MEASUREMENT AND INTERPRETATION OF NITROGEN IN TREE RINGS

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ABSTRACT

Give the recent doubling of the transfer from the vast and unreactive atmospheric pool to biologically available forms on land, it is increasing important to better understand the effects of N deposition on the growth of mature trees (GCTE, 1999). A retrospective view would be helpful to know N availability and uptake by trees through time, preferably prior to the onset of anthropogenic impacts on N deposition. The paleoenvironmental subdiscipline of dendrochemistry might apply in this regard, but past dendrochemical research has led to the conclusion that N concentration variation in tree rings cannot provide information on past conditions of the environmental availability of N. Our objective here is to test strategies of wood extraction pretreatments to remove mobile N and thereby remove temporal variation in ring N concentration that relates to the heartwood-sapwood boundary and/or recently formed rings. Such extraneous variation could obscure the environmental signal of N availability and uptake in tree rings.

Three mature, dominant trees of Douglas-fir and of ponderosa pine of the Catalina Mountains of southern Arizona were studied. One increment core of each tree was left untreated, referred to as CONTROL. A second core from each tree was extracted for four hours in a 50:50 mixture of toluene and ethanol, then for four hours in ethanol, and then for one hour in distilled water, referred to as EXTRACT. A third core from each tree was also extracted, but each solvent was used for 24 hours, resulting in a total extraction time of three days, referred to as 3-DAY. For total N determination on decadal groups of rings, a semi-micro Kjeldahl method modified to recover NO^{-3} was used.

Average N concentration of EXTRACT cores was significantly less than that of CONTROL, and the coefficient of variation of EXTRACT cores was also significantly less than that of CONTROL. Five of the six CONTROL cores showed substantial change in N concentration between heartwood and sapwood rings and/or between recently formed versus older rings. In

contrast, four of the six EXTRACT cores showed no substantial change in N concentration between heartwood and sapwood rings and/or between recently formed versus older rings. Two of the six EXTRACT CORES retained some variation that may associate with the heartwood-sapwood boundary and/or most recently formed rings. The 3-DAY ponderosa pine cores confirmed, but did not improve upon, results obtained with extraction using the typical, shorter time duration. Pre-treating wood by extraction appears to substantially reduce the ambiguities of interpreting N concentration in tree rings as relative indicators of environmental availability at the time of ring formation. Further research on this topic is merited to investigate yet other pre-treatment strategies as well as the behavior of N in rings of trees with known differences in N availability.

Keywords: dendrochemistry, dendrochronology, nitrogen, tree rings

INTRODUCTION

Ecological research on the global nitrogen cycle has been emphasized recently (Mansfield et al., 1998), and this research emphasis extends from two notable features of N in the environment. One, anthropogenically enhanced atmospheric deposition of N has been occurring across many ecosystem types at continental to hemispheric scales (Mayewski et al., 1986) due to pollution from internal combustion engines (Russell et al., 1985) as well as from intensive agricultural activities (Galloway et al., 1995). Indeed, the most fundamental human-caused change in the global N cycle is the recent doubling of the transfer from the vast and unreactive atmospheric pool to biologically available forms on land (Vitousek et al., 1997).

Two, N is typically the most-limiting nutrient for tree growth because it is required in relatively large quantities while its inorganic ionic forms in soil are relatively rare (Pritchett and Fisher, 1987). Accumulation rates of biomass in terrestrial ecosystems are generally limited by N supply (Vitousek et al., 1997), and most temperate forest ecosystems therefore have a significant capacity to assimilate and retain additional N (Aber, 1992). Intuitively, increased N availability from atmospheric deposition should lead to increased tree growth (Norby, 1998), as has been shown in many forestry fertilization experiments (Kenk and Fisher, 1988; Johnson, 1992).

However, the details of how increased N deposition has affected tree growth and, by extension, how it might affect future tree growth and forest productivity, are still debatable. Hemisphere-scale N deposition represents a continuous addition to background N availability to natural forests, which is quite different from instantaneous applications of N as a fertilizer to intensively managed forests (Skeffington and Wilson, 1988; Aber et al., 1989; Johnson and Ball, 1990/91; Johnson, 1992). Additionally, too much N for too long a period may lead to a "satura-

tion" status, whereby trees may show no change in growth or even decline as a result of excessive N (Aber et al., 1989, 1998; Johnson and Ball, 1990/91).

An additional concern is how the effects of increased N deposition on tree growth might interact with those of CO_2, which is also increasing in the atmosphere (Hansen et al., 19998) and is also a limiting factor on tree growth (Kramer and Kozlowski, 1979). Some recent dendrochronological studies have shown increasing tree growth during the 20th century (Innes, 1991). This has been attributed that to increasing atmospheric CO_2 (LaMarche et al., 1984; Nicolussi et al., 1995), variable soil nutrient availability (Sheppard et al., in review), or some interaction of multiple single effects (Hättenschwiler et al., 1996). It is important to better understand these simple and interactive causes of widespread changes in tree growth and forest productivity (Norby, 1998), and for that it would be helpful to better understand the effects of N deposition on the growth of mature trees (GCTE, 1999).

In general, retrospective views of environmental processes help establish "normal" processes of the past against which to assess current and possibly abnormal departures (Arbaugh et al., 1999; Biondi, 1999). For example, it would be helpful to know N availability and uptake by trees through time, preferably prior to the onset of anthropogenic impacts on N deposition. Unfortunately, century-length time series of soil N availability at the tree level in natural forests do not exist readily, if at all. To overcome this limitation in general, the paleoenvironmental subdiscipline of dendrochemistry, which is the measurement, analysis, and environmental interpretation of elemental concentrations in tree rings (Lewis, 1995), seeks to place present nutrient or pollution phenomena in context of the past (Smith and Shortle, 1996). With specific respect to N, if its environmental availability and uptake through time were accurately and unambiguously reflected in its concentration in tree rings, it would be possible to assess the synchroneity and

possible dependence of well dated changes in N availability with well dated changes in tree growth.

Past dendrochemical research has led to the conclusion that N concentration variation in tree rings cannot provide information on past conditions of the environmental availability of N (Poulson et al., 1995). This conclusion has resulted from the general characteristic that N is highly mobile in xylem (Cutter and Guyette, 1993; Colin-Belgrand et al., 1996; Lévy et al., 1996). Specifically, temporal variation of N concentration in tree rings is typically associated with the heartwood-sapwood boundary and/or the most-recently formed rings (Merrill and Cowling, 1966; De Visser, 1992; Lévy et al., 1996).

However, various extraction and digestion pre-treatments have been tested on wood to remove soluble, mobile forms of N from wood prior to measuring N (Merrill and Cowling, 1966), and such pre-treatment strategies might reduce the extraneous effects of radial translocation that plague dendrochemistry in general (DeWalle et al., 1995). Our objective here is to test strategies of wood extraction pre-treatments to remove mobile N and thereby remove temporal variation in ring N concentration that relates to the heartwood-sapwood boundary and/or recently formed rings. Such extraneous variation could obscure the environmental signal of N availability and uptake in tree rings. Measuring only those nitrogenous compounds that are integral to the wood itself may result in dendrochemical analyses that are more valid (Cutter and Guyette, 1993). Merely succeeding at this would be a prerequisite for additional integrative research that evaluates ring N responses to increasing N deposition and ring width responses to increasing atmospheric CO_2 (Kiefer and Fenn, 1997).

METHODS

Study Sites

Two stands of trees were selected at ~2000 meters elevation on the south-facing side of the Catalina Mountains (32° 30' N, 110° 45' W) of southern Arizona. Douglas-fir (*Pseudotsuga menziesii*) trees were chosen from near the Bear Wallow Campground, and ponderosa pine (*Pinus ponderosa*) trees were chosen from near the Palisades Ranger Station. These two species were rated as "recommendable" for dendrochemistry based on various xylem features (Cutter and Guyette, 1993). According to weather records from nearby Tucson (32° 16' N, 111° 00' W, 788 meters elevation), the climate of the area is semiarid (mean annual precipitation of 300 mm with a summer maximum) and warm (mean annual temperature of 20° C). Because the selected stands are higher in elevation than Tucson, they have a climate that is both wetter and cooler.

Field Sampling

Field sampling took place in January 1998. From each stand, three mature, dominant trees without any outward evidence of past disturbances such as fires, insect attacks, etc., were sampled. Using a 5.2-mm-diameter tree increment borer, three cores were collected from each sampled tree, all from breast height. All three cores were along the same radius but were offset vertically from each other by two centimeters. Thus, the three cores of each tree were nearly identical to each other with respect to the rings that were collected. The cores were transported from field to lab in paper straws.

Dendrochronological Laboratory Analysis

The cores were temporarily mounted without gluing using a vise-like holder (Phipps, 1985). Using steel razor blades, small wood shavings were cut off a transverse surface in order to see all tracheids using a microscope. All samples were crossdated by matching temporal patterns of

ring-width variation between cores within each species (Douglass, 1941), and the dating was verified by comparing it to existing dendrochronologies from the International Tree-Ring Data Bank (Grissino-Mayer and Fritts, 1997).

Extraction Pre-Treatment

One core of each tree was left untreated and referred to as CONTROL. One of the remaining cores was extracted for four hours in a 50:50 mixture of toluene and ethanol, then for four hours in ethanol, and then for one hour in distilled water (Park et al., 1992). Each solvent was alternately vaporized and condensed in a Soxhlet extraction apparatus. These cores were referred to as EXTRACT. The third core of each tree was also extracted, but each solvent was used for 24 hours, resulting in a total extraction time of three days. These cores were referred to as 3-DAY.

All cores were separated into decadal to multi-decadal groups of rings so that each group weighed at least 100 mg, which we calculated to be the minimum sample mass necessary for measuring total N using the Kjeldahl digestion method (Bremner, 1996). Using a Wiley mill, each group of rings was ground into chips that passed through a 40-mesh screen. Milled samples were stored in paper envelopes and oven dried at 60° C.

Nitrogen Measurement Using Kjeldahl

For total N determination, a semi-micro Kjeldahl method modified to recover NO^{-3} was used (Bremner, 1996). The digestion block contained slots for 40 tubes, 30 of which were allocated for tree-ring samples while the remaining 10 were reserved for replicate blanks and two levels of known standards (0, 0.090, and 5.0 mg N). In sequence, 1 mL of $KMnO_4$, 2 mL of 50% H_2SO_4, 0.5 g of reduced iron, 1.1 g of catalyst (K_2SO_4, $CuSO_4$, and Se), and 3 ml of concentrated H_2SO_4 were added to all tubes and subsequently mixed and heated. A refluxing manifold was placed

over all test tubes and the block temperature was raised slowly to ~400° C, where it remained for several hours.

Following the acid digestion step, resultant residues were re-hydrated with 15 mL of de-ionized water and 20 mL of 10M NaOH. The N in each test tube was then distilled as $NH_{3(g)}$ into a boric acid indicator solution titrated with 0.0025M H_2SO_4. N concentration of each tree-ring sample was then calculated as the ratio of N mass to oven dry wood mass.

The 30 tree-ring samples per Kjeldahl batch run were allocated such that 10 samples of each of 3 cores were measured at a time. Separate digestion batches were conducted by tree species and wood pre-treatment strategy. That is, all Douglas-fir CONTROL samples were measured in one Kjeldahl run, all ponderosa pine EXTRACT samples were measured in another run, and so on. With 10 groups of rings per pre-treatment, 3 pre-treatments per tree, 3 trees per species, and 2 species, a total of 180 measurements were made in 6 Kjeldahl batch runs (Table 1).

RESULTS

Because 100 mg of wood were needed for analysis, the temporal resolution of N concentrations of tree-ring increment core samples ranged from one to four decades (Figures 1 and 2). Average N concentration of all CONTROL cores ranged from 846 to 1859 mg Kg^{-1}, and the coefficient of variation ranged from 53 to 116%. The Douglas-fir B core showed an anomalous value of 8442 mg Kg^{-1} N for the 1880s sample (Figure 2), which was considered to be a measurement error and therefore was not included in subsequent analyses or interpretations. Almost all of the measured rings of the three ponderosa pines were sapwood, making it impossible to evaluate any association between variation in N concentration and the heartwood-sapwood boundary (Figure 1) in those samples. Nonetheless, CONTROL cores of pines A and C showed relatively high N concentration since 1950 and 1970 respectively. CONTROL cores of all three

Douglas-firs showed higher and/or rising N concentration in sapwood rings relative to those of heartwood (Figure 2).

Average N concentration of EXTRACT cores ranged from 649 to 958 mg Kg^{-1}, which was significantly less than that of CONTROL ($p < 0.05$ for two-tailed test of paired differences, $n = 6$) (Figures 1 and 2). The coefficient of variation of EXTRACT cores ranged from 11 to 58%, which was also significantly less than that of CONTROL ($p < 0.05$ for two-tailed test of paired differences, $n = 6$). EXTRACT cores of Douglas-firs A and C showed no substantial change in N concentration between heartwood and sapwood rings, but the EXTRACT core of Douglas-fir B showed an anomalously high N concentration just after the heartwood-sapwood boundary (Figure 2). EXTRACT cores of pines A and B showed no substantial change in N concentration between recently formed versus older rings, but the EXTRACT core of pine C showed a slight increase in N concentration since 1980 (Figure 1).

Average N concentration of the ponderosa pine 3-DAY cores ranged from 679 to 959 mg Kg^{-1}, which was nearly identical to the EXTRACT values of 751 to 958 mg Kg^{-1} (Figure 1). The coefficient of variation of ponderosa pine 3-DAY cores ranged from 33 to 52%, which again was nearly identical to the EXTRACT values of 27 to 52%.

During the Kjeldahl run of the Douglas-fir 3-DAY samples, we did not reasonably succeed at measuring the standards, as we came within ± 20% of known values for only half of the 10 replicates of blanks or standards. By contrast, we achieved reasonable success with the other five Kjeldahl batch runs, coming within ± 20% of the known values for most of the 10 blanks or standards. Consequently, we considered the results from the Douglas-fir 3-DAY samples to be invalid and they are not included in Figure 2.

DISCUSSION

Extraction of wood prior to Kjeldahl measurement significantly reduced temporal variation of N concentration, much of which was probably biogenically due to translocation of nitrogenous substances across rings after they were formed. More specifically, extraction eliminated most of the variation in N concentration that could be attributed to the heartwood-sapwood boundary (as in the case of the Douglas-firs) or to the most-recently formed rings (as in the case of the ponderosa pines). The three-day extraction pre-treatment of the ponderosa pine cores confirmed, but did not improve upon, results obtained with extraction using the typical, shorter time duration (Park et al., 1992).

The non-extractable N of the wood tissues was probably less soluble in tree sap and therefore less mobile across rings, and as such it is potentially interpretable as a relative measure of environmental N availability at the time of ring formation. Thus, pre-treating wood by extraction appears to substantially reduce the ambiguities of interpreting N concentration in tree rings as relative indicators of environmental availability at the time of ring formation. Further research on this topic should investigate yet other pre-treatment strategies as well as the behavior of N in rings of trees with known differences in N availability, such as those in long-term N fertilization experiments. Analyses of trees that have received fertilizer applications with labeled ^{15}N would also help confirm the extent to which wood pre-treatment solves the issue of N translocation across rings (Rolfe, 1974).

Our N concentrations using the Kjeldahl digestion method were generally close to those of past researchers who used either Kjeldahl (Merrill and Cowling, 1966; Lévy et al., 1996) or other methods (De Visser, 1992; Poulson et al., 1995), suggesting that our values are valid for analysis. However, the Kjeldahl digestion method has some drawbacks that make it unattractive for large-

scale dendrochemical studies of N. Most importantly, the need for 100 mg of wood squanders the original annual resolution of tree rings, at least when increment cores versus whole cross sections are collected from trees (Hall and Naumann, 1984). Given that many of the soil physical, chemical, and biological processes that combine to regulate nutrient availability vary quite slowly, the loss of temporal resolution from annual to decadal, or even multi-decadal, scales might not substantially degrade general dendrochemical studies of environmental N. However, some soil processes, for example various responses to surface fire, cause rapid changes in soil nutrient availability, including that of N (Raison, 1979), and dendrochemical analysis of nutrient and tree growth responses to such processes might be impossible with the decadal resolution of Kjeldahl measurements of increment core samples.

Additionally, each run of the entire Kjeldahl process of digestion, distillation, acid titration, and cleaning of glassware required two person-days of work, all to measure just N in just 30 tree-ring samples. As such, Kjeldahl measurement of N concentration is more time-consuming and labor-intensive than many other dendrochronological measurement tasks, and it is probably not tenable for measuring full site collections–and certainly not networks of sites–of dendrochemical samples. Furthermore, without ring carbon measurements, it is not possible to dendrochronologically analyze C:N, which would be pertinent to integrative research on tree growth responses to changes in N and carbon availability (Comins, 1997). The Kjeldahl method also requires strong acids and bases as well as strong oxidizers and known carcinogens. Consequently, the Kjeldahl method generates toxic wastes and potentially exposes analysts to dangerous reagents (Artiola, 1990).

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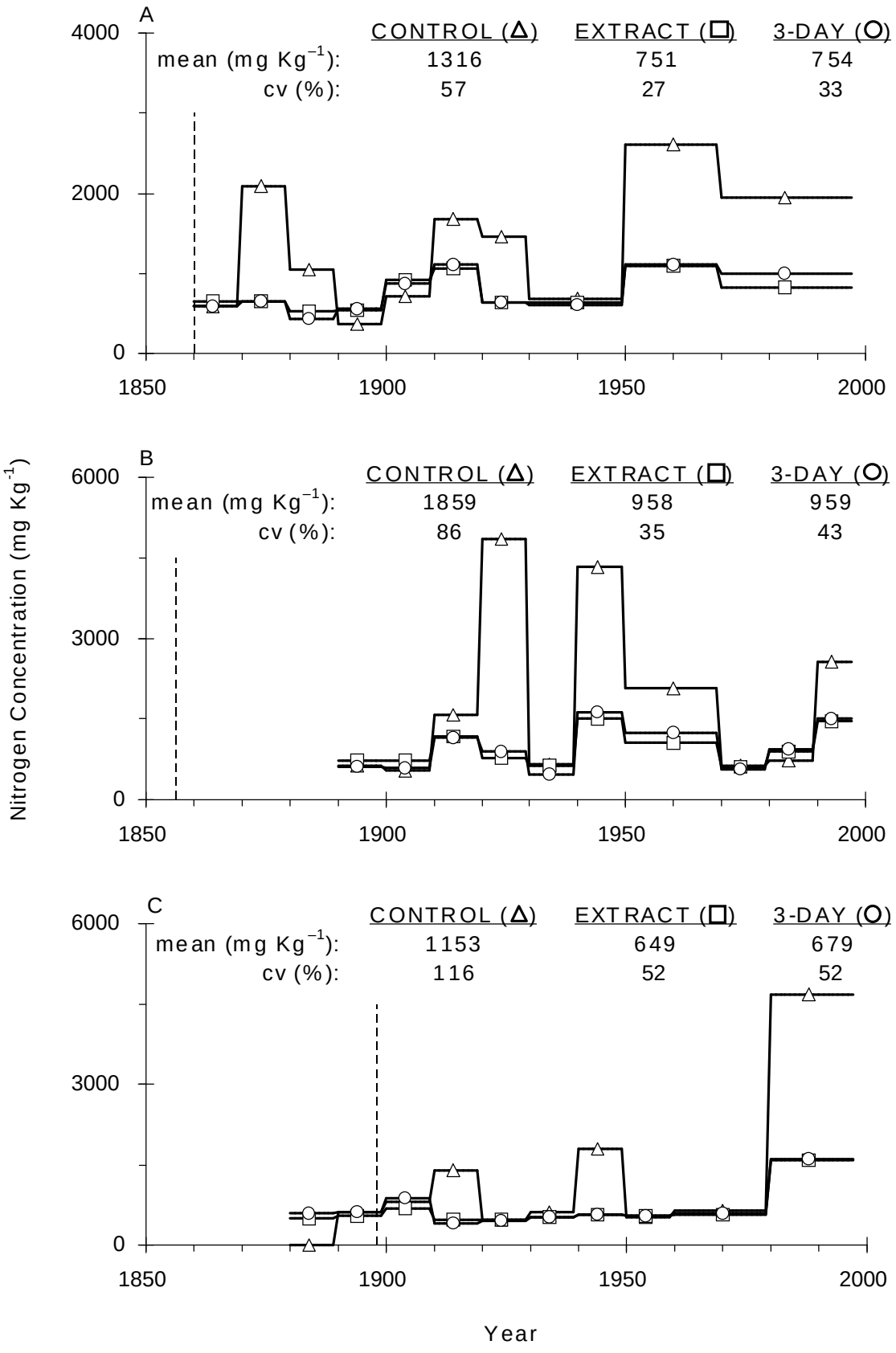
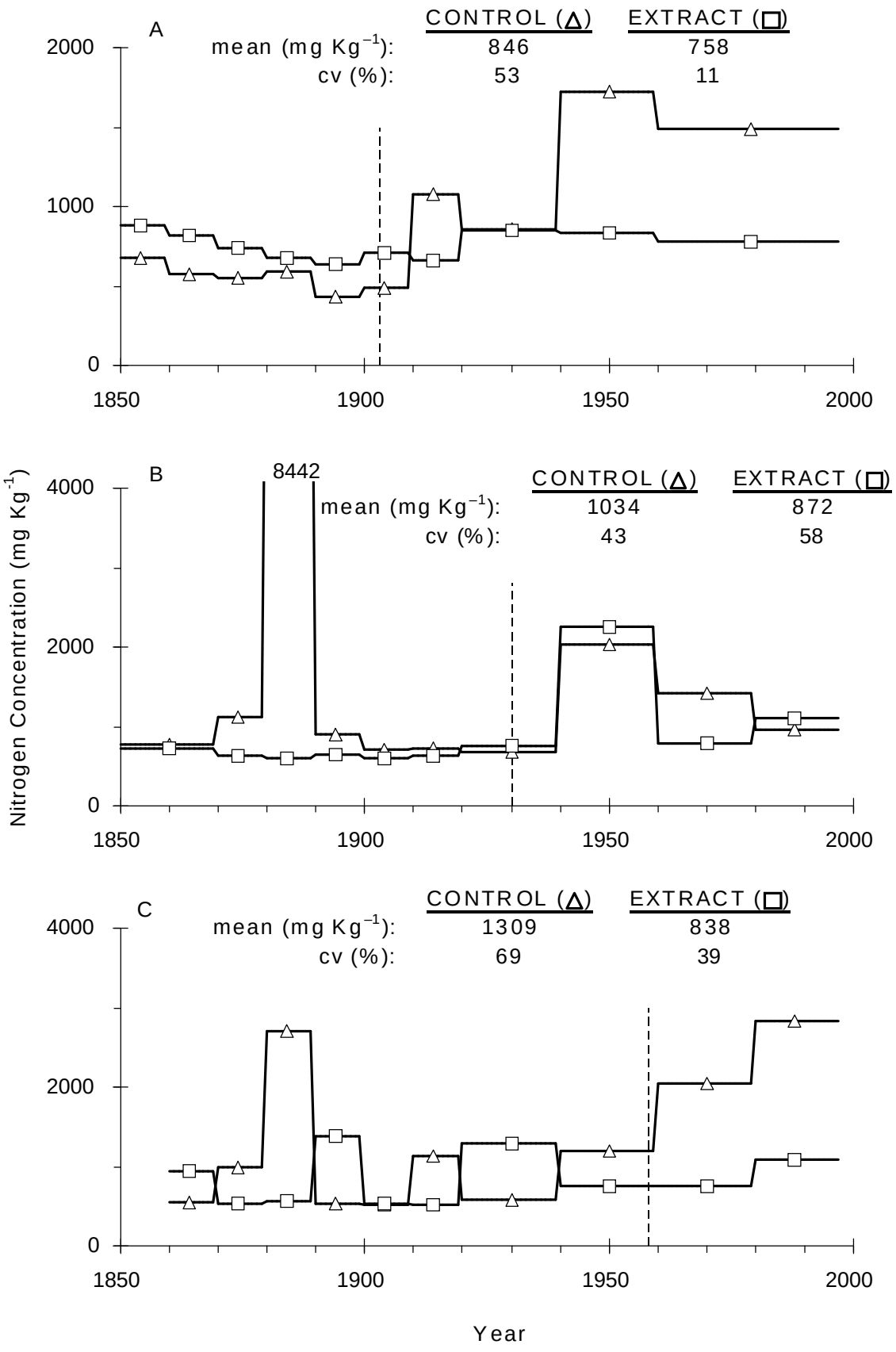
Table 1. Experimental Research Design to Test Pre-Treatment Strategies of Kjeldahl Measurement of Nitrogen in Tree Rings.

	CONTROL Pre-Treatment	EXTRACT Pre-Treatment	3-DAY Pre-Treatment
Douglas-fir	Trees A, B, and C 1 core/tree 10 samples/core	Trees A, B, and C 1 core/tree 10 samples/core	Trees A, B, and C 1 core/tree 10 samples/core
Ponderosa Pine	Trees A, B, and C 1 core/tree 10 samples/core	Trees A, B, and C 1 core/tree 10 samples/core	Trees A, B, and C 1 core/tree 10 samples/core

Figure 1. Time series of nitrogen concentration for ponderosa pine samples (trees A, B, and C). Solid lines with symbols represent CONTROL (–△–), EXTRACT (–□–), and 3-DAY (–○–) pre-treatments, and vertical dashed lines represent the hardwood-sapwood boundaries, which were determined visually by wood color. The means and coefficients of variation were calculated using just one value per group of tree rings, i.e., with a sample size of 10.

Figure 2. Same as in Figure 1, but for Douglas-fir samples. For the statistics of Douglas-fir B, the anomalous value of 8442 mg Kg^{-1} was excluded.


A
CONTROL (Δ)
EXTRACT (□)
3-DAY (○)
mean (mg Kg⁻¹):
1316
751
754
cv (%):
57
27
33
4000
2000
0
1850
1900
1950
2000
B
CONTROL (Δ)
EXTRACT (□)
3-DAY (○)
mean (mg Kg⁻¹):
1859
958
959
cv (%):
86
35
43
6000
3000
0
1850
1900
1950
2000
Nitrogen Concentration (mg Kg⁻¹)
C
CONTROL (Δ)
EXTRACT (□)
3-DAY (○)
mean (mg Kg⁻¹):
1153
649
679
cv (%):
116
52
52
6000
3000
0
1850
1900
1950
2000
Year


A
CONTROL (Δ)
EXTRACT (□)
mean (mg Kg⁻¹): 846 758
cv (%): 53 11
2000
1000
0
1850
1900
1950
2000
B
8442
CONTROL (Δ)
EXTRACT (□)
mean (mg Kg⁻¹): 1034 872
cv (%): 43 58
Nitrogen Concentration (mg Kg-1)
4000
2000
0
1850
1900
1950
2000
C
CONTROL (Δ)
EXTRACT (□)
mean (mg Kg⁻¹): 1309 838
cv (%): 69 39
4000
2000
0
1850
1900
1950
2000
Year