

Intra-annual variability in isotopic and total nitrogen in tree rings of old growth Sitka spruce from coastal British Columbia

T.E. Reimchen and Estelle Arbellay

Abstract: Tree-ring studies using increment cores have increasingly measured elemental (N) and isotopic ($\delta^{15}\text{N}$) nitrogen values to evaluate environmental changes in the nitrogen cycle. The paucity of nitrogen in wood has constrained tree-ring analyses to annual resolution. Based on 77 rings and 310 sub-rings, we provide evidence for substantial intra-annual variability in N and $\delta^{15}\text{N}$ values in the heartwood of 11 geographically widely separated, old growth Sitka spruce [*Picea sitchensis* (Bong.) Carrière] trees from coastal British Columbia. The range of N and $\delta^{15}\text{N}$ values within rings (intra-annual level) was on average equal to or up to seven-times the range among rings (inter-annual level). Most rings showed a seasonal reduction in N values from early to late growth and a corresponding increase to the early growth of the following season ($P < 0.05$ to 0.001 , Mann–Whitney–Wilcoxon test). By contrast, intra-annual changes in $\delta^{15}\text{N}$ values were highly variable among years and among trees, ranging from pronounced oscillations (4.0‰) to reasonable consistency. Our results allude to the potential importance of such intra-ring data for interpreting seasonal trends in nitrogen use and increasing understanding of ecological processes in the marine–terrestrial interface.

Key words: intra-annual, total nitrogen, nitrogen isotope, Sitka spruce, tree rings.

Résumé : Les valeurs d'azote élémentaire (N) et isotopique ($\delta^{15}\text{N}$) sont de plus en plus souvent mesurées dans les carottes prélevées lors d'études des anneaux de croissance des arbres, afin d'évaluer les changements environnementaux du cycle de l'azote. La rareté de l'azote du bois a limité les analyses des anneaux de croissance à une résolution annuelle. À partir de 77 anneaux et 310 parties d'anneaux, les auteurs apportent la preuve d'une variabilité intra-annuelle importante des valeurs de N et de $\delta^{15}\text{N}$ dans le bois de cœur de 11 épinettes de Sitka [*Picea sitchensis* (Bong.) Carrière] de forêts anciennes de la côte de la Colombie-Britannique, largement séparées géographiquement. L'étendue des valeurs de N et de $\delta^{15}\text{N}$ à l'intérieur des anneaux (niveau intra-annuel) était en moyenne égale ou jusqu'à sept fois l'étendue entre les anneaux (niveau inter-annuel). La plupart des anneaux présentaient une réduction saisonnière des valeurs de N de la croissance précoce à la croissance tardive et une augmentation correspondante jusqu'à la croissance précoce de la saison suivante ($P < 0,05$ à $0,001$, test de Mann–Whitney–Wilcoxon). Par contre, les changements intra-annuels des valeurs de $\delta^{15}\text{N}$ étaient hautement variables selon les années et entre les arbres, allant d'oscillations prononcées (4,0 ‰) à une assez bonne constance. Les résultats des auteurs font référence à l'importance potentielle de telles données intra-anneaux pour interpréter les tendances saisonnières de l'utilisation de l'azote et mieux comprendre les processus écologiques à l'interface marine–terrestre. [Traduit par la Rédaction]

Mots-clés : intra-annuel, azote total, azote isotopique, épinette de Sitka, anneaux de croissance.

Introduction

Elemental (N) and isotopic ($\delta^{15}\text{N}$) nitrogen values from tree rings have been extensively used to investigate environmental changes in nitrogen availability (Doucet et al. 2011; Gerhart and McLauchlan 2014; Gessler et al. 2014; van der Sleen et al. 2017). However, nitrogen content is exceptionally low in wood (Meerts 2002) and reconstructing N and $\delta^{15}\text{N}$ values from increment cores with annual,

let alone sub-annual, resolution remains a challenge for the scientific community (Poulson et al. 1995). Nevertheless, finer-scale analyses such as intra-annual resolution have yielded further ecological and physiological insights (Helle and Schleser 2004; DeSoto et al. 2011; Li et al. 2011; Wood and Smith 2015).

Intra-annual variations in N and $\delta^{15}\text{N}$ values have mainly been assessed in fundamental studies on tree phenology

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Table 1. Coastal watersheds from British Columbia and old growth Sitka Spruce trees in this study.

Trees	MA (years)	DBH (cm)	Rings (n)	Sub-rings (n)	Rings subdivided in sub-rings for intra-annual analysis
Amback Creek (51°41'51"N, 127°02'35"W)					
A05	80	113	3	13	1920, 1921, 1923
Bag Harbour Creek (52°20'42"N, 131°22'15"W)					
B06	63	121	5	25	1938–1942
B17	83	80	3	14	1964–1966
B19	66	126	19	73	1951–1959, 1975–1978, 1980–1985
B24	77	75	10	36	1950–1952, 1954, 1980–1985
B25	75	70	9	29	1953, 1954, 1975–1981
B26	93	96	5	15	1980, 1981, 1983–1985
Gardner Creek (53°19'24"N, 127°55'26"W)					
G01	77	88	4	17	1943–1946
Kostan Creek (52°34'48"N, 131°42'52"W)					
K07	65	180	6	29	1944–1949
Salmon River (52°42'14"N, 131°48'58"W)					
S15	52	140	6	24	1955–1957, 1972–1974
S16	33	118	7	35	1971–1974, 1981–1983
Totals			77	310	

Note: MA, minimum age; DBH, diameter at breast height.

dealing with the internal cycling of nitrogen in the stem, twigs, and roots (von Fircks et al. 2001; Brereton et al. 2014; Zhang et al. 2016). Moreover, seasonal changes in non-structural nitrogen compounds in the stem of mature trees have only recently been published and are restricted to the sapwood of oak and beech (El Zein et al. 2011; Bazot et al. 2013). To our knowledge, similar studies have not been undertaken in the heartwood of any species. This may be due to the fact that heartwood has an even lower nitrogen content than sapwood, owing to translocation of mobile nitrogen across the sapwood at the time of heartwood formation when parenchyma cells die and lose nitrogen to growing regions (Meerts 2002; Pallardy 2008). The paucity of nitrogen in wood, especially heartwood, thus makes ring width a major limiting factor for measuring N and $\delta^{15}\text{N}$ values at the intra-annual level.

In this study, we report, for the first time, evidence for substantial intra-annual variability in N and $\delta^{15}\text{N}$ values in the heartwood of old growth Sitka spruce [*Picea sitchensis* (Bong.) Carrière] from coastal British Columbia. Large annual rings are characteristic of some Sitka spruce in this region due to the important fertilization of terrestrial vegetation by spawning salmon (Reimchen et al. 2003; Reimchen and Fox 2013). As a consequence, tree-ring series contain rings sufficiently wide to allow analysis of wood nitrogen at the intra-annual level. The aim of this research is to identify and quantify changes in N and $\delta^{15}\text{N}$ values during the growing season in the heartwood of a coniferous species.

Materials and methods

Sample procurement

This study is based on 11 Sitka spruce trees from five coastal watersheds of British Columbia (Table 1) including three systems located on Moresby Island in southeastern Haida Gwaii (Bag Harbour Creek, Kostan Creek, and

Salmon River) and two systems located about 300 km away on the mid-coast of mainland British Columbia (Amback Creek and Gardner Creek). The five watersheds are composed of old growth forest dominated by Sitka spruce (*Picea sitchensis*), western hemlock (*Tsuga heterophylla*), and red cedar (*Thuja plicata*). Increment cores (12 mm) were sampled in autumn 1998–2002 during a coast-wide field survey of riparian vegetation in British Columbia (Reimchen et al. 2003). The cores were prepared using standard dendro-chronological methods (Stokes and Smiley 1996) and the rings were measured and crossdated using CooRecorder and CDendro 9.2 (Cybis Elektronik & Data AB, Saltsjöbaden, Sweden). The five watersheds were chosen because these were geographically diverse and had trees with annual rings of sufficient width to allow intra-annual resolution.

Sample analysis

We measured elemental (N) and isotopic ($\delta^{15}\text{N}$) nitrogen values in a total of 77 annual rings (Table 1). We chose 3–19 of the widest rings of the heartwood in each tree. Care was taken to only select heartwood rings, where no further nitrogen translocation occurs (Merrill and Cowling 1966; Tomlinson et al. 2014). Based on results from Reimchen and Fox (2013), we estimated the heartwood–sapwood transition to be in the early 1990s and accordingly chose rings prior to 1985 (Table 1). According to their width, the entire rings were subdivided into three, four, or five radial segments (average of 1.5 mm) using a scalpel blade under the microscope, resulting in 310 sub-rings in total (Table 1).

Each sub-ring was powdered, then 30 mg of the powder was packaged in tin capsules and analyzed at the University of California – Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spec-

Table 2. Mean and maximum deviation from the mean (range) of N and $\delta^{15}\text{N}$ values for each tree.

		Mean		Range among rings		Range within rings		Range diff. (%)	
Trees	Rings (<i>n</i>)	N (%)	δ ¹⁵ N (‰)	N (%)	δ ¹⁵ N (‰)	N (%)	δ ¹⁵ N (‰)	N	δ ¹⁵ N
Amback Creek									
A05	3	0.061	0.6	0.019	0.4	0.026	3.0	+38	+654
Bag Harbour Creek									
B06	5	0.044	4.0	0.007	0.7	0.029	1.2	+306	+86
B17	3	0.049	−3.9	0.007	0.4	0.029	3.0	+337	+597
B19	19	0.049	−0.6	0.026	2.0	0.020	1.0	−25	−49
B24	10	0.035	1.7	0.026	3.1	0.016	2.2	−39	−31
B25	9	0.039	−1.2	0.014	1.2	0.014	1.1	−3	−11
B26	5	0.044	3.6	0.014	2.1	0.014	0.9	0	−57
Gardner Creek									
G01	4	0.053	−1.7	0.004	0.8	0.033	1.5	+716	+97
Kostan Creek									
K07	6	0.052	−0.7	0.013	1.2	0.023	1.4	+81	+13
Salmon River									
S15	6	0.064	2.3	0.020	1.4	0.025	1.0	+23	−29
S16	7	0.072	1.8	0.025	1.1	0.040	1.0	+58	−10
Mean		0.051	2.3	0.016	1.3	0.024	1.6	+136	+115

Note: The range represents the maximum deviation from the mean, obtained by subtracting the lowest value from the highest value. The range difference represents the difference in range between the two levels examined: range among rings (inter-annual level) and range within rings (intra-annual level). The range within rings, i.e., among sub-rings, was calculated for each ring and averaged to a single value for each tree.

trometer (Sercon Ltd., Cheshire, UK). N values represent percentage of total nitrogen, whereas $\delta^{15}\text{N}$ values were calculated by:

$$\delta^{15}\text{N}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}}) - 1$$

where R equals the ratio of ^{15}N : ^{14}N stable isotopes. R_{standard} is the ratio of ^{15}N : ^{14}N stable isotopes in atmospheric N_2 . The repeatability of N and $\delta^{15}\text{N}$ values from the wood reference samples ($n = 135$) averaged $\pm 0.012\%$ for N and $\pm 1.0\%$ for $\delta^{15}\text{N}$ (T.E. Reimchen, unpublished data). In addition, we examined $\delta^{15}\text{N}$ signatures of sequential rings processed sequentially within a single mass spectrometer run, which gave us a value of $\pm 0.4\%$ based on 42 comparisons (T.E. Reimchen, unpublished data).

Statistical analysis

First, we calculated the mean and maximum deviation from the mean (range) of N and $\delta^{15}\text{N}$ values for each tree (Table 2). Second, we used the Mann–Whitney–Wilcoxon test to determine whether N and $\delta^{15}\text{N}$ values changed significantly ($P < 0.05$) during the growing season. This non-parametric test is analogous to the t test but is distribution-free (Sprent and Smeeton 2007), providing more robust results when the number of samples analyzed does not exceed 30. The test was run to quantify changes in N and $\delta^{15}\text{N}$ values in three groups of rings of distinct intra-annual resolution (IAR), i.e., in rings subdivided into three, four, and five sub-rings (Table 3). In each group of rings, we investigated differences in N and $\delta^{15}\text{N}$ values between sub-rings to assess the type and magnitude of seasonal changes in wood nitrogen. For that purpose, we compared the first sub-ring (early growth) with the subsequent ones, and the last sub-ring (late growth)

with the first sub-ring of the following season. Finally, we tested for co-variation between N and $\delta^{15}\text{N}$ values by calculating Spearman's correlation coefficients. All of the data were processed with SPSS 24 (IBM).

Results

Extent of variation in N and $\delta^{15}\text{N}$ values

N and $\delta^{15}\text{N}$ values vary substantially among watersheds, among trees within a watershed, among rings within a tree, and among sub-rings within a ring (Table 2). N values range from 0.035% to 0.072% among watersheds, whereas $\delta^{15}\text{N}$ values range from -3.9‰ to 4.0‰. We found that the range difference between the range within rings and the range among rings was on average +136% for N and +115% for $\delta^{15}\text{N}$ (Table 2). This means that the range of N and $\delta^{15}\text{N}$ values within rings (intra-annual level) was on average equal to the range among rings (inter-annual level). Nevertheless, we also found that the range within rings could be up to seven-times the range among rings (Table 2).

Trends in intra-annual variations in N values

Changes in N values during the growing season were similar at IAR of three, four, and five sub-rings (Table 3). Comparisons of the first sub-ring (early growth) with the subsequent ones showed significant decreases in N values (of up to 0.016%) during the growing season. At IAR of three sub-rings, the decrease was highly significant ($P < 0.001$) between the first sub-ring (early growth) and the last sub-ring (late growth). A seasonal reduction is evident in most rings of this group (Fig. 1A). At IAR of four and five sub-rings, the decrease could be detected earlier. Significant ($P < 0.05$) to highly significant ($P < 0.01$) results were found from the third sub-ring (mid growth) to the last sub-ring (late growth). A seasonal reduction is

Table 3. Changes in N and $\delta^{15}\text{N}$ values at the intra-annual resolution of three, four, and five sub-rings.

Sub-ring comparisons	Samples (<i>n</i>)	N		δ ¹⁵ N	
		Diff. (%)	<i>P</i> value	Diff. (‰)	<i>P</i> value
Intra-annual resolution of three sub-rings					
1–2	24	–0.002	0.16	–0.1	0.89
1–3	24	–0.011	<0.001	+0.4	0.64
3–1f	13	+0.012	<0.001	–0.3	0.76
Intra-annual resolution of four sub-rings					
1–2	27	–0.003	0.62	+0.3	0.49
1–3	27	–0.008	<0.05	+0.2	0.74
1–4	27	–0.009	<0.05	+0.2	0.90
4–1f	12	+0.005	0.63	+0.1	0.84
Intra-annual resolution of five sub-rings					
1–2	26	–0.006	0.30	+0.1	0.89
1–3	26	–0.016	<0.01	–0.2	0.80
1–4	26	–0.011	<0.05	+0.2	0.71
1–5	26	–0.013	<0.05	+0.0	0.97
5–1f	17	+0.018	<0.05	+0.1	0.84

Note: The Mann–Whitney–Wilcoxon test was used to compare the first sub-ring (early growth) with the subsequent ones (1–2; 1–3; 1–4; 1–5), and the last sub-ring (late growth) with the first sub-ring of the following season (3–1f; 4–1f; 5–1f). Significant changes ($P < 0.05$) appear in **bold**.

also evident in most rings of these groups (Figs. 1B and 1C). In addition, comparisons of the last sub-ring (late growth) with the first sub-ring of the following season showed significant ($P < 0.05$) to very highly significant ($P < 0.001$) increases in N values (of up to 0.018%). The increase was significant at IAR of three and five sub-rings, but not at IAR of four sub-rings. We note that at the higher IAR of five sub-rings the increase can be visually detected late in the current season, namely between the two last sub-rings (e.g., S16–1972 and 1974, Fig. 1C).

Trends in intra-annual variations in $\delta^{15}\text{N}$ values

At IAR of three, four, and five sub-rings (Table 3), comparisons of the first sub-ring (early growth) with the subsequent ones showed no significant changes in $\delta^{15}\text{N}$ values during the growing season. Similarly, comparisons of the last sub-ring (late growth) with the first sub-ring of the following season showed no significant changes in $\delta^{15}\text{N}$ values. These results highlight that intra-annual variations in $\delta^{15}\text{N}$ values, unlike those in N values, are much less consistent among rings and among trees (Fig. 1). At one end of the range are pronounced intra-annual oscillations (4.0‰) (e.g., B24–1950) through more modest effects (1.0–3.0‰), while at the opposite end of the range there is reasonable intra-annual consistency from the first sub-ring (early growth) to the last sub-ring (late growth) (e.g., S15–1956).

Relationship between N and $\delta^{15}\text{N}$ values

Based on 310 sub-rings in total, we tested for co-variation between N and $\delta^{15}\text{N}$ values for each watershed. At Amback Creek ($n = 13$), Bag Harbour Creek ($n = 192$), and Salmon River ($n = 59$), we found negative low correlations between N and $\delta^{15}\text{N}$ values that were non-significant. At Gardner Creek ($n = 17$) and Kostan Creek

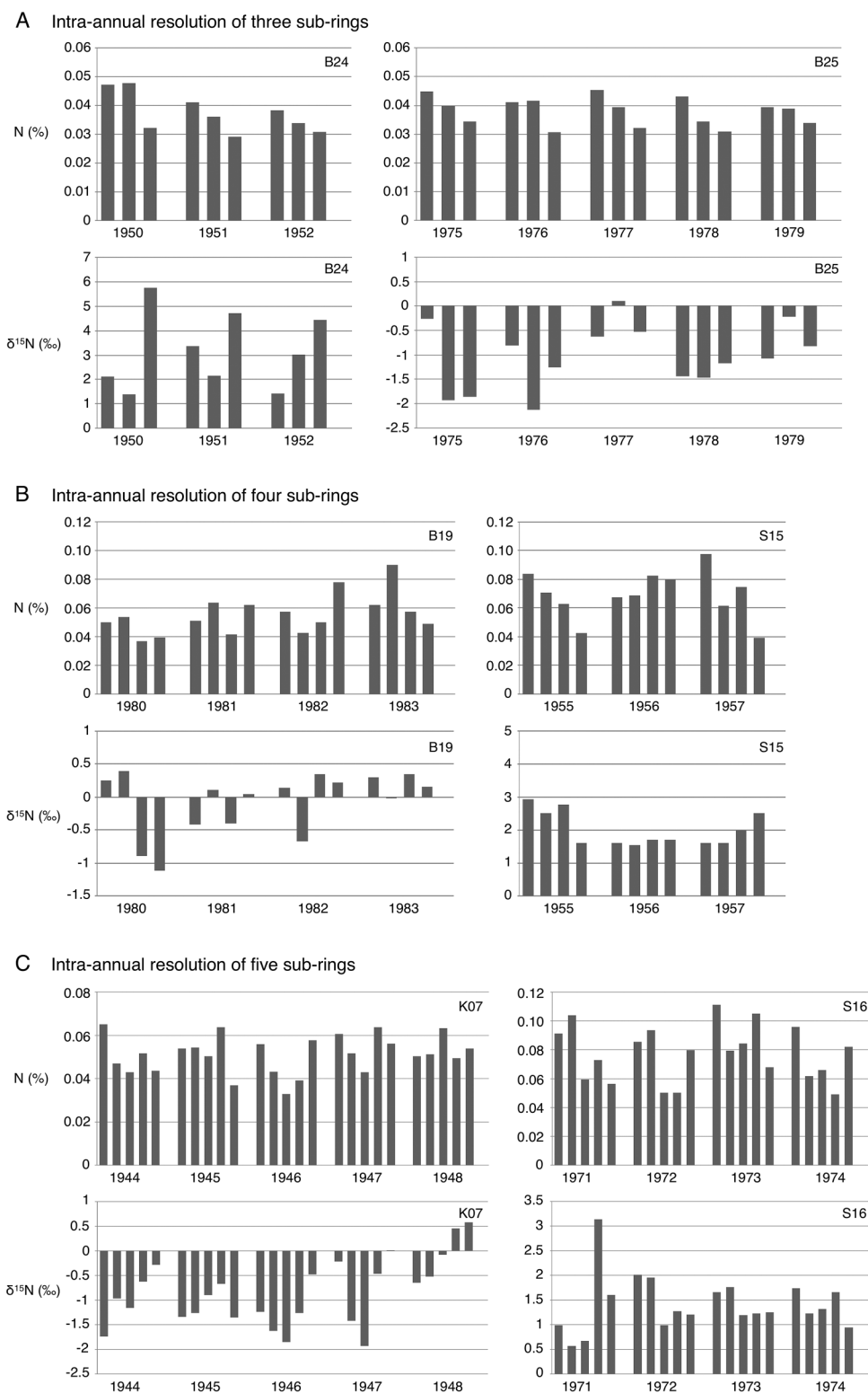
($n = 29$), N and $\delta^{15}\text{N}$ values were positively correlated with $r = 0.73$ ($P < 0.01$) and $r = 0.47$ ($P < 0.05$), respectively.

Discussion

Through fine-scale analyses of tree rings, we provide evidence for substantial intra-annual variability in N and $\delta^{15}\text{N}$ values in the heartwood of old growth Sitka spruce from coastal British Columbia. We found that the range of N and $\delta^{15}\text{N}$ values within rings (intra-annual level) was on average equal to or up to seven-times the range among rings (inter-annual level). Most rings showed an intra-annual decrease in N values (of up to 0.016%) from early to late growth, and an increase (of up to 0.018%) from late growth to the early growth of the following season. By contrast, changes in $\delta^{15}\text{N}$ values were highly variable among rings and among trees during the growing season, ranging from pronounced intra-annual oscillations (4.0‰) to reasonable intra-annual consistency from early to late growth. We assume that some of this variability, potentially from $\pm 0.4\%$ up to $\pm 1.0\%$, could be an artefact of mass spectrometer measurement.

The seasonal pattern in N values that we observed in most rings corresponds to the internal cycling of nitrogen previously described in the sapwood of broad-leaved and coniferous trees (Pallardy 2008; Millard and Grelet 2010; El Zein et al. 2011; Bazot et al. 2013): nitrogen reserves are (i) remobilized in spring for new growth in late spring-early summer (translating into a decrease in N during earlywood development) and (ii) newly synthesized in late summer-early autumn and stored in winter (translating into an increase in N during latewood formation and dormancy). Remobilization at the onset of spring growth depends upon the amount of nitrogen stored in the previous year but is independent of the

Fig. 1. Changes in N and $\delta^{15}\text{N}$ values at the intra-annual resolution of three (A), four (B), and five (C) sub-rings. A total of 24 rings from six Sitka spruce trees are illustrated.



amount of new growth or current nitrogen uptake from roots (Millard and Proe 1993; Weatherall et al. 2006). Interestingly, at the higher IAR of five sub-rings we could capture the late summer-early autumn synthesis of nitrogen reserves (corresponding to an increase in N dur-

ing latewood formation) presumably because latewood made up the major fraction of the last sub-ring analyzed.

While we illustrate that intra-annual variations in N values are strongly driven by physiological processes within the tree, the interpretation of variations in $\delta^{15}\text{N}$

values is more complex. At annual resolution, fluctuations of $\delta^{15}\text{N}$ values in tree rings are generally associated with environmental changes in nitrogen availability and can be either uncorrelated or correlated with fluctuations of N values (Poulson et al. 1995; Reimchen and Fox 2013; Reimchen et al. 2013; Gerhart and McLauchlan 2014). At sub-annual resolution, we demonstrate that tree-ring N and $\delta^{15}\text{N}$ values are uncorrelated in some watersheds but positively correlated in others, pointing to an environmental influence on nitrogen proxies. In foliar tissues, N and $\delta^{15}\text{N}$ values are positively correlated (Mathewson et al. 2003; Wilkinson et al. 2005; Craine et al. 2012; Reimchen 2017). Hence, the lack of consistent correlation between tree-ring N and $\delta^{15}\text{N}$ values might be attributed to nitrogen reallocation towards foliage during the growing season (Gerhart and McLauchlan 2014; Tomlinson et al. 2016) when lignification of wood cells releases high amount of nitrogen (Cantón et al. 2005). Nitrogen recycling towards the needles results in more enriched ^{15}N in the stem (Couto-Vázquez and González-Prieto 2010; Tomlinson et al. 2016) due to isotopic discrimination against ^{15}N (Evans 2001).

We determined that intra-annual variations in $\delta^{15}\text{N}$ values, unlike those in N values, are much less consistent among rings and among trees, suggesting that both ecological and physiological factors influence isotopic signatures in tree rings. Pronounced intra-annual oscillations in $\delta^{15}\text{N}$ values might reflect seasonal shifts in nitrogen demand between the needles and the stem, with peak $\delta^{15}\text{N}$ values in late growth likely corresponding to nitrogen reallocation towards foliage for storage (Millard and Proe 1992). At the same time, four out of the five watersheds in this study have salmon runs and are characterized by consistent bear-mediated uploading of salmon-derived nutrients to the riparian zone (Fisheries and Oceans Canada 1999; Hocking and Reimchen 2009). In this context, distance into the forest and upstream were found to be the strongest predictors of $\delta^{15}\text{N}$ values in Sitka spruce tree rings (Reimchen and Fox 2013). Further ecological factors influencing nutrient uploading, such as salmon abundance, bear, and scavenger activity as well as edaphic conditions, might account for differences in intra-annual variations in $\delta^{15}\text{N}$ values among rings and among trees (Hocking and Reimchen 2009; D'Amore et al. 2011; Reimchen 2017). We presume that years of exceptionally high density of spawning salmon provide trees with an elevated nitrogen pool such that they could operate a constant utilization of nitrogen reserves during the growing season, leading to reasonable intra-annual consistency from early to late growth.

This study successfully identified and quantified changes in N and $\delta^{15}\text{N}$ values during the growing season in the heartwood of a coniferous species. Old growth Sitka spruce from coastal British Columbia produces rings sufficiently wide to allow analysis of wood nitrogen at a finer temporal scale than achieved previously: at the

intra-annual level (sub-annual resolution). Although we do not determine the ecological or physiological origins to these seasonal changes in wood nitrogen, we demonstrate that N and $\delta^{15}\text{N}$ values vary substantially within rings, among rings, and among trees. We found that the range of N and $\delta^{15}\text{N}$ values within rings (intra-annual level) was on average equal to or up to seven-times the range among rings (inter-annual level). Our results allude to the potential importance of such intra-ring data for interpreting seasonal trends in nitrogen use and increasing understanding of ecological processes in the marine-terrestrial interface. Our findings serve as a foundation for future research on the seasonal cycling of salmon nutrients in coastal watersheds.

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