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Effect of Extraction Pretreatment on Radial Variation of Nitrogen Concentration in Tree Rings

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Abstract

Past research in the paleoenvironmental subdiscipline of dendrochemistry has concluded that N concentration variation in tree rings cannot provide information on past conditions of environmental availability of N. The objective of this study was to test wood extraction pretreatments to remove wood extractives and sap, both of which may obscure the environmental signal of N availability in tree rings. Three increment cores were collected from each of six trees (three ponderosa pines and three Douglas-firs). Within each tree, the first core was left untreated (referred to as CONTROL), the second core was extracted for several hours in organic solvents and distilled water (referred to as EXTRACT), and the third core also was extracted but for a total time of 3 d (referred to as 3-DAY). A semimicro Kjeldahl method was used to determine total N on decadal groups of rings. Average N concentration of EXTRACT cores was significantly less than that of CONTROL, and the coefficient of variation of EXTRACT cores also was significantly less than that of CONTROL. Most CONTROL cores showed substantial temporal variation in N concentration related to heartwood and sapwood and/or recently formed rings. In contrast, most EXTRACT cores showed no substantial change in N concentration related to heartwood and sapwood and/or recently formed rings. The 3-DAY cores confirmed, but did not improve upon, results obtained with extraction using the shorter time duration. Thus, pretreating wood by extraction appears to substantially reduce the variation in N concentration of tree rings, which is a necessary first step toward interpreting ring N as an indicator of past environmental N availability.

Atmospheric deposition of N has been enhanced anthropogenically across many ecosystems 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). When other nutrients are not limiting, increased N availability from atmospheric deposition can lead to increased plant growth (Norby, 1998), as has been shown for trees in many forestry fertilization experiments (Johnson, 1992). This is because N is typically the most-limiting nutrient for tree growth, as it is required in relatively large quantities (Taiz and Zeiger, 1991), while its inorganic ionic forms in soil are relatively rare (Pritchett and Fisher, 1987). 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 is a continuous addition to background N availability to natural forests, which is quite different from when N fertilizer is instantaneously applied to managed forests (Skeffington and Wilson, 1988; Aber et al., 1989; Johnson and Ball, 1990/91; Johnson, 1992).

Retrospective views of such environmental processes help establish "normal" processes of the past against which to assess current and possibly abnormal departures (Arbaugh et al., 1999). 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 by trees through time were accurately and unambiguously reflected in its concentration in growth rings, it would be possible to assess the synchroneity and possible dependence of well-dated changes in N availability with 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 observation that N is highly mobile in xylem (Cutter and Guyette, 1993; Colin-Belgrand et al., 1996; Lévy et al., 1996). In particular, radial 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 pretreatments have been shown to remove soluble forms of N from wood prior to measuring N (Merrill and Cowling, 1966), and such pretreatment might reduce the effects of extraneous variation that interferes with dendrochemical analysis (DeWalle et al., 1995). Our objective here is to test wood extraction pretreatments to remove mobile N and/or other extraneous compounds and thereby remove radial variation in ring N that is associated with the heartwood–sapwood boundary and/or recently formed rings. Such extraneous variation in wood

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N could obscure the environmental signal of N availability and uptake in tree rings. Measuring only N that is integral to the wood itself should result in dendrochemical analyses that are more valid (Cutter and Guyette, 1993), which would be a prerequisite for additional integrative research that evaluates ring N and ring-width responses to increasing N deposition (Kiefer and Fenn, 1997).

Materials and Methods

Study Sites

Two stands of trees were selected at ~2400 m elevation on the south-facing side of the Catalina Mountains (32°30′ N, 110°45′ W) of southern Arizona. These stands were chosen for their local convenience and the availability of existing tree-ring chronologies to facilitate crossdating (Grissino-Mayer and Fritts, 1997). Southern Arizona is not in a zone of extraordinarily high N deposition (Galloway et al., 1994) nor are the Catalina Mountains downwind of any intense point source of atmospheric N pollution. Accordingly, N availability at these sites probably has not varied substantially in the past. Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] trees were chosen from near the Bear Wallow Campground, and ponderosa pine (*Pinus ponderosa* P. Lawson & C. Lawson) 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 Palisades Ranger Station (for years 1965–1995), mean annual temperature is 9°C and mean annual precipitation is 762 mm with distinct winter and summer peaks.

Field Sampling

Field sampling took place in January 1998. From each stand, three mature, dominant trees with no outward evidence of past disturbances (such as fires or insect attacks) were sampled. Using a 5.2-mm-diameter tree increment borer, three cores were collected from each sampled tree, all from breast height (1.4 m). All three cores were along the same radius but were offset vertically from each other by 2 cm. Thus, the three cores within 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 from a transverse surface in order to see the rings clearly 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 with existing dendrochronologies.

Extraction Pretreatment

One core of each tree was left untreated and referred to as CONTROL. One of the remaining cores was extracted for 4 h in a 50:50 mixture of toluene and ethanol, then for 4 h in ethanol, and then for 1 h in distilled water (Park et al., 1992). This extraction strategy conforms to Tappi standards for removing secondary plant compounds, inorganic salts, and low-molecular-weight polysaccharides from wood (Pettersen, 1984; Fengel and Wegener, 1984). 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 also was extracted, but each solvent was used for 24 h, resulting in a total extraction time of 3 d. These cores were referred to as 3-DAY.

All cores were separated into groups of rings so that each group weighed at least 100 mg, which we estimated to be the minimum sample mass necessary for measuring total N using the Kjeldahl digestion method. Because of this minimum sample mass, the temporal resolution of N concentrations of tree-ring increment core samples ranged from one to four decades. 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 semimicro 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 rehydrated with 15 mL of deionized water and 20 mL of 10 *M* NaOH. The N in each test tube was then distilled as $NH_{3(g)}$ into a boric acid indicator solution and titrated with 0.0025 *M* H_2SO_4. Nitrogen concentration of each tree-ring sample was then calculated as the ratio of N mass to oven-dry wood mass. The method detection limit was approximately 50 mg kg^{-1}.

The 30 tree-ring samples per Kjeldahl batch run were allocated such that 10 samples of each of three cores were measured at a time. Separate digestion batches were conducted by tree species and wood pretreatment 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 pretreatment, three pretreatments per tree, three trees per species, and two species, a total of 180 measurements were made in six Kjeldahl batch runs. Nitrogen concentrations of the Douglas-fir 3-DAY samples were not included in subsequent analyses because measurements exceeded ±20% of known values of blanks and standards.

Quantitative Analysis

Data were displayed as time series in order to view radial variation relative to the heartwood–sapwood boundary and the most-recently formed rings. Mean values and coefficients of variation within cores were compared within trees and across treatments using two-tailed *t*-tests of means.

Results

Average N concentration of CONTROL cores ranged from 846 to 1859 mg kg^{-1}, which is similar to values of other research using nonextracted wood (Allison et al., 1963; Cotrufo, 1983; Cotrufo and Wells, 1984; Weetman and Wells, 1990; De Visser, 1992; Poulson et al., 1995) (Fig. 1 and 2). The coefficient of variation of CONTROL cores ranged from 53 to 116%. The Douglas-fir

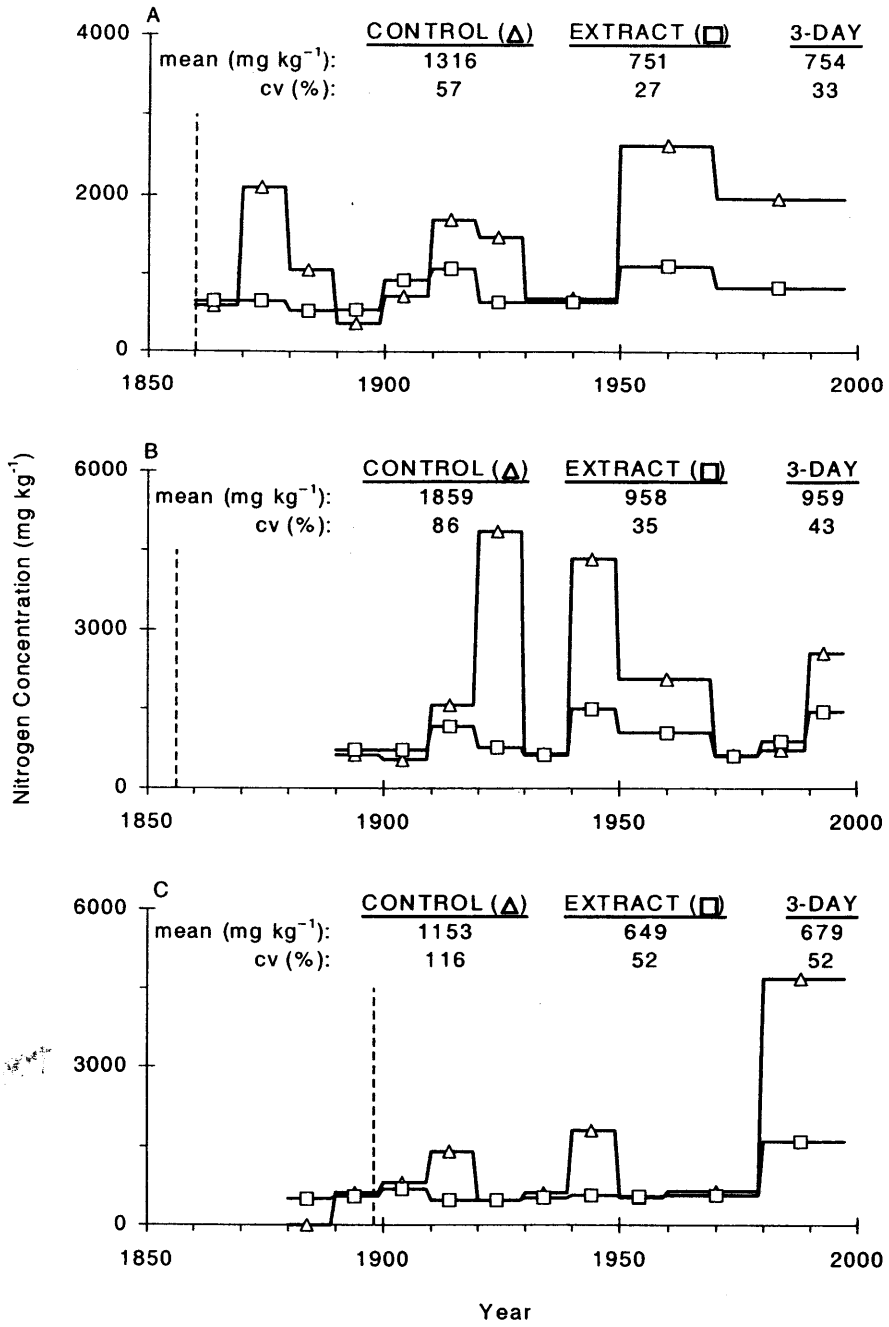

Fig. 1. Time series of nitrogen concentration for ponderosa pine samples (Trees *A*, *B*, and *C*). Solid lines with symbols represent CONTROL (△) and EXTRACT (□) pretreatments, and vertical dashed lines represent the heartwood–sapwood boundaries, which were determined visually by wood color. The means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

B core showed an anomalous value of 8442 mg kg^{-1} N for the 1880s sample (Fig. 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 in those samples (Fig. 1). 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 (Fig. 2).

Average N concentration of EXTRACT cores ranged from 649 to 958 mg kg^{-1}, which was significantly less than that of CONTROL ($p < 0.05$, $n = 6$) (Fig. 1 and 2). The coefficient of variation of EXTRACT cores ranged from 11 to 58%, which also was significantly less than that of CONTROL ($p < 0.05$, $n = 6$). 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

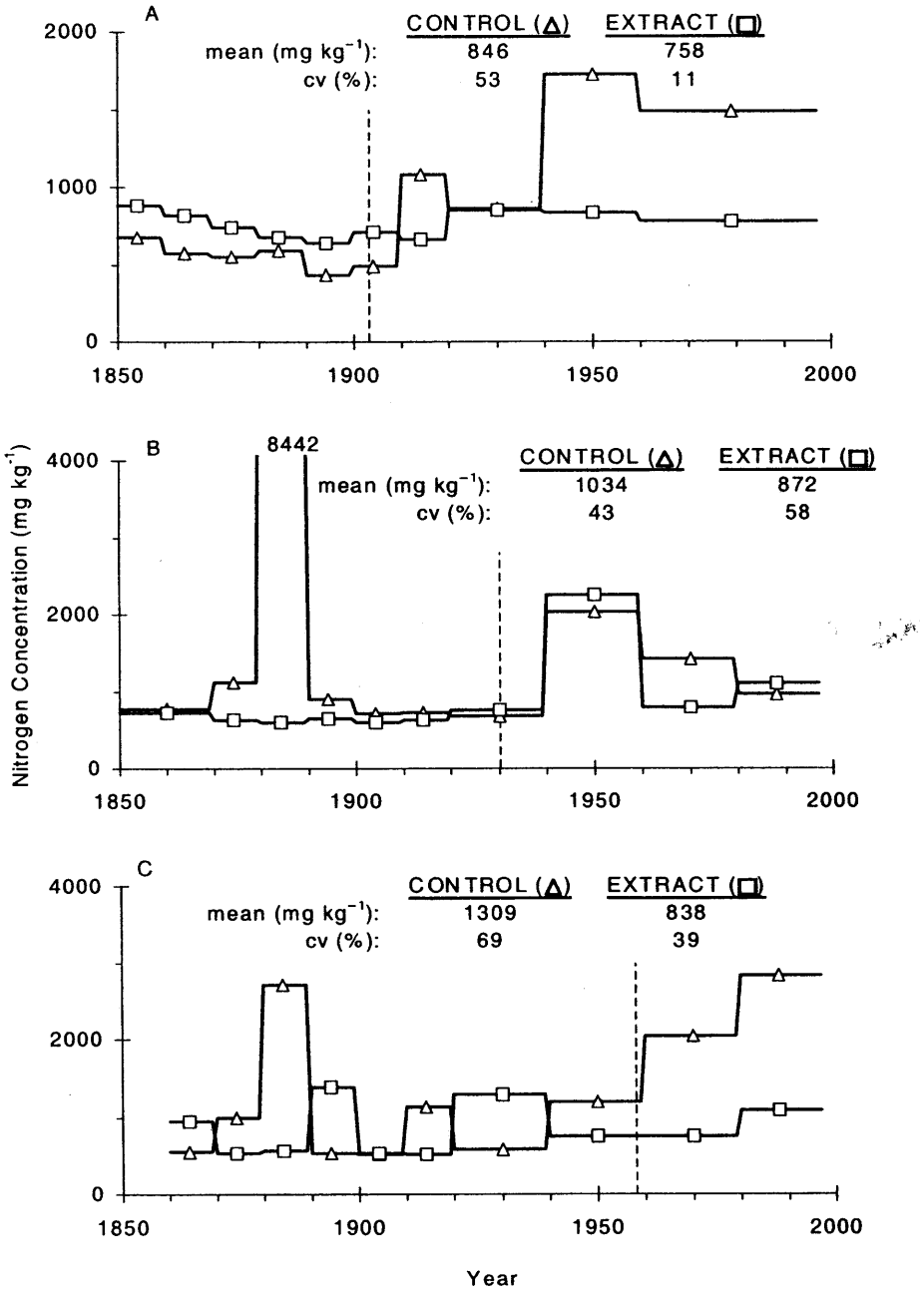

Fig. 2. Time series of nitrogen concentration for Douglas-fir samples (Trees *A*, *B*, and *C*). Solid lines with symbols represent CONTROL (△) and EXTRACT (□) pretreatments, and vertical dashed lines represent the heartwood–sapwood boundaries, which were determined visually by wood color. The means and coefficients of variation were calculated using just one value per group of tree rings (i.e., with a sample size of 10). For the statistics of Douglas-fir *B*, the anomalous value of 8442 mg kg^{-1} was excluded.

showed an anomalously high N concentration just after the heartwood–sapwood boundary (Fig. 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 (Fig. 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} (Fig. 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%. The 3-DAY time series overlaid nearly identically with those of EXTRACT and therefore were not included in Fig. 1.

Discussion

Our results conform to theoretical expectations based on much research on the chemistry of wood, extractives, and xylem sap. Xylem sap contains nitrogenous compounds, almost all of which are organic in form within

trees (Bollard, 1958; Barnes, 1963). The nitrogen concentration of xylem sap can be as high as ~200 mg kg^{-1} (Bollard, 1958), but is known to vary by as much as 10-fold intra-annually in response to phenological events and changes in N availability (Bollard, 1958; Barnes, 1963; Carter and Larsen, 1965; Cotrufo and Wells, 1984). This seasonal N variation has been shown specifically for sap of ponderosa pine (Stark and Spitzner, 1985) and Douglas-fir (Stark et al., 1985). Thus, while the extent of xylem sap as a source of error in dendrochemical analysis of tree rings is variable, it is clear that xylem sap and its solutes should be routinely removed from tree-ring samples prior to measuring N. This is especially true because moisture content, which indicates the amount of sap in wood, typically varies between heartwood and sapwood, with sapwood usually being wetter (Hillis, 1962). Removing sap and its solutes should lower N concentrations of rings of sapwood, as occurred in this study.

Plant extractives can be classified into three groups: nonstructural polyphenols, which are variably soluble in organic solvents or water; terpenes, which are soluble in water; and nitrogenous compounds (e.g., alkaloids), which are soluble in water (Taiz and Zeiger, 1991). The content of extractives in wood can be as high as 5%, but they are typically more abundant in heartwood than in sapwood (Hillis, 1962; Mutton, 1962; Browning, 1963; Sjöström, 1981; Kai, 1991). Wood extractives are not strongly bound to cell walls, but rather they are typically located within the lumina of tracheids or in specific structures such as resin canals (Kai, 1991). Thus, regardless of what extractives any particular tree species has or whether or not those extractives contain N, it is clear that they also should be routinely removed from tree-ring samples prior to measuring N. Even if extractives contained no N, they would be an indirect source of error because of their extra mass (Cowling and Merrill, 1966). In the case of temperate conifers, the dominant wood extractive is resin, which includes many different compounds (Mutton, 1962; Fengel and Wegener, 1984), while alkaloids are rare (McNair, 1935; Buchanan, 1963). Thus, extracting resins, which are non-nitrogenous, could actually raise N concentration of rings of heartwood, as occurred in one of the Douglas-firs of this study (Fig. 2A).

As for the wood tissue itself, it is composed chiefly of cellulose, hemicelluloses, and lignin (Dadswell and Hillis, 1962; Browning, 1963; Taiz and Zeiger, 1991). Most of the cell wall material is insoluble in organic solvents or water (Browning, 1963), though some hemicelluloses dissolve in boiling water (Pettersen, 1984; Sjöström, 1981). In addition to those carbon-based compounds, protein is an integral part of plant cell walls (Lamport and Northcote, 1960; Browning, 1963; Buchanan, 1963; Merrill and Cowling, 1966), where it is thought to control cell wall extension by providing cross-linkages between cellulose microfibrils (Lamport, 1962). In particular, a cell-wall protein called extensin functions as a structural network to strengthen the wall (Cowling and Merrill, 1966; Taiz and Zeiger, 1991). Most cell wall proteins are fully incorporated into the walls (Lamport, 1962) and therefore may be considered insoluble in organic solvents and water. Thus, cell-wall N should remain in tree-ring samples even after pretreatment with organic solvents and water (Cowling and Merrill, 1966).

Nitrogen concentration values in extracted wood of this study generally agree with those of other research using only ethanol extraction (Merrill and Cowling, 1966). Extraction of wood significantly reduced radial variation of N concentration, much of which was probably due to the effects of xylem sap solutes in sapwood and/or extractives in heartwood. 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 3-d extraction pretreatment of the ponderosa pine cores confirmed, but did not change, results obtained with extraction using the shorter duration (Park et al., 1992), which can be interpreted as evidence that extraction removes soluble N compounds without affecting cell-wall N. Our extraction strategies did not remove all radial variation in N concentration, and further research on extraction might include adding other solvents (e.g., acetone and alcohol [Buchanan, 1963]) as well as grinding the wood samples prior to extraction to maximize surface area contact with the solvents (Browning, 1963).

These results do not speak directly to whether or not the concentration of cell-wall N in tree rings reflects N availability at the time of ring formation, but other research provides hope in this regard. Nitrogen content of living cells within the cambial zone has been shown to correlate directly with N availability (Cowling and Merrill, 1966). If new xylem cells initiated by the cambium reflected the N content of the cambial cells, then the N content of the cell walls of any given ring would reflect N availability for that year. Further research on this topic should include trees for which nutrient availability through time has been tightly controlled and recorded (e.g., forestry fertilization trials) to calibrate ring N responses to known changes in N availability. Analysis of trees that have received fertilizer applications with labeled ^{15}N also would help confirm the extent to which wood pretreatment improves the interpretability of N in tree rings (Rolfe, 1974).

Conclusions

If N concentration of wood can be dendrochemically interpreted for N availability at the time of ring formation, it will probably need to be done on extracted wood. Measuring N in nonextracted wood is not recommended because of the presence of xylem sap solutes and extractives. Fortunately, extracted wood still contains measurable quantities of N and its concentration has at least a theoretical basis for relating to N availability at the time of ring formation.

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