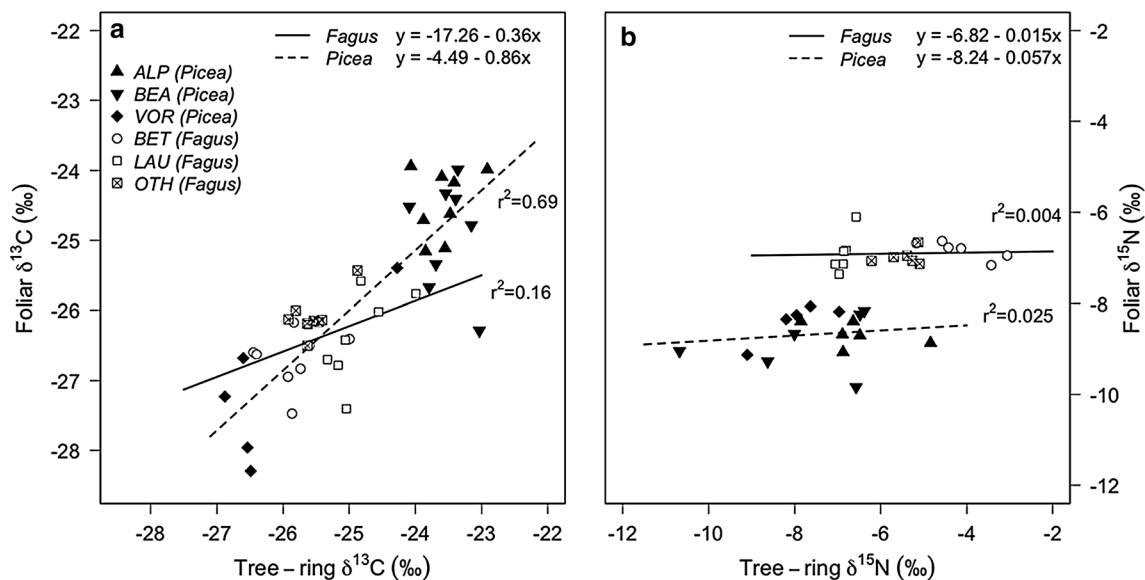
**Fig. 1** Relationships between, **a** tree-ring and foliar $\delta^{13}\text{C}$, and **b** tree-ring and foliar $\delta^{15}\text{N}$ for *P. abies* (dashed line) and *F. sylvatica* (solid line). *F. sylvatica* of Vordemwald is excluded due to lack of foliage data

Table 2). In contrast, tree-ring $\delta^{13}\text{C}$ of *P. abies* was significantly less negative than that of *F. sylvatica* (~ 1.98 ‰; $p \leq 0.001$), showing a similar pattern as foliar $\delta^{13}\text{C}$, which was also significantly less negative in *P. abies* compared to *F. sylvatica* (1.54 ‰; $p \leq 0.001$) (Table 2).

Temporal variations and environmental drivers

Between 1997 and 2011, no significant temporal trends in either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in tree-rings were observed for any of the sites (Table 3; Fig. 2a, c). However, large among-site variations were present for both $\delta^{13}\text{C}$ in tree-rings (with maximum differences of 3.0 and 1.6 ‰ for *F. sylvatica* and *P. abies*, respectively) and $\delta^{15}\text{N}$ in tree-rings (maximum differences of 5.0 and 5.8 ‰ for *F. sylvatica* and *P. abies*, respectively). In contrast, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in foliage significantly decreased between 1997 and 2011, by up to 1.5 ‰ for $\delta^{13}\text{C}$ and 1.8 ‰ for $\delta^{15}\text{N}$ (Table 3; Fig. 2b, d). At the same time, variations of both foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

among sites for the same species were lower than seen in tree-rings. Standardized TRW (corrected for tree age) also decreased significantly between 1997 and 2011 (Table 3; Fig. 3a), in contrast to $\text{DRI}_{\text{Apr–Oct}}$ ($p = 0.65$; Fig. 3b). Bulk N deposition also decreased significantly within this period ($p \leq 0.001$; Fig. 3c). Foliar $\delta^{13}\text{C}$ values were affected by these environmental factors and became less negative with increasing soil C/N values and increasing N deposition (Table 3). Conversely, tree-ring $\delta^{13}\text{C}$ values and both $\delta^{15}\text{N}$ values in tree-rings and foliage were not affected by any of the environmental factors considered (Table 3).

Discussion

Intra-tree and inter-species isotopic variations

Tree-rings showed less negative $\delta^{15}\text{N}$ values relative to foliage for both species (~ 1.0 ‰). Bukata and Kyser

Table 3 Summary of the significance of explanatory variables in explaining variation in tree-ring width (TRW), foliage and tree-ring $\delta^{15}\text{N}$ chronologies, and foliage and tree-ring $\delta^{13}\text{C}$ chronologies from the linear mixed-effect models

Variable	Tree-ring width (TRW)		Foliar $\delta^{15}\text{N}$		Tree-ring $\delta^{15}\text{N}$		Foliar $\delta^{13}\text{C}$		Tree-ring $\delta^{13}\text{C}$	
	<i>t</i> value	<i>p</i> value	<i>t</i> value	<i>p</i> value	<i>t</i> value	<i>p</i> value	<i>t</i> value	<i>p</i> value	<i>t</i> value	<i>p</i> value
Year	−2.301	0.016*	−2.538	0.008**	0.331	0.878	−3.114	0.002**	0.295	0.787
Species	−0.589	0.464	−9.074	<0.001***	−1.685	0.053	5.005	<0.001***	16.858	<0.001***
Soil C/N	0.173	0.818	−0.442	0.628	−0.874	0.203	−1.81	0.026*	−1.075	0.196
N deposition	1.321	0.084	0.214	0.818	−0.429	0.08	−1.374	0.021*	0.381	0.251
DRI _{Apr–Oct}	−1.372	0.091	−1.451	0.117	−0.232	0.432	−0.125	0.932	1.407	0.135
<i>R</i> ²	0.14		0.91		0.21		0.74		0.75	

Explanatory variables considered were year, species, soil C/N, bulk deposition mineral N (N deposition) and growing-season drought response index (DRI_{Apr–Oct}). *t*, *p*, and *R*² values given with significant variables marked in bold

*, ** or *** for $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively

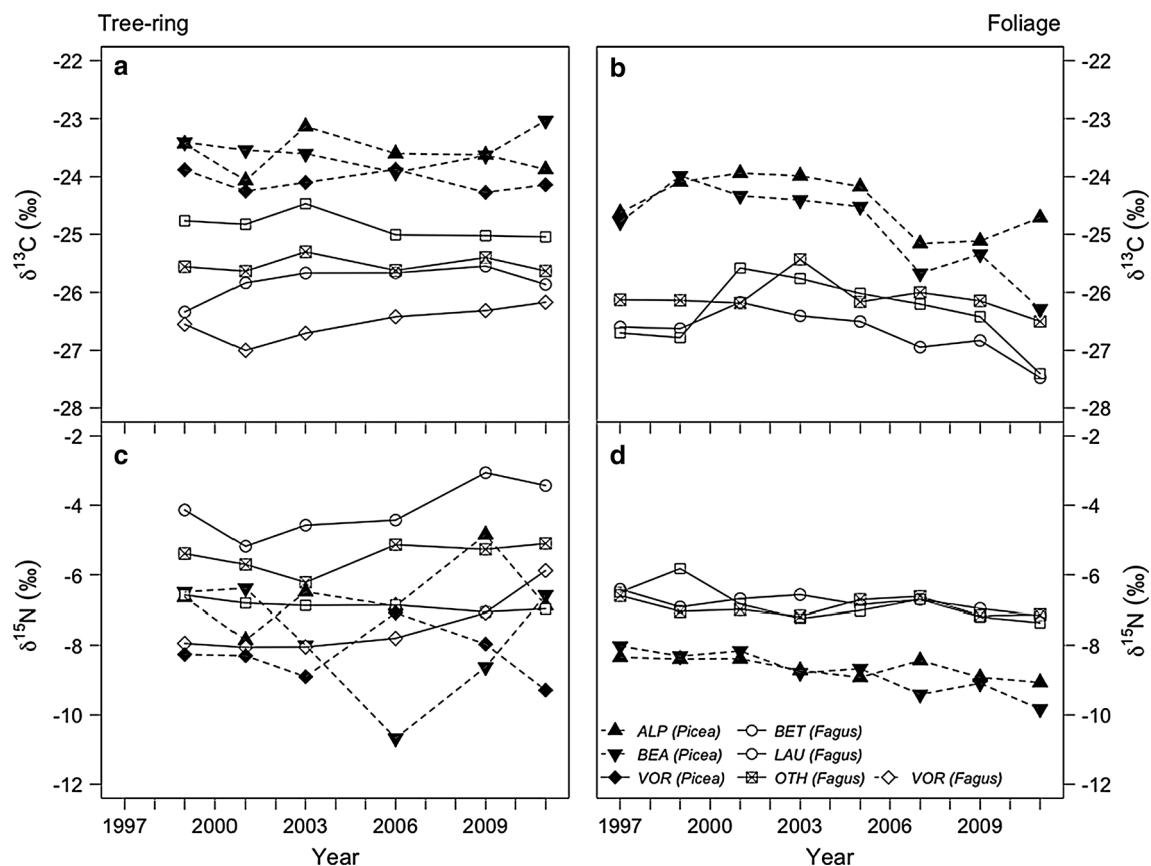


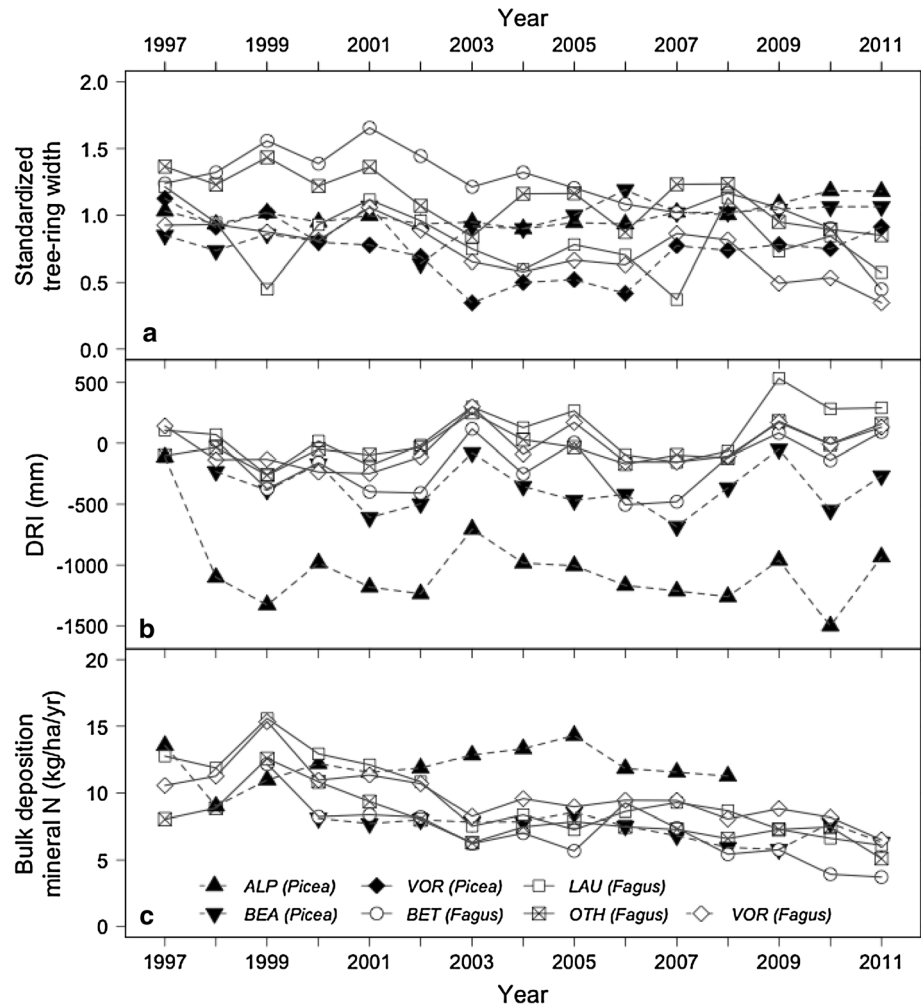
Fig. 2 Chronologies of **a** tree-ring $\delta^{13}\text{C}$, **b** foliar $\delta^{13}\text{C}$, **c** tree-ring $\delta^{15}\text{N}$, and **d** foliar $\delta^{15}\text{N}$ for both *P. abies* (dashed lines) and *F. sylvatica* (solid lines) between 1997 and 2011 for six sites across

Switzerland. Annual tree-ring $\delta^{13}\text{C}$ data have been averaged to match the tree-ring $\delta^{15}\text{N}$ data (i.e. the 10-year split pool approach; 1998–2000, 2001, 2002–2004, 2005–2007, 2008–2010, 2011)

(2007) found the same pattern for a number of *Quercus* and *Betula* species (with differences of about 0.5 ‰). In our investigation, we found no significant relationship between $\delta^{15}\text{N}$ in tree-rings and foliage. Furthermore, N concentrations within the tree stem were particularly low compared to those in foliage for both *F. sylvatica* (average values of

0.12 ‰ and 2.36 ‰, respectively) and *P. abies* (0.06 ‰ and 1.13 ‰, respectively). This marked difference in N concentration between the tree stem and the foliage highlights the greater need for N in the foliage than in the tree-rings. Tree foliage is a large N sink within a tree as N is a key component of the photosynthetic enzyme ribulose-1,5-

Fig. 3 Chronologies of, **a** standardized tree-ring widths, **b** growing-season (April–October) drought response index (DRI), and **c** bulk deposition mineral N for six *P. abies* (dashed lines) and *F. sylvatica* sites (solid lines) sites between 1997 and 2011



bisphosphate carboxylase/oxygenase (RuBisCO) in leaves and needles (Takashima et al. 2004). With low tree N concentrations and a high foliar N demand, N recycling within the tree is an essential physiological process to ensure that all N demands within the tree are met, primarily in the foliage. This process of N recycling can occur within the foliage, before leaf abscission in deciduous trees or between different age-classes of needles in conifers (Gebauer and Schulze 1991; Kolb and Evans 2002). Further N recycling processes occur within the tree stem (Tomlinson et al. 2014) and between the tree stem and foliage. Notably, foliar $\delta^{15}\text{N}$ values measured in our investigation showed very little variation compared to $\delta^{15}\text{N}$ in tree-rings from the same site. Similarly, Gerhart and McLauchlan (2014) highlighted a lack of a consistent correlation between tree-ring $\delta^{15}\text{N}$ and tree-ring N concentrations, as often seen in the foliage. They attributed the lack of a relationship between tree-ring $\delta^{15}\text{N}$ and tree-ring N concentrations to non-environmental processes, such as N recycling to fulfill the high N demands in the foliage. Recycling of N will most likely result in more enriched (less negative) $\delta^{15}\text{N}$ in

the source tissues since molecules containing heavier isotopes are discriminated against and are left behind, a pattern indeed observed in tree-rings in our study.

The comparison of $\delta^{15}\text{N}$ between species revealed less negative $\delta^{15}\text{N}$ values in tree-rings and foliage for *F. sylvatica* than for *P. abies* (by 1.6 and 1.9 ‰, respectively). These differences might be due to a substantial difference in the biochemical composition of the foliage between the two species. For example, *P. abies* allocates far less N to compounds used in photosynthesis than *F. sylvatica*, instead allocating more N (in relative terms) to cell wall structures to increase foliage toughness and longevity (Takashima et al. 2004). The two species also differ in rooting depth (Table 1), which could result in beech taking up N also from deeper soil N pools than spruce. $\delta^{15}\text{N}$ values are known to increase with increasing soil depth due to fractionation during mineralisation and litter decomposition (Gebauer and Schulze 1991; Högberg 1997). As a result, differences in N uptake from different soil depths may explain the higher $\delta^{15}\text{N}$ for beech compared to spruce. Furthermore, differences in mycorrhizal relationships may

influence species-specific $\delta^{15}\text{N}$ values, as mycorrhizae typically transfer ^{15}N -depleted N to the tree (Craine et al. 2009). Different species-specific uptake preferences for nitrate and ammonium may also contribute to species differences in both tree-ring and foliar $\delta^{15}\text{N}$. However, the uptake of ammonium is known to be significantly greater than the uptake of nitrate for both tree species (Gessler et al. 1998). Furthermore, we do not have any data on the isotopic composition of either soil nitrate or ammonium at any of our sites. There was, however, no effect of site-specific soil C/N values (which are inversely correlated with nitrification rates; Bengtsson et al. 2003) on either tree-ring or foliar $\delta^{15}\text{N}$ values.

Tree-ring $\delta^{13}\text{C}$ was less negative than foliar $\delta^{13}\text{C}$ by 0.91 ‰ on average. A multi-species review by Badeck et al. (2005) also found less negative $\delta^{13}\text{C}$ values in woody stems relative to foliage (by 1.9 ‰). Other studies have suggested this intra-tree variation to primarily being the result of (1) differences in the biochemical composition between tree-rings and foliage, and (2) the growth of tree-rings and foliage occurring during periods of different environmental conditions and thus photosynthetic discrimination (Badeck et al. 2005; Cernusak et al. 2009). Our investigation found a significant positive correlation between $\delta^{13}\text{C}$ values of tree-rings and foliage for *P. abies* but not so for *F. sylvatica*. Species-specific differences between evergreen and deciduous tree $\delta^{13}\text{C}$ values have generally been attributed to differences in tree physiology, primarily to lower stomatal conductance and higher intrinsic water-use efficiency of *P. abies* relative to *F. sylvatica* (Brooks et al. 1997; Cernusak et al. 2009). Moreover, the intra-tree difference between $\delta^{13}\text{C}$ values of tree-rings vs. foliage was nearly twice as large for *P. abies* than for *F. sylvatica* (1.13 and 0.69 ‰, respectively). This can additionally be attributed to species-specific differences in wood biochemical composition. Softwoods, such as *P. abies*, typically have lower lignin and higher cellulose concentrations than hardwoods, such as *F. sylvatica*. This is significant, given that cellulose is often ^{13}C -enriched and lignin ^{13}C -depleted relative to the organic mass of the measured plant component (Badeck et al. 2005; Saka 2001).

Temporal trends and environmental influences

Tree-ring $\delta^{15}\text{N}$ did not show any significant temporal trends, whilst foliage $\delta^{15}\text{N}$ significantly decreased over the measurement period by about 0.9 ‰. Internal N recycling and a smaller sink strength than growing photosynthesizing tissues might explain the lack of temporal trends in the $\delta^{15}\text{N}$ of tree-rings. A temporal decrease of up to 4 ‰ in foliar $\delta^{15}\text{N}$ has previously been observed by Högberg et al. (1992) in current-year foliage between 1966 and 1989.

These decreasing $\delta^{15}\text{N}$ values over time have been suggested to be the result of changes in atmospheric N isotopic composition, which is strongly dependent on emission sources (Ammann et al. 1999; Saurer et al. 2004). However, there are no long-term datasets of the N isotopic signature of atmospheric N deposition available that would allow us to determine whether this was the case at our study sites.

Similarly, we found no significant temporal trends in the $\delta^{13}\text{C}$ values of tree-rings for this relatively short time period. Foliar $\delta^{13}\text{C}$ values, however, became more negative over the measurement period for both species, even after correcting for the declining atmospheric $^{13}\text{C}/^{12}\text{C}$ caused by the production of ^{13}C -depleted fossil fuel emissions (McCarroll et al. 2009). While none of the environmental variables considered provided an explanation for the variation in tree-ring $\delta^{13}\text{C}$, both soil C/N and N deposition were partially able to explain the site differences in foliar $\delta^{13}\text{C}$ (Table 3). Less negative foliar $\delta^{13}\text{C}$ values were found at sites with higher soil C/N values (i.e. decreasing soil N availability), while foliar $\delta^{13}\text{C}$ also became less negative with increasing N deposition. Investigating the water-use efficiency of poplar leaves under different soil N and atmospheric NO_2 regimes, Siegwolf et al. (2001) also found a decrease in foliar $\delta^{13}\text{C}$ with increasing soil N availability and decreasing NO_2 exposure. In that study, the effect of decreasing $\delta^{13}\text{C}$ in foliage with increasing soil N availability was attributed to an increase in stomatal conductance. Increasing $\delta^{13}\text{C}$ values with increasing NO_2 , however, was attributed to an increase in CO_2 assimilation, but with a more moderate increase in stomatal conductance. In our study, the lack of soil C/N or N deposition affecting tree-ring $\delta^{13}\text{C}$ and TRW suggests that both spatial and temporal variations in N availability were not strong enough to significantly alter either tree-ring $\delta^{13}\text{C}$ or TRW.

Are tree-ring $\delta^{15}\text{N}$ values suitable proxies for foliar $\delta^{15}\text{N}$ values?

Our analyses for the period 1997–2011 clearly demonstrated a significant uncoupling between tree-ring and foliar $\delta^{15}\text{N}$, suggesting that tree-ring $\delta^{15}\text{N}$ represents an inappropriate proxy for foliar $\delta^{15}\text{N}$ or $\delta^{15}\text{N}$ in atmospheric N deposition. Contrary to our results, Bukata and Kyser (2007) reported a significant positive correlation between the $\delta^{15}\text{N}$ values in both tissues for two *Quercus* species. However, their analyses included foliar and tree-ring samples from one year only (2006) and thus did not include a time series of foliar and tree-ring isotopic composition, such as in our investigation. The lack of a time series in the investigation of Bukata and Kyser (2007) becomes significant when N recycling processes within the tree are taken into account. While all foliage samples analyzed in our

investigation were current year foliage samples (subsequently archived), all tree-ring samples were collected together during one sampling procedure in 2012 (i.e. the tree-rings from 1997 were already 15 years old). Consequently, the tree-rings included in our investigation would have been subjected to N recycling processes to meet the strong N demand from sinks within the foliage (and younger tree-rings). The fact that N can be assimilated into the tree from both the soil and directly from the atmosphere also contributes to this uncoupling effect, as direct foliar uptake of atmospheric reactive N has previously been suggested to contribute to 16 % of plant N demand (Valano and Sparks 2007). This uncoupling process is further supported by: (1) the fact that foliar $\delta^{15}\text{N}$ values significantly decreased over our measurement period, while there was no significant difference in tree-ring $\delta^{15}\text{N}$, (2) the much greater variation in tree-ring $\delta^{15}\text{N}$ relative to foliar $\delta^{15}\text{N}$, and (3) the large differences in N concentrations between tree-rings and foliage in both *F. sylvatica* and *P. abies*.

Furthermore, our analyses demonstrated that tree-ring $\delta^{15}\text{N}$ values were not affected by various site-specific environmental conditions over a 15-year period, such as soil N availability or changing rates of bulk N deposition. This is in contrast to a number of previous investigations that have shown that tree-ring $\delta^{15}\text{N}$ values might be able to provide information on anthropogenic sources of forest N inputs (Elhani et al. 2005; Guerrieri et al. 2009; McLauchlan and Craine 2012; Saurer et al. 2004; Savard et al. 2009; Sun et al. 2010). For example, a number of these studies demonstrated a gradual decrease in tree-ring $\delta^{15}\text{N}$ values over time, thought to be due to changes in the isotopic composition of atmospheric N (e.g. McLauchlan and Craine 2012; Poulson et al. 1995; Savard et al. 2009). Unlike our investigation, however, many of these studies sampled trees that were either exposed to high levels of N deposition (Guerrieri et al. 2009; Saurer et al. 2004; Sun et al. 2010) or from N fertilization experiments that simulated high doses of N deposition (Elhani et al. 2005; Guerrieri et al. 2011). Furthermore, a recent review by Gerhart and McLauchlan (2014) on the potential application and limitations of using wood $\delta^{15}\text{N}$ to study terrestrial nutrient cycles, highlighted the speculative nature of the causes of these temporal trends of tree-ring $\delta^{15}\text{N}$. This is primarily due to a lack of both long- and short-term data on the isotopic composition of deposited N, and the large regional and seasonal variability of the isotopic signature of N deposition (Gerhart and McLauchlan 2014). Moreover, the high bidirectional mobility of N within the tree stem of *P. abies*, demonstrated by Tomlinson et al. (2014) for a period of 70 years, suggests that any potential ^{15}N -deposition signal within the tree-ring $\delta^{15}\text{N}$ chronology must be interpreted with great care. This movement of N

within the tree stem also contributes to the large inter-annual variability in tree-ring $\delta^{15}\text{N}$ seen in our investigation, a systematic feature found within many investigations on tree-ring $\delta^{15}\text{N}$. Thus, the results of our investigation have shown that, over a 15-year period and at levels of low to moderate N deposition, physiological processes within the tree mask any environmental signals that were present within the tree-ring $\delta^{15}\text{N}$ chronology.

In conclusion, using tree-ring $\delta^{15}\text{N}$ as a proxy for foliar $\delta^{15}\text{N}$ for both *F. sylvatica* and *P. abies* is inappropriate for site conditions with low to moderate N deposition. Furthermore, the large variation in tree-ring $\delta^{15}\text{N}$ found for the same species and among sites make them also unsuitable as archives for the nitrogen isotopic composition of low to moderate atmospheric N deposition, most likely due to internal N recycling processes and/or differences in tree N assimilation. As a consequence of such recycling processes, particularly within the tree stem, any potential N deposition signal—once present in tree-ring N isotopic composition—will unfortunately not be preserved within a given tree-ring year.

Author contribution statement Study concept formed by PWe, and developed by PWe, PWa, RS, NB and AT. Foliage samples and environmental data provided by PWa, AT, EGP, MS and MS, tree-ring samples taken by PWe and GT and measured by GT, isotope analyses performed by RS and data analyses performed by GT. GT wrote the first draft of the manuscript, and all co-authors contributed significantly to revisions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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