# Ionic balance and organic acids in western redcedar, western hemlock, and Douglas-fir seedlings grown in low- and high-N soils[1]

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**Abstract**. Seedlings of western redcedar (*Thuja plicata* Donn ex. D. Don), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were transplanted into soils with low and high levels of available $NO_3^-$ (and total N). Current-year foliage was sampled after 10 weeks to determine the effect of N availability on foliar cation–anion balance (C–A) and the concentrations of low molecular weight organic acids of the three species. Carboxylate concentrations were estimated by using the difference between sums of cations and anions (C–A): 750 mequiv.·kg$^{-1}$ for western redcedar, 351 mequiv.·kg$^{-1}$ for western hemlock, and 266 mequiv.·kg$^{-1}$ for Douglas-fir. Quinic acid was a primary constituent, accounting for 40% of the total for western redcedar and 75% for western hemlock and Douglas-fir. Oxalic acid was present in greatest concentration in the foliage of western redcedar (65 mequiv.·kg$^{-1}$) but was a minor constituent in western hemlock and Douglas-fir. The quantified acids accounted for only 15% of the C–A of western redcedar but >80% of the C–A of western hemlock and Douglas-fir. A considerable portion of the C–A balance not accounted for in redcedar may be associated with the accumulation of $CaCO_3$. Litterfall deposition of $CaCO_3$ may lead to the consumption of $H^+$ ions and enrichment of exchangeable soil Ca in the rooting zone of long-lived western redcedar trees. No statistically significant differences among the soils were detected with regard to C–A or the concentration of organic acids.

**Résumé** : Des semis de thuya géant (*Thuja plicata* Donn. ex D. Don), de pruches de l'Ouest (*Tsuga heterophylla* (Raf.) Sarg.) et de Douglas vert (*Pseudotsuga menziesii* (Mirb.) Franco) ont été transplantés dans des sols dont les niveaux de $NO_3^-$ disponible (et de N total) étaient faibles ou élevés. Le feuillage de l'année courante a été échantillonné après 10 semaines pour mesurer l'effet de la disponibilité de N sur la balance cations–anions (C–A) et la concentration d'acides organiques de faible poids moléculaire chez les trois espèces. La concentration d'acides carboxyliques a été estimée par la différence entre la somme des cations et des anions (C–A) : 750 méquiv.·kg$^{-1}$ chez le thuya géant, 351 méquiv.·kg$^{-1}$ chez la pruche de l'Ouest et 266 méquiv.·kg$^{-1}$ chez le Douglas vert. L'acide quinique était le principal constituant, représentant 40% du total chez le thuya géant et 75% chez la pruche de l'Ouest et le Douglas vert. L'acide oxalique était présent en concentration la plus forte (65 méquiv.·kg$^{-1}$) dans le feuillage du thuya géant, mais il s'agissait d'un constituant mineur chez la pruche de l'Ouest et le Douglas vert. Les acides qui ont été quantifiés représentaient seulement 15% de la balance cations–anions chez le thuya géant, mais plus de 80% chez la pruche de l'Ouest et le Douglas vert. Une portion considérable de la balance cations–anions reste inexpliquée chez le thuya géant et pourrait être associée à l'accumulation de $CaCO_3$. Le dépôt de $CaCO_3$ via la chute de litière pourrait entraîner la consommation d'ions $H^+$ et l'augmentation de Ca échangeable du sol dans la zone d'enracinement des thuya géants qui ont une longue durée de vie. Aucune différence statistiquement significative dans la balance cations–anions ou la concentration des acides organiques n'a été observée entre les sols.

[Traduit par la rédaction]

## Introduction

Uptake of N from soils by plants usually involves the inorganic N forms ammonium ($NH_4^+$) and nitrate ($NO_3^-$). Because a plant's requirement for N is greater than that for all other nutrients, the form of N exerts the primary influence on the ionic balance of the cells. Plants must regulate ionic balance to maintain cellular pH and electrochemical potential (Raven and Smith 1976), and their ionic status has been inferred from the balance of inorganic cations and anions (C–A) (Ingestad 1976; Israel and Jackson 1982). The C–A in the tissue of plants grown on $NH_4^+$ has been found to be substantially lower than for plants of the same species supplied with $NO_3^-$ (Kirkby and Mengel 1967; Nelson and Selby 1974; Raven and Smith 1976).

Western redcedar (*Thuja plicata* Donn ex D. Don), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) are coniferous tree species associated with each other throughout the Pacific Northwest across a range of soils that vary greatly with respect to physical and chemical properties (Radwan and DeBell 1980; Radwan and Harrington 1986; Turner et al.

1993). The pH and Ca concentrations of litter and foliage tend to be highest for western redcedar, lowest for western hemlock, and intermediate for Douglas-fir (Daubenmire 1953; Alban 1969). High rates of nitrification have been observed in the litter and soils beneath western redcedar, whereas it has been suggested that western hemlock actively inhibits nitrification (Turner and Franz 1985; Turner et al. 1993).

Excess base concentrations (C–A) of the needles and stems of 26 conifer species were reported by Crooke et al. (1964), who found 720 mequiv.·kg$^{-1}$ in western redcedar, 280 mequiv.·kg$^{-1}$ in western hemlock, and 210 mequiv.·kg$^{-1}$ in Douglas-fir. The high C–A of western redcedar may have resulted from the assimilation of $NO_3^-$ in the foliage. The typically high concentrations of Ca in the foliage of western redcedar (e.g., Daubenmire 1953; Crooke et al. 1964; Imper and Zobel 1983; Radwan and Harrington 1986) may in part be related to charge balance associated with the uptake of $NO_3^-$. Nitrate assimilation in the foliage would result in the production of organic acid anions to maintain ionic balance. Plant species that accumulate excess Ca and have the potential to reduce $NO_3^-$ in their foliage have been shown to accumulate oxalic acid (Chandler 1937; Franceschi and Horner 1980). The high C–A of western redcedar (Crooke et al. 1964) may be associated with foliar accumulation of oxalic acid, but oxalic acid has rarely been identified in conifers (Chandler 1937). Furthermore, few investigations have attempted to correlate C–A with the actual content of low molecular weight organic acids.

In the current study, western redcedar, western hemlock, and Douglas-fir were grown on soils of low and high $NO_3^-$ availability so that the influence of N nutrition on growth and C–A could be assessed. The objectives of this study were to determine (*i*) the effect of N availability on the foliar C–A of western redcedar, western hemlock, and Douglas-fir; (*ii*) whether C–A in the foliage is balanced by the presence of low molecular weight organic acids; and (*iii*) the identity of organic acids associated with C–A balance of the three conifers.

## Methods

### Site and soil descriptions

Two soils representative of low and high N status were collected in two areas in early May 1987 from the upper 15 cm of the A horizon. The nitrogen-deficient Wind River soil (designated "low-N soil" here) was obtained from site IV land adjacent to a Douglas-fir spacing trial established in 1925 near Carson, Wash. Curtis and Reukema (1970) describe the soil as "a loose sandy loam with sporadic admixture of basaltic gravel and cobble developing on pumiceous alluvium underlain by lightly fractured basaltic rock." A fire in 1924 consumed much of the duff and debris, exposing mineral soil. The intensity of the burn led to formation of extremely stable soil aggregates that comprise approximately 20% of the soil mass. The area is currently occupied by a 60-year-old stand of Douglas-fir; associated vegetation is typical of the PSME/GASH community type (*Pseudotsuga menziesii / Gaultheria shallon* Pursh) (Corliss and Dyrness 1965).

The nitrogen-rich soil (designated "high-N soil") was obtained near the Oregon coast at the Cascade Head Experimental forest in a 45-year-old ALRU/RUSP/POMU community type (*Alnus rubra* Bong. / *Rubus spectabilis* Pursh / *Polystichum munitum* (Kaulf.) Presl.) (Corliss and Dyrness 1965) dominated by *Pseudotsuga*

*menziesii*, *A. rubra*, and *Picea sitchensis* (Bong.) Carr. The organically rich silt to silty clay loam Typic Dystrandept is most likely of the Hembre or Astoria series (Bowlsby and Swanson 1964).

At the two sites, soils were pushed through a large framed 4-mm sieve as they were collected to remove coarse material and plant roots. Nylon feed bags were used to transport the soils to the Oregon State University (OSU) campus, where they were stored in a cold room at 4°C for a week until seedlings were delivered. Samples of the soils were taken immediately for determination of soil C and N content, mineralizable N, potential N mineralization and nitrification, and pH.

### Soil chemical analyses and incubations

Chemical properties of the soils were determined by the following methods: soil pH, at a soil : distilled–deionized water ($H_2O_{dd}$) ratio of 1:2 (g·g$^{-1}$); total N, by Kjeldahl digestion; mineralized $NH_4^+$ and $NO_3^-$, by extraction from soil with 2M KCl (Horneck et al. 1989); and organic C, by combustion of soil samples in a Leco induction furnace. Laboratory incubations were used to determine potential N mineralization and nitrification of the two soils. Sixty 20-g samples of field-moist soil (oven-dried basis) were incubated in 100-mL cups with lids. Samples of each soil were assigned randomly to one of two treatments: (*i*) control or (*ii*) amendment with 77 mg·kg$^{-1}$ N as $(NH_4)_2SO_4$. A stock solution of $(NH_4)_2SO_4$ was prepared (3.51 g·L$^{-1}$). Four millilitres of $H_2O_{dd}$ or 2 mL each of $H_2O_{dd}$ and the stock solution were applied to the surface of the soil in the cups. Additional $H_2O_{dd}$ was added to bring the soils to field capacity (gravimetric water content: 0.31 g·g$^{-1}$ low-N soil; 0.55 g·g$^{-1}$ high-N soil). Samples were incubated in the dark at 25°C, aerated daily, and weighed at 3- or 4-day intervals. Water was added as needed to maintain the original mass. Twelve samples, three randomly selected replicates from each of the four soil by N amendment treatments, were extracted with 50 mL of 2 M KCl at 3, 7, 21, 35, and 49 days. Solution concentrations of $NH_4^+$-N and $NO_3^-$-N were determined on an autoanalyzer (Scientific Instruments CFA 200) by the Soil Testing Laboratory at OSU ($NH_4^+$ by salicylate–nitroprusside; $NO_3^-$ by diazotization following Cd reduction).

### Growth chamber study

Three 1-year-old plug seedlings of each species (western redcedar, western hemlock, and Douglas-fir) were planted in 48 paperpots (30.5 cm diameter lip by 46 cm depth), with half the pots containing low-N and half high-N soil. To minimize variation attributable to soil heterogeneity and seedling interspecific competition, the trees were positioned with a circular template such that one member of each species was planted for each of the three triangular pie-shaped subdivisions within each pot (three seedlings of each species per pot). Distilled water (control) or ammonium sulfate (60 mg·kg$^{-1}$ N) was applied in solution to the surface of the soil of half the pots. The $(NH_4)_2SO_4$ treatment was included in the experiment to increase the availability of N, especially in the low-N soil. The solutions were allowed to percolate in before more water was added to bring the soils to field capacity. Ultimately, each of the four treatments (2 soils × 2 levels of N amendment) was replicated in 12 pots. Forest soils and $(NH_4)_2SO_4$ were used to prevent possible effects of chloride on seedling growth or on the C–A balance; this problem was inherent in an earlier study (Krajina et al. 1973) where N was added as $NH_4Cl$ to a sandy soil. The pots were randomly positioned in the growth chamber (16 h light (140 µmol·m$^{-2}$·s$^{-1}$, 20°C, RH 45%) : 8 h dark (15°C)). To minimize leaching losses, approximately 1.5 L of $H_2O$ was applied to the soil in each pot every 3–7 days when the base of the pot was dry to the touch. After 8 weeks, seedlings from 6 of the 12 replicate pots for each treatment were sampled for dry matter and N (unpublished data).

The concentrations of cations (Ca, K, Mg, Na, Al, Fe, and Mn), anions (from P and S; $NO_3^-$), and low molecular weight organic acids in the current-year foliage were determined after 10 weeks of seedling growth. A 3-g sample of foliage was collected from each species from the six additional pots per treatment. Half of each sample (1.5 g) was placed in an envelope and oven-dried at 70°C for 72 h prior to dry mass determination and subsequent ion analysis. The replicate 1.5 g portions from each pot were stored in 9.6 mL of acidified ethanol (0.375% $H_2SO_4$) (Phillips and Jennings 1976) in polypropylene tubes that were immediately placed in liquid $N_2$ and later moved to a –80°C freezer. This tissue was utilized for quantitative determination of organic acids by high performance liquid chromatography (HPLC).

### Ion analysis

The dried tissue was ground in a Wiley mill (20 mesh), and 250 mg of the dry material was placed in a clean 250 mL Erlenmeyer flask and subjected to nitric–perchloric acid digestion by the Soil Analysis Laboratory at OSU (Horneck et al. 1989). In addition to the original 72 preparations (3 species × 2 soils × 2 levels of N × 6 replications), five replicate samples of pine needle tissue (SCM 1575) certified by the National Bureau of Standards (1976) were analyzed. The solutions were refluxed for 1 h to enhance the retention of sulfur. The extracts were decanted and brought to 50 mL final volume in preparation for quantitation of Ca, K, Mg, P, S, Na, Al, Fe, and Mn by ion coupled argon plasma atomic emission spectroscopy (ICAP-AES) (Jones 1977), performed by the Plant Analysis Laboratory, OSU. Standard solutions were prepared with AES materials. Acid content (6% $H_4ClO_4$) of blank and standard solutions was carefully matched to the matrix in which the digested tissue was dissolved.

The equivalent concentrations of cations and P ($H_2PO_4^-$ at physiological pH of plant cells) were calculated directly from the tissue concentrations found with ICAP, and inorganic sulfate was estimated from total S and N by the following equation (Turner 1979):

$$SO_4^{2-}\text{-}S = [\text{total S moles} - (0.03)$$

$$\times (\text{total N moles})](32.06\ \text{g·mol}^{-1}\ S)$$

Nitrate-N was determined colorimetrically with a spectrophotometer (410 nm) after extraction of 100 mg of dried foliage in hot $H2O_{dd}$ (Cataldo et al. 1975). Cation–anion difference was estimated as the sum of the equivalent amounts of cations (Ca, K, Mg, Na, Al, Fe, and Mn) minus the sum of the equivalent amounts of anions ($H_2PO_4^-$, inorganic $SO_4^{2-}$, and $NO_3^-$).

### Extraction of organic acids

Initial attempts to extract organic acids by elution from anion-exchange resins with acetic acid (Phillips and Jennings 1976) were unsuccessful. The labor-intensive method yielded small or no traces of the constituent acids. The protocol was changed to that documented by Libert (1981). Prior to extraction, the ethanol was evaporated from the vials in a vacuum desiccator, and the dried tissue was ground with mortar and pestle in the presence of liquid $N_2$ to produce a fine powder. The powder was weighed, then immersed in 6 mL of 0.02 M HCl. Nonvolatile low molecular weight organic acids were extracted by continuously shaking the solution overnight. The pH of the extract solutions after 12 h was approximately 1.30 and did not vary among the three conifer species. The solutions were centrifuged at 8000 r/min for 50–60 min to pellet out the solids (dried tissue). The supernatant was taken up in a syringe and passed through a preconditioned Sep-pak C-18 cartridge (Waters Associates) attached in series to a 0.22-µm filter to remove nonpolar compounds and particulates. This process yielded 1.0–2.0 mL of extract for analysis by HPLC.

Extract solutions were diluted 1:3 to 1:15 (usually 1:4) with 0.02 M HCl prior to injection of at least two separate 10-µL aliquots of each sample into the HPLC system. The mobile phase (0.02 M $H_2SO_4$) continuously flowed at 0.4 mL·min$^{-1}$ (970 000 psi; 1 psi = 6.895 kPa). Acids were detected and quantified on the basis of retention times and concentrations of known standards at 210 nm (Fig. 1). Standard solutions were prepared with salts of nine organic acids in 0.02 M HCl (oxalic, citric, tartaric, malic, quinic, succinic, shikimic, acetic, and fumaric). Fumaric acid was displaced from the column at 23 min; therefore, the selected interval between sample injections was 32 min. Citric acid, which was not found in tissue extracts during preliminary analyses, was used as an internal standard at a solution concentration of 0.15 mg·mL$^{-1}$.

The HPLC system included a Beckman controller (No. 420), solvent delivery module (No. 112), variable wavelength detector (No. 164), and autosampler (No. 406A). A strong cation exchange Bio-Rad Aminex HPX-87H organic acid column (10 µm × 250 mm, No. 125–0140) was protected in series by a Bio-Rad micro-guard column (No. 125–0129). Separation was achieved by ion exclusion and partition chromatography mechanisms leading to acid emergence from the column in order of increasing pKa (Turkelson and Richards 1978). Maxima chromatography software integrated through a Keithley interface was used to collect and analyze data.

Apparent co-elution of malic with quinic acid and succinic with shikimic acid on the HPX-87H column necessitated confirmation of peak identification. Therefore, a set of tissue extracts was sent to the OSU Food Science Laboratory for analysis on a reverse-phase HPLC system modified from Coppola and Starr (1986). A phosphate buffer was used as the mobile phase flowing in series through a Bio-Rad reverse-phase micro-guard column, a 25-cm Supelco Spherosorb ODS-2 high carbon load well-end-capped column, and a 25-cm Spherosorb ODS-1 low carbon load no-end-capped column. Retention times for the paired acids in standard solution (malic:quinic and succinic:shikimic) differed by more than 4 min (Fig. 1). Neither malic nor succinic acid was detected in the conifer tissue extracts run on the reverse-phase system (data not shown).

### Statistical analysis

Treatment effects of this split-plot experiment were analyzed by using PROC MIXED in SAS, version 6.10 (soil as the main plots; N amendment as sub-plots; species as sub-sub-plots) (SAS Institute Inc. 1987). If the whole-plot mean square error term was smaller than the sub-plot mean square error terms, it was removed from the model and whole-plot effects were tested with the larger sub-plot effect. Comparisons of means were based on the $F$ statistics generated in PROC MIXED. Analyses of the organic acid concentration data required $Log_e$ transformation because preliminary observations of the residual plots revealed increasing error variance with increased concentration. Means for the organic acid concentration data in the tables and figures are the result of back transformation.

## Results

### Soil chemical properties

Chemical properties of the soils, including concentrations of C, N, and extractable $NH_4^+$ and $NO_3^-$, are included in Table 1. The C/N ratios were relatively low for each of the soils, suggesting active N mineralization. The exceptionally low C and N values in the low-N soil reflect a low organic matter content that is consistent with the fire history at the adjacent Douglas-fir spacing trial site.

**Fig. 1.** Representative chromatograms (HPX-87H) of organic acids from a standard solution and current-year foliage of western redcedar, western hemlock, and Douglas-fir. The solvent used was degassed 0.01 M $H_2SO_4$ at a flow rate of 0.4 mL·min$^{-1}$ and ambient temperature.
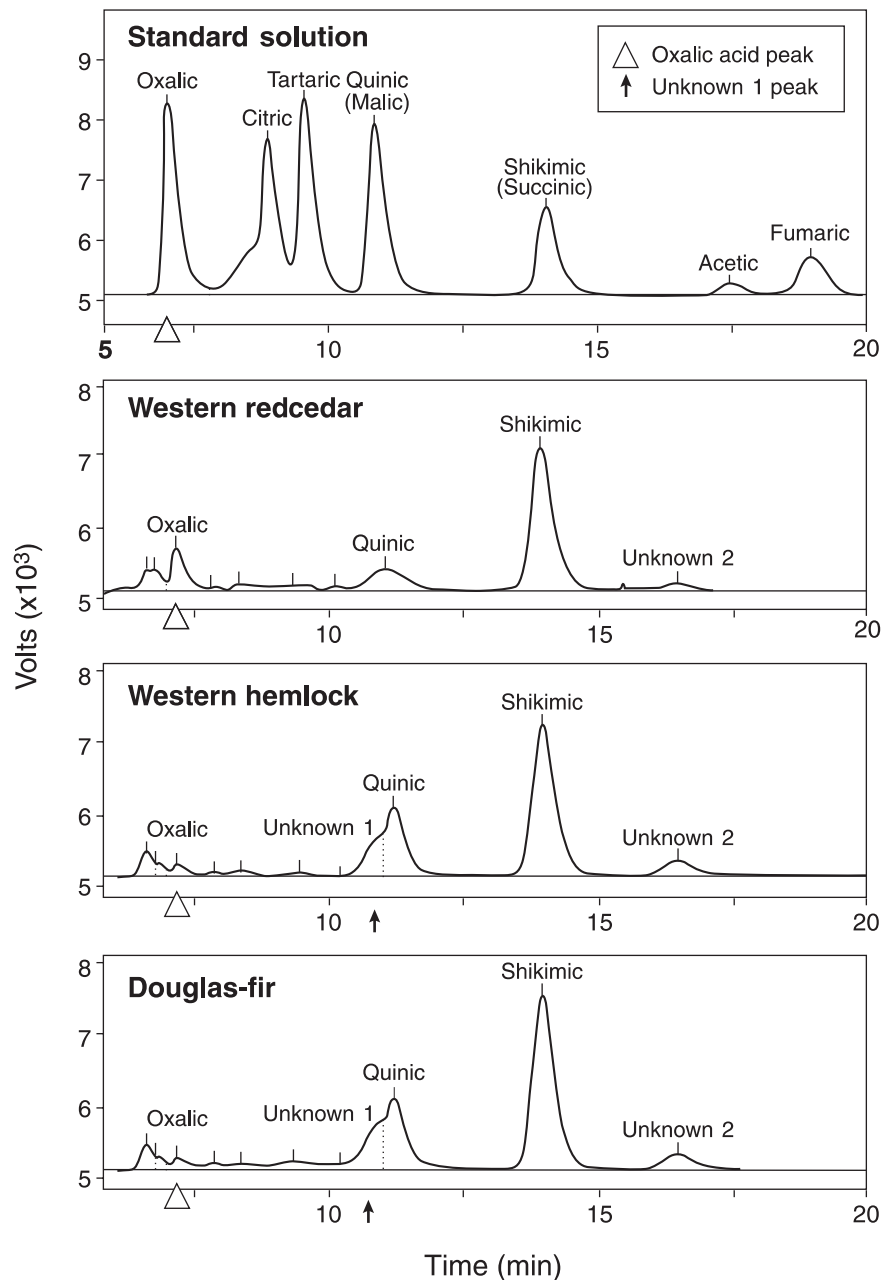


The low-N soil maintained a potential mineralization rate that was only 18% of that in the high-N soil during the first 21 days of incubation (Table 1). Little or no nitrification occurred during the incubation period, and the concentration of $NH_4^+$ was stable or declined in the unamended low-N soil from day 21 to day 49 (Fig. 2A). In the high-N soil, in contrast, production of N was sustained continuously at a rate of approximately 1.5 mg N·kg$^{-1}$/day through 49 days (Fig. 2B). More than 85% of the N mineralized was present as $NO_3^-$. Whereas two thirds of the N added to the low-N soil was immobilized by the soil biomass and $NO_3^-$ production was not stimulated (Fig. 2A), mineralized N in high-N soil was rapidly nitrified (Fig. 2B).

**Evaluation of methods for nutrient analysis**

The analytical techniques, nitric–perchloric digestion and analysis of the extracts by ICP, underestimated the concentrations of Ca, K, P, and Al by 10% relative to the certified values for NBS pine needles (National Bureau of Standards 1976). Because other element values have not been certified, data for those elements are reported as obtained. Standard errors of the element means were less than 1% for the

**Table 1.** Chemical properties of the low-N and high-N soils.

| Property | Low-N soil Wind River | High-N soil Cascade Head |
|---|---|---|
| Total C (mg·g$^{-1}$) | 15 | 137 |
| Total N (mg·g$^{-1}$) | 0.7 | 10.7 |
| C/N | 20.8 | 12.8 |
| Extractable NH$_4^+$ (mg·kg$^{-1}$) | 0.19 | 0.77 |
| Extractable NO$_3^-$ (mg·kg$^{-1}$) | 0.01 | 11.0 |
| N mineralization (mg N·kg$^{-1}$·d$^{-1}$)[a] | 0.37 | 2.00 |
| Nitrification (mg N·kg$^{-1}$·d$^{-1}$)[a] | 0.001 | 1.73 |
| N mineralization (N-amended soil)[a] | 1.11 | 2.68 |
| Nitrification (N-amended soil)[a] | 0.001 | 2.31 |

[a]Rates of nitrogen mineralization and nitrification from soils incubated for 21 days in the laboratory with and without N amendment (77 mg·kg$^{-1}$ (NH$_4$)$_2$SO$_4$).

**Fig. 2.** Potential N mineralization and nitrification (aerobic incubations) in (*a*) low-N soil (from Wind River) and (*b*) high-N soil (from Cascade Head). Amended treatment included 77 mg·kg$^{-1}$ N as (NH$_4$)$_2$SO$_4$. Soil moisture content was maintained at field capacity (0.31 g·g$^{-1}$ and 0.55 g·g$^{-1}$, respectively). Concentration of NO$_3$-N in low-N soil did not exceed 0.6 mg·kg$^{-1}$.
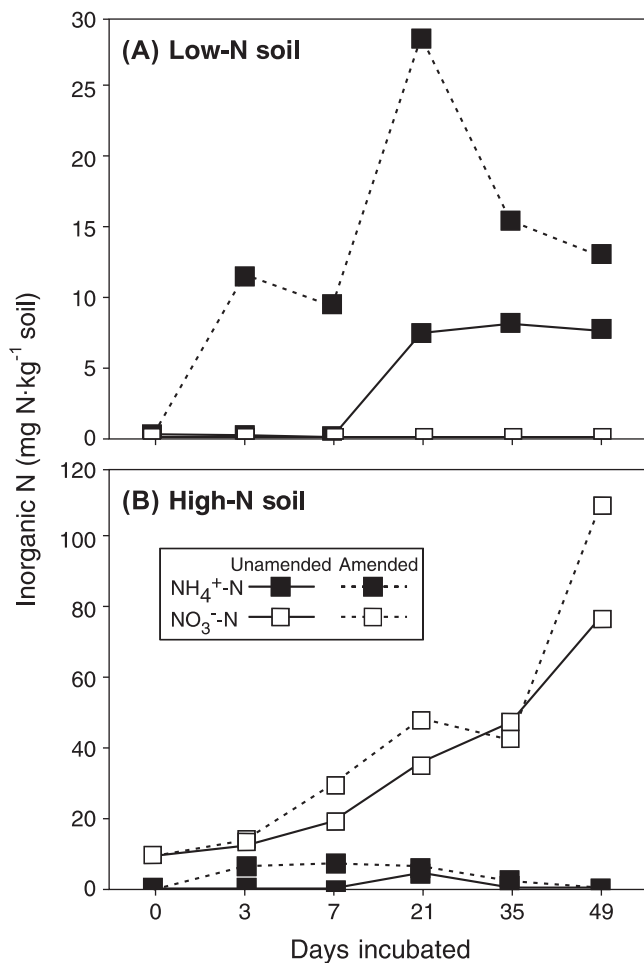


concentrations of both NBS-certified (Fe, Mn, and the above) and non-certified (N, Mg, S, Na) elements.

**Nutrient concentrations**

Current-year foliage concentrations of Ca, K, H$_2$PO$_4$, and

Al differed in response to soil-by-species interaction effects (Table 2). In each of the two soils, western redcedar seedlings had the highest concentrations of Ca, K, and Mg. For western hemlock and Douglas-fir, only the concentration of Mg differed. Western redcedar tended to have lower concentrations of Al in its foliage than the other two species, whereas western hemlock accumulated the highest concentrations. The foliar concentration of soluble NO$_3^-$ for western redcedar seedlings grown in high-N soil was greater than for western hemlock, Douglas-fir, or low-N western redcedar (Table 2). Amendment of the soils with (NH$_4$)$_2$SO$_4$ did not affect the nutrient or the organic acid concentrations in the foliage of the seedlings.

**Cation and anion equivalents and C–A**

Differences in equivalent cation concentrations in current-year foliage were found for both soil and species (Table 2). Foliage of seedlings grown on low-N soil accumulated a greater concentration of cation equivalents than did plants in the high-N soil (540 vs. 465 mequiv.·kg$^{-1}$). Western redcedar foliage had cation concentrations nearly twice as high as those of western hemlock and Douglas-fir. Both differences were primarily attributable to Ca. Four to seven times as much Ca was accumulated in the current-year foliage of western redcedar as in that of the other two species, comprising almost 50% of the total equivalent concentration of cations. Calcium contributed only 15–20% of the cation equivalents in the foliage of western hemlock and Douglas-fir. Current-year foliage of western hemlock accumulated one third more Mg ($P < 0.01$) and Ca, and one sixth more K, than found in Douglas-fir. The total concentration of inorganic anions (H$_2$PO$_4^-$, SO$_4^{2-}$, and NO$_3^-$) was very small relative to that of inorganic cations (Table 2). Foliage of the three species maintained significantly different C–A levels (Fig. 3).

**Extractable organic acids**

Western redcedar had the lowest concentration of extractable organic acids of the three species ($P < 0.01$; Fig. 3). No significant differences were observed among soils. Only three organic acids (oxalic, quinic, and shikimic) were extracted and identified from current or 1-year-old foliage of the three conifers; differences among the species were statistically significant for each of them ($P < 0.01$). Oxalic acid accounted for 50% of the organic acid quantified

**Table 2.** Concentrations of N, major cations and anions, and aluminum in current-year foliage.

| Variable | Low-N soil | | | High-N soil | | |
|---|---|---|---|---|---|---|
| | Western redcedar | Western hemlock | Douglas-fir | Western redcedar | Western hemlock | Douglas-fir |
| **Nitrogen concentrations (%)** | | | | | | |
| N** | 1.48*bc* | 1.38*bc* | 1.24*c* | 2.11*a* | 1.51*b* | 1.56*b* |
| **Cation concentrations (mequiv.·kg$^{-1}$)** | | | | | | |
| Ca** | 436*a* | 103*c* | 73*cd* | 358*b* | 74*cd* | 57*d* |
| K* | 229*a* | 203*b* | 171*cd* | 245*a* | 177*c* | 150*d* |
| Mg$^{a}$ | 163*a* | 119*b* | 89*c* | 169*a* | 114*b* | 86*c* |
| Total$^{b}$ | 851*a,x* | 458*b,x* | 354*c,x* | 799*a,y* | 413*b,y* | 316*c,y* |
| **Anion concentrations (mequiv.·kg$^{-1}$)** | | | | | | |
| $H_2PO_4^-$** | 40*b* | 48*a* | 40*b* | 40*b* | 32*c* | 26*c* |
| $SO_4$-S$^{a}$ | 2*b* | 3*b* | 20*a* | 2*b* | 1*b* | 8*a* |
| $NO_3$-N* | 8*b* | 7*b* | 7*b* | 16*a* | 10*b* | 9*b* |
| Total** | 50*ab* | 60*a* | 66*a* | 58*a* | 41*b* | 44*b* |
| **Aluminum (mg·kg$^{-1}$)** | | | | | | |
| Al** | 110*de* | 378*b* | 147*cd* | 48*e* | 475*a* | 213*c* |

**Note:** Means with different letters are statistically significant different within a row based on analyses using PROC MIXED (*, $P < 0.05$; **, $P < 0.01$) (SAS Institute Inc. 1987).

$^{a}$Results of PROC MIXED for Mg and $SO_4^{2-}$-S indicated that species means were significantly different ($P < 0.01$), but not soil means (no interaction effect).

$^{b}$Results of PROC MIXED for total cations indicated that fixed effects soil (*x, y*) and species (*a, b, c*) were significant ($P < 0.01$; no interaction effect).

from western redcedar. In contrast, quinic acid represented nearly 80% of the total organic acid for western hemlock and Douglas-fir. Shikimic acid contributed about 10% of the total acid concentration.

Two unidentified peaks were eluted from the HPX-87H column at retention times just prior to quinic acid (10.9 min) and subsequent to shikimic acid (16.8 min). The first peak was not observed for western redcedar, but the latter was present on traces from each of the three species (Fig. 1). For western hemlock, the area of the peak eluted at 10.9 min was three times as large as the peak at 16.8 min. For Douglas-fir, it was somewhat greater. The mean areas of the peaks at 16.8 min were one sixth as large as the oxalic acid peak of western redcedar but nearly equivalent to those of oxalic acid for western hemlock and Douglas-fir (Fig. 1). Corresponding peaks were observed at 16.1 and 18.6 min on the reverse-phase system. Extracts of western redcedar were not subjected to analysis on the reverse-phase HPLC system, so peak correspondence between the HPX-87H and reverse-phase systems for these two unknowns cannot be determined.

## Discussion

### Nutrient concentrations

Calcium concentration differences between low-N and high-N seedlings of each species reflect dilution, as the Ca content of current-year foliage did not differ significantly between soils (9 and 11 mg, respectively, on a per-pot basis). The small differences in Ca concentration between low- and high-N western redcedar foliage (Table 2) suggest that Ca accumulation is probably not related to the form of N acquired from the soil or that the N form acquired did not differ between the soils.

Phosphorus availability was clearly limited in both the low- and high-N soils. Foliar P concentrations for all three species (Table 2) were indicative of deficiency (Ballard and Carter 1985). Between-soil differences in P concentrations for western hemlock and Douglas-fir foliage (Table 2) were attributable to increased production of foliage dry matter in the high-N soil that diluted the greater amount of P accumulated in the current-year foliage (an average content of 2.5 mg of P in seedlings in low-N pots; 3.6 mg in seedlings in high-N pots). In contrast, the concentration of P in redcedar foliage did not differ between soils (Table 2).

Concentrations of S in current-year foliage (Table 2) are indicative of at least moderate levels of deficiency (Ballard and Carter 1985). Estimates of mean $SO_4^{2-}$-S concentrations for western redcedar and western hemlock in both soils were <1 mg·kg$^{-1}$, suggesting either that the equation (Turner 1979) is inappropriate or that S was especially limiting for these two species. Estimates of $SO_4^{2-}$-S based on organic N and S concentrations for Douglas-fir suggest that S was a limiting nutrient in the high-N soil (estimated $SO_4^{2-}$-S: 316 mg·kg$^{-1}$ in low N grown seedlings vs. 131 mg·kg$^{-1}$ in high N grown seedlings). Nitrate concentrations of current-year foliage (Table 2) were greater than those reported previously for conifers (Radwan and DeBell 1980; Margolis et al. 1988), but they were of little significance to the C–A balance.

### Cation and anion equivalents and C–A

The C–A values in current-year foliage of the three species (Fig. 3) were nearly identical to the "excess base concentrations" documented by Crooke et al. (1964). The greater C–A of western redcedar compared with that of western hemlock or Douglas-fir was mainly attributable to a greater concentration of Ca, but K and Mg also contributed to the overall differences (Table 2). The C–A values for seedlings grown in the high-N soil did not exceed those for the low-N soil for any of the three species. In the present

**Fig. 3.** C–A and actual organic acid concentration (extracted with 0.01 M $H_2SO_4$) of current-year foliage of western redcedar, western hemlock, and Douglas-fir. Experimental units included 3 seedlings/species: $n = 12$ units/mean. Reported means for organic acid concentrations resulted from back transformation following comparisons with PROC MIXED in SAS. Comparable bars with the same letter are not significantly different ($P < 0.01$).
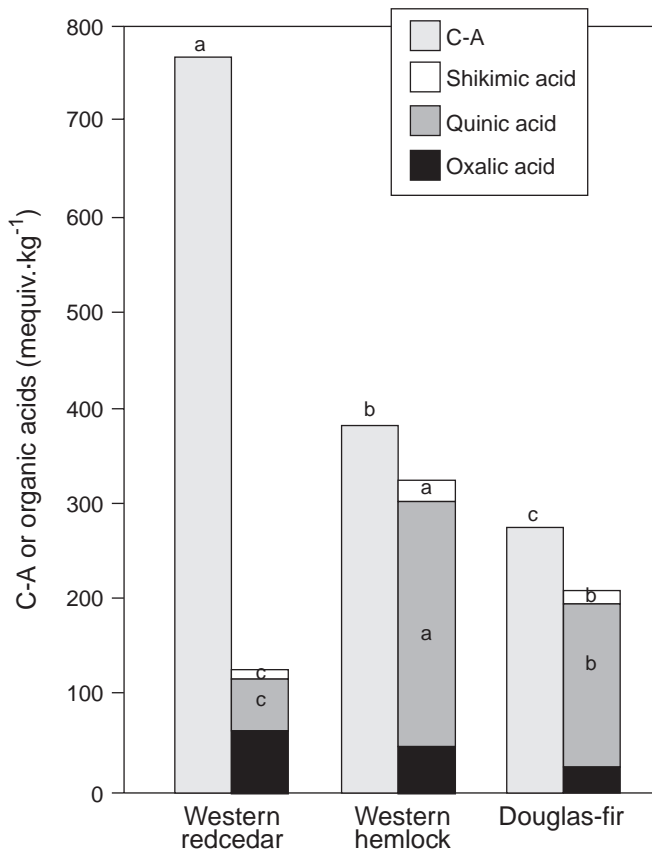


study, greater N availability for seedlings grown in the high-N soil resulted in a smaller ratio of cation:N uptake than for those grown on the low-N soil. Consequently, the concentrations of cation equivalents, and therefore C–A, were lower in high-N than in low-N seedlings. Gijsman (1990) reported that Douglas-fir seedlings grown on $NO_3^-$ or $NH_4NO_3^-$ had whole-shoot C–A concentrations ranging from 310 to 575 mequiv.·$kg^{-1}$, compared with 260 mequiv.·$kg^{-1}$ in the current study. The C–A values of both low-N- and high-N-grown seedlings were within the range of those reported for Douglas-fir grown only on $NH_4^+$-N (Gijsman 1990). The low light level in the growth chamber in the current study (140 µmol·$m^{-2}$·$s^{-1}$) may have reduced the potential for $NO_3^-$ uptake and foliar $NO_3^-$ reduction by the seedlings (e.g., Rao and Rains 1976).

**Extractable organic acids**

Nearly equal concentrations of oxalic and quinic acid were obtained from the foliage of western redcedar (Fig 3). Quinic acid was the principal organic acid extracted from the foliage of western hemlock and Douglas-fir. The concentrations of quinic and shikimic acid are consistent with data for the Pinaceae in the literature (Goldschmid and Quimby

1964; Sarkar and Malhotra 1979; Lüthy-Krause et al. 1990). The greater concentrations of quinic and shikimic acids in foliage of western hemlock support speculation by Turner (1984) that relatively low soil pH values beneath western hemlock might in part be a function of organic acids leached from the canopy and decomposing litter.

The tricarboxylic acid cycle intermediates citrate, malate, succinate, and fumarate were not detected in the foliage extracts of the three conifers in this study. Initial analysis with the HPX-87H indicated that malic and succinic acids were constituents in the foliage of these conifer species. Subsequent analysis of the extracts on the reverse phase system showed that these peaks had been misidentified. Coelution of malic with quinic and succinic with shikimic acids on the HPX-87H column has not been reported previously. Soluble organic acids extracted from plant tissues and soils have been identified as malic and succinic acids, apparently without consideration that they may be quinic and shikimic acids (Pohlman and McColl 1988; Fox and Comerford 1990; Tam and McColl 1991). Lüthy-Krause et al. (1990) reported that concentrations of citric and malic acids did not exceed 10 mequiv.·$kg^{-1}$ in the foliage of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.). Because of the prominence of the oxalic and quinic peaks in the present study and the large peak for shikimic acid, undiluted extract solutions were not subjected to HPLC. Presumably, citric and malic acids were present in the foliage of redcedar, hemlock, and Douglas-fir, but at relatively low concentrations. Syringic acid has been reported as a trace constituent in the foliage of jack pine (*Pinus banksiana* Lamb.) (Sarkar and Malhotra 1979), but no attempt was made to identify it in this study.

**The C–A imbalance in western redcedar**

The total concentrations of organic acids determined from the results of HPLC were only 15% of C–A for redcedar. In contrast, concentrations for western hemlock and Douglas-fir were more than 80% of C–A (Fig. 3). The C–A values were greater, and the organic concentrations were lower for western redcedar than for western hemlock or Douglas-fir. Values for both C–A and total organic acid concentrations were slightly greater for western hemlock than for Douglas-fir (Fig. 3). Because of western redcedar's clearly documented capacity to accumulate Ca and its potential to assimilate $NO_3^-$-N in leaves (Smirnoff et al. 1984; supported by our unpublished data), the relatively low concentrations of oxalic acid found in the foliage were unexpected. Reagent-grade calcium oxalate was readily dissolved in dilute HCl (0.02 M), and extraction with 0.4 M HCl brought no additional oxalate into solution. Therefore, it seems likely that a large proportion of the C–A imbalance for western redcedar must be associated with something other than oxalic acid. The C–A imbalances may be due to (*i*) the apoplastic cation exchange capacity (CEC) (Crooke et al. 1964; Turner and van Broekhuizen 1992); (*ii*) small quantities of organic acids that were not identified or not detected (e.g., citric and malic acids; the unknowns); (*iii*) the presence of chloride (Eaton 1966); and (or) (*iv*) the accumulation of insoluble precipitates (Pobequin 1954; Arnott and Pataud 1970).

The apoplastic CEC may account for up to 120–160 mequiv.·$kg^{-1}$ (Crooke et al. 1964; Turner and van Broekhuizen

1992) and is sufficient to balance entirely the C–A of western hemlock and Douglas-fir. In contrast, apoplastic CEC for western redcedar (160 mequiv.·kg$^{-1}$; Crooke et al. 1964) would account for only 25% of the residual C–A balance in the current study.

A Cl$^-$ concentration of 9000 mg·kg$^{-1}$ (0.9%) would be required to balance the remaining C–A of western redcedar foliage. Such a concentration is in the range generally associated with plant toxicity (Eaton 1966). However, it is an order of magnitude greater than the maximum concentrations previously reported for conifers (Crooke et al. 1964; Beaton et al. 1965; Radwan and DeBell 1980). In addition, if redcedar accumulated Cl$^-$ from the soils used in this study, similar Cl$^-$ concentrations (and associated C–A imbalance) would be expected in the foliage of western hemlock and Douglas-fir. To completely eliminate Cl$^-$ from consideration, however, analysis of Cl$^-$ concentrations would be necessary.

Calcium pectates are major constituents of cell walls (Bangerth 1979). Analyses of leaves of tomato plants by Kirkby and Mengel (1967) revealed that C–A and organic acid content were not in balance; calcium was bound as 50% insoluble complexes with oxalate and 50% uronic acids. Crooke et al. (1964) attempted to quantify uronic acids in conifer foliage by using the same method of extraction (acid decarboxylation (Tracey 1948)). They reported that uronic acids accounted for 75, 125, and 170% of the C–A in the foliage of western redcedar, western hemlock, and Douglas-fir, respectively. Clearly, some other constituent was decarboxylated, yielding an overestimate of the pectic fraction. The method Crooke et al. (1964) used to quantify uronic acids is nearly identical to that currently employed to determine total carbonates in soil (Horneck et al. 1989). Acid decarboxylation cannot be used to differentiate among pectic acids and carbonates and may yield $CO_2$ from other constituents (proteins, lignins, etc.). The accumulation of calcium in western redcedar foliage may be associated with the formation of $CaCO_3$, a common phenomenon in deciduous angiosperms (Cooil 1948; Pobequin 1954; Arnott and Pataud 1970). Alternative methods of analysis must be developed to determine whether $CaCO_3$ is present in the foliage of western redcedar.

### Potential role of calcium carbonate

Turner (1984) reported a pH of 6.6 for distilled water extracts of air-dried leaf tissue of western redcedar, whereas the pH was 4.4 for corresponding extracts of western hemlock. The high pH of the redcedar extracts (Turner 1984) is greater than the equilibrium constant (pKa) for the reversible chemical reaction:

carbonic acid ($H_2CO_3$) ⇌ bicarbonate ($HCO_3^-$) + H$^+$
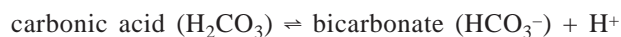
providing indirect evidence that $CaCO_3$, and not Ca-oxalate, accumulates in the foliage of redcedar. Supporting evidence for the presence of $CaCO_3$ can be inferred from the results of Howard and Howard (1990), who reported that leaf extracts of western redcedar were among a group of deciduous species with the lowest titrable acidity and the highest contents of directly titrable bases and ash bases. In contrast, foliage of western hemlock and Douglas-fir had one tenth the concentration of titrable bases and one fifth the concentration of ash bases. Scanning electron microscopy should be performed to detect the presence of $CaCO_3$ crystals in the foliage of western redcedar and other species that have been found to have similarly high titrable base and ash base contents (Howard and Howard 1990; Côté and Fyles 1994).

Soil properties beneath long-lived western redcedar trees (Alban 1969; Turner and Franz 1985; Turner et al. 1993) may have been influenced by litter rich in $CaCO_3$. The high concentration of Ca from western redcedar litter and the potential role of carbonic acid as the missing anion component of C–A suggest that $CaCO_3$ could be a critical regulator of chemical activity, especially N transformations, in the soil beneath western redcedar. Litterfall deposition of $CaCO_3$ might lead to consumption of H$^+$ ions, preventing large changes in soil pH that might occur with excessive Ca leaching, nitrification, or $NH_4^+$ uptake. Enrichment of exchangeable soil Ca in the rooting zone would also occur.

## Conclusions

Western redcedar, western hemlock, and Douglas-fir were found to have distinct C–A values, confirming the earlier results obtained by Crooke et al. (1964). These values were not influenced by differences in soil N availability, as no differences among soils were identified. The C–A of western redcedar was not balanced by the low molecular weight organic acids. In contrast, the C–A values of western hemlock and Douglas-fir are nearly balanced by the presence of quinic, oxalic, and shikimic acids. We speculate that the difference is attributable to the presence of $CaCO_3$ in current-year foliage of redcedar. Further research is necessary to (*i*) examine the ultrastructure of redcedar foliage via scanning electron microscopy for the presence of calcium carbonate; (*ii*) identify the source of foliar $CaCO_3$ in western redcedar, if it is present (e.g., direct uptake (H$^{14}CO_3^-$) and (or) plant metabolism associated with the reduction of $NO_3^-$); (*iii*) characterize organic matter from soils beneath western redcedar and western hemlock (including fractionation (e.g., Prescott and Preston 1994); and (*iv*) determine the effect of western redcedar litter on soil pH.

Finally, because of coelution of malic with quinic and succinic with shikimic acids, quantitation of organic acids in conifer tissue should be performed with an HPLC analytical column other than the HPX-87H. The alternative reverse-phase system effectively discriminated between the acids of each pair, and neither malic nor succinic acid was detected in the foliage extracts run through the column. Coelution of these acid pairs in analysis of soil and plant extracts apparently has not been recognized previously.

## Acknowledgements

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