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## Availability of residual $^{15}\text{N}$ in a coniferous forest soil: a greenhouse bioassay and comparison with chemical extractions

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### Abstract

The assessment of soil N availability by chemical extraction methods often needs to be checked by methods which directly measure plant N uptake such as a greenhouse bioassay. In this paper, the recovery of residual  $^{15}\text{N}$ , from humus material samples with  $^{15}\text{N}$  labelled for 24-h, seven-month, and 31-month, in western redcedar (*Thuja plicata* Donn ex D. Don) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) seedlings was investigated in a 342-day greenhouse incubation study and was compared to chemical extraction studies on the same samples. Apparently higher N availability in the 24-h treatment resulted in greater shoot mass in that treatment than in the other two treatments. However, root and whole plant mass were not significantly different among treatments and there were no differences between the species in any of the above measurements. Plants in the 24-h treatment also proportionally took up more residual  $^{15}\text{N}$  from the humus material than those in the other two treatments and thus significantly greater availability ratios were obtained in the former than in the latter two treatments. At the end of the 342-day incubation, a significant amount of  $^{15}\text{N}$  had been immobilised by the soil in the 24-h treatment compared to net  $^{15}\text{N}$  mineralisation in the other two treatments. The high soil mineral N and  $^{15}\text{N}$  contents in the 24-h treatment at the end of the 342-day incubation compared to the low soil mineral N and  $^{15}\text{N}$  contents in the seven-month treatment at the beginning of greenhouse incubation means immobilisation of fertiliser N in the greenhouse incubation was dramatically reduced compared to field situations. Nitrification was negligible before day 182 but was detected at the end of the incubation. Correlation analysis showed that  $^{15}\text{N}$  released during a two-week anaerobic incubation or in a 42-day aerobic incubation,  $^{15}\text{N}$  extracted by 0.01 M  $\text{KMnO}_4$  or 2 M  $\text{KCl}$ ,  $^{15}\text{N}$  released by autoclaving or fumigation (-extraction), and even  $^{15}\text{N}$  abundance in the fulvic acid fraction of the organic matter all seemed good indicators of soil residual N availability. © 1999 Elsevier Science B.V. All rights reserved.

### 1. Introduction

The use of chemical extraction methods and indices to quantify N availability is sometimes criticised for

artefacts introduced by sampling and incubation regimes not characteristic of the site where the samples were collected (Adams and Attiwill, 1986), although these indices and methods are usually a practical alternative to field methods of assessing soil N availability (Maimone et al., 1991). Although, chemical extraction and laboratory incubation methods may seem to be quite artificial, chemical indices of

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N availability were often found to be correlated with plant uptake of N or tree growth (Carlyle and Malcolm, 1986a; Keeney, 1980; Powers, 1980).

Field based methods for assessing N availability in forestry situations can be extremely difficult and can be very costly because of the long-term nature of tree growth, the remoteness of many forest stands and the large variability in forest soils. Bioassay methods represent a direct measurement of soil N availability to plants under a controlled environment. A greenhouse-based bioassay certainly reduces some of the variabilities (spatial and temporal) discussed by Binkley and Hart (1989). With costs associated with totally field based methods to evaluate N availability and the time it takes to observe any response in plant growth rate changes due to manipulation, bioassay methods are very good alternatives to both laboratory and field based N availability evaluation methods.

In previous studies Chang and Preston (1998) and Chang et al. (1997) compared results from chemical extraction (2 M KCl, 0.5 M K<sub>2</sub>SO<sub>4</sub>, autoclaving with 0.01 M CaCl<sub>2</sub>, acidic permanganate of different strength and fumigation–extraction), organic matter fractionation and laboratory incubation (both aerobic and anaerobic) methods to evaluate the availability of residual fertiliser N (<sup>15</sup>N-labelled) in a coniferous forest soil with <sup>15</sup>N labelled for different periods (24 h, seven months and 31 months). These studies showed that one year after fertiliser N application, residual N was as unavailable as that applied three years ago, supporting observations in field <sup>15</sup>N studies (Preston and Mead, 1994a, b). The objective of this study was to verify the chemical extraction and laboratory incubation results with a greenhouse incubation study.

## 2. Materials and methods

### 2.1. Field labelling and sampling

Samples for this study were collected from a site in the very wet maritime subzone of the Coastal Western Hemlock (CWH) biogeoclimatic zone (Pojar et al., 1991), located near Port McNeill (50° 36'N, 127° 15'W) on northern Vancouver Island, British Columbia, Canada. More detail on climate and topography for the site are available in Chang et al. (1997). The

original vegetation cover before clearcutting and slash-burning in 1985 was a very old forest of western redcedar (*Thuja plicata* Donn ex D. Don) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg). The mineral soil is an Orthic Humo–Ferric Podzol (Agriculture Canada Expert Committee on Soil Survey, 1987), with unconsolidated glacial moraine and fluvial outwash as its parent material (Lewis, 1982). The plots used for the field labelling and sampling were located within an area of ca. 50×100 m and thus had relatively uniform conditions.

Nitrogen-15 labelled fertiliser (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (200 kg N ha<sup>-1</sup>) was applied to microplots (1 m radius) on April 16, 1991, April 24, 1993, and December 1, 1993. Sampling was carried out on December 2, 1993, to obtain H humus material with <sup>15</sup>N residence times of 24 h, seven months, and 31 months. Nitrogen-15 enrichment in the fertiliser solution applied was 3.38044% for the 31 months and 2.37753% for the seven months and 24-h treatments. At each application, fertiliser solutions (in 2 l water) for each plot were applied using a watering can after removing the thin L/F layer (Chang et al., 1997).

The whole top 10-cm layer of the H horizon was collected from each plot in the field, each of which formed one sample (ca. 12 kg each, fresh weight). In the laboratory, the samples were picked free of visible roots, sieved through an 8 mm sieve, thoroughly mixed using a Monarch (Winnipeg, Canada) cement mixer, and subsampled (≈1 kg) for chemical extraction, organic matter fractionation and laboratory incubation studies. The remainder was used for the greenhouse study described below.

### 2.2. Greenhouse incubation

The greenhouse experiment comprised treatment (duration of <sup>15</sup>N labelling), species (western redcedar, western hemlock and control) and three replicates. Extra pots without plants (i.e., the control) were set up to allow for sampling at half the length of the greenhouse incubation. The redcedar seeds were PFC# 1646 from Black Creek, B.C., with 88% viability and the hemlock seeds were PFC# 586 from Holberg, B.C., with 89% viability. The 15 cm diameter pots were placed in flat-bottom saucers and filled with 145 g (oven-dry basis) of the H horizon material. The fresh weight of the sample in each pot was ca. 700 g because

the organic material can hold water up to five times its own weight. Three germinants of each species were planted in each pot on January 5, 1994. For some reason, the western hemlock plants grew poorly and many pots had zero plants. These pots with hemlock were planted again on April 18, 1994, without disturbing the soil in the pots. Because of mortality in some pots, N uptake was calculated on a per plant basis. Replication for statistical analysis was still at the pot level.

The pot experiment was designed to have no fertiliser additions. Watering was on a daily basis and tap water was used for the entire experiment. Plants were grown under natural daylengths. The temperature regime was adjusted every two weeks to reflect seasonal changes and ranged from 10 to 20°C daytime (16 h), and 3 to 18°C nighttime (8 h). A set of no-plant pots were extracted with 2 M KCl to check N mineralisation and nitrification on July 7, 1994. Final harvesting of all the pots took place on December 13, 1994, a little over 11 months (342 days). The number of plants in each pot was recorded and the shoot portion of the seedlings was cut off and roots separated

200 ml plastic bottle and ca. 50 ml of 2 M KCl was added. The mixture was shaken for 1 h and filtered through a Whatman #42 filter on a vacuum filtration system. The extracts were kept in a freezer until analysed for total N and  $^{15}\text{N}$ . Extracts were analysed for  $\text{NH}_4^+-\text{N}$  by steam distillation with MgO and titration (Keeney and Nelson, 1982). After the  $\text{NH}_4^+-\text{N}$  was measured, the mixture was treated with Devarda's alloy to reduce  $\text{NO}_3^-\text{N}$  to  $\text{NH}_4^+-\text{N}$  and the amount of  $\text{NH}_4^+-\text{N}$  measured as above. The distillates from the above inorganic or total N measurements, collected in boric acid-ethanol, were dried at 70°C and analysed for  $^{15}\text{N}$  abundance as described in the previous paragraph. Spiking with a standard was used to raise the total N content for some of the samples before  $^{15}\text{N}$  analysis.

For a description of the methods of laboratory analyses used for the chemical indices listed in Table 1, please refer to Chang et al. (1997) and Chang and Preston (1998).

Recovery rate of residual  $^{15}\text{N}$  from fertiliser applied in the field was calculated using the following formula:

$$\text{Recovery}(\%) = \frac{[^{15}\text{N}_{\text{ex}}(\%) \text{ in plant}] \times (\text{total N content in plant})}{[^{15}\text{N}_{\text{ex}}(\%) \text{ in soil}] \times (\text{total N content in soil})}$$

from the soil. The saucer was washed with a few small aliquots (ca. 0.01 l each time) of distilled water which was added to the soil.

### 2.3. Laboratory and statistical analyses

The plant samples were dried in an oven at 65°C, weighed, and then ground in a Wiley mill. Total N concentration and  $^{15}\text{N}$  per cent abundance were analysed by Kjeldahl analysis with steam distillation. The  $\text{NH}_4^+-\text{N}$  collected in the boric acid was then titrated to determine N content and dried at 70°C for  $^{15}\text{N}$  abundance measurement using a Vacuum Generators Sira 9 mass spectrometer (Preston et al., 1990).

Fresh humus samples were extracted with 2 M KCl. This included samples of the three treatments before the incubation, about half way through the incubation for pots without plants and at the final harvesting for pots with and without plants. For each extraction,  $\approx 50$  g (wet weight) sample was weighed into a

Statistical analysis was performed using the commercial Statistical Analysis System (SAS) software (SAS Institute Inc., 1989). Group means of independent variables were compared between treatments for each species (or sampling date) or between species (or sampling dates) of each treatment using Scheffe's multiple range test if interactions were significant. Otherwise, multiple comparisons were performed for treatment and species (or sampling date) means using Scheffe's multiple range test. Correlation analysis was also performed using SAS.

## 3. Results

### 3.1. Mineral N dynamics

Over the 342-day incubation period, mineral N content for the 24-h treatment in the control pots remained high although the net effect of incubation

Table 1

Simple correlations between N availability indices and plant N uptake. Pearson correlation coefficient ( $r$ ) with significance levels

N availability index	Pot experiment plant N uptake and biomass				
	Shoot N (g/kg)	Shoot $^{15}\text{N}$ abundance (%)	$^{15}\text{N}$ mineralised ( $\mu\text{g/g}$ )	Root $^{15}\text{N}$ abundance (%)	Plant biomass (g/plant)
<i>Western redcedar</i>					
ANA $^{15}\text{N}^{\text{a}}$	0.67 <sup>j</sup>	0.99 <sup>1</sup>	-0.98 <sup>1</sup>	0.70 <sup>j</sup>	0.38
Min 42-day $^{15}\text{N}^{\text{b}}$	0.71 <sup>j</sup>	0.97 <sup>1</sup>	-0.92 <sup>1</sup>	0.76 <sup>j</sup>	0.47
KMnO <sub>4</sub> $^{15}\text{N}^{\text{c}}$	0.67 <sup>j</sup>	0.99 <sup>1</sup>	-0.99 <sup>1</sup>	0.70 <sup>j</sup>	0.38
FA $^{15}\text{N}^{\text{d}}$	0.66 <sup>j</sup>	0.98 <sup>1</sup>	-0.99 <sup>1</sup>	0.63	0.30
HA $^{15}\text{N}^{\text{e}}$	0.22	0.24	-0.18	0.39	0.42
Humin $^{15}\text{N}^{\text{f}}$	-0.16	-0.62	0.59	-0.50	-0.28
Flush of $^{15}\text{N}^{\text{g}}$	0.69 <sup>j</sup>	0.99 <sup>1</sup>	-0.96 <sup>1</sup>	0.76 <sup>j</sup>	0.45
KCl $^{15}\text{N}^{\text{h}}$	0.67 <sup>j</sup>	0.99 <sup>1</sup>	-0.99 <sup>1</sup>	0.69 <sup>j</sup>	0.38
Autocla $^{15}\text{N}^{\text{i}}$	0.68 <sup>j</sup>	0.99 <sup>1</sup>	-0.98 <sup>1</sup>	0.71 <sup>j</sup>	0.39
<i>Western hemlock</i>					
ANA $^{15}\text{N}^{\text{a}}$	0.71 <sup>j</sup>	0.99 <sup>1</sup>	-0.95 <sup>1</sup>	0.88 <sup>k</sup>	0.69 <sup>j</sup>
Min 42-day $^{15}\text{N}^{\text{b}}$	0.69 <sup>j</sup>	0.95 <sup>1</sup>	-0.88 <sup>k</sup>	0.91 <sup>1</sup>	0.73 <sup>j</sup>
KMnO <sub>4</sub> $^{15}\text{N}^{\text{c}}$	0.71 <sup>j</sup>	0.99 <sup>1</sup>	-0.95 <sup>1</sup>	0.88 <sup>k</sup>	0.69 <sup>j</sup>
FA $^{15}\text{N}^{\text{d}}$	0.71 <sup>j</sup>	0.98 <sup>1</sup>	-0.97 <sup>1</sup>	0.83 <sup>k</sup>	0.65
HA $^{15}\text{N}^{\text{e}}$	-0.05	0.28	-0.15	0.41	0.60
Humin $^{15}\text{N}^{\text{f}}$	-0.49	-0.62	0.59	-0.59	-0.38
Flush of $^{15}\text{N}^{\text{g}}$	0.73 <sup>j</sup>	0.99 <sup>1</sup>	-0.91 <sup>1</sup>	0.91 <sup>1</sup>	0.71 <sup>j</sup>
KCl $^{15}\text{N}^{\text{h}}$	0.71 <sup>j</sup>	0.99 <sup>1</sup>	-0.95 <sup>1</sup>	0.88 <sup>k</sup>	0.69 <sup>j</sup>
Autocla $^{15}\text{N}^{\text{i}}$	0.72 <sup>j</sup>	0.99 <sup>1</sup>	-0.94 <sup>1</sup>	0.88 <sup>k</sup>	0.70 <sup>j</sup>

<sup>a</sup>  $^{15}\text{N}$  mineralised in two-week anaerobic incubation at 30°C.<sup>b</sup>  $^{15}\text{N}$  mineralised during a 42-day aerobic incubation.<sup>c</sup>  $^{15}\text{N}$  extracted by 0.01 M KMnO<sub>4</sub>.<sup>d</sup>  $^{15}\text{N}$  content in the fulvic acid fraction of the organic matter.<sup>e</sup>  $^{15}\text{N}$  content in the humic acid fraction.<sup>f</sup>  $^{15}\text{N}$  content in the humin fraction.<sup>g</sup>  $^{15}\text{N}$  extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub> after chloroform fumigation.<sup>h</sup>  $^{15}\text{N}$  extracted by 2 M KCl.<sup>i</sup>  $^{15}\text{N}$  released through autoclaving in 0.01 M CaCl<sub>2</sub>.Note: All the above measurements were expressed as  $\mu\text{g } ^{15}\text{N/g}$  soil.<sup>j</sup>  $p < 0.05$ .<sup>k</sup>  $p < 0.01$ .<sup>1</sup>  $p < 0.001$ .

was a net immobilisation of mineral N at the end of the incubation (Fig. 1(a)). Mineral N measured was mainly  $\text{NH}_4^+-\text{N}$  as  $\text{NO}_3^--\text{N}$  was negligible in the early stage but did appear at the day 342 sampling (see Fig. 2(c)). However, for the 31- and seven-month treatments, net mineralisation resulted from the incubation, and mineral N content continuously increased from day 1 to day 342 (Fig. 1(a)). The mineral N fraction was always enriched (greater than the background level) with  $^{15}\text{N}$  and the enrichment was greater than that of the bulk soil (with  $^{15}\text{N}$  abundances of

0.4830, 0.4569 and 0.6648% for the 31-month, seven-month and 24-h labelling treatments, respectively) regardless of the labelling history. Over time, the  $^{15}\text{N}$  abundances decreased from day 1 to day 342, regardless of the previous labelling treatment (Fig. 1(b)).

At the final harvesting, the effect of trees planted in the pots on mineral N ( $\text{NH}_4^+-\text{N} + \text{NO}_3^--\text{N}$ ) content was obvious, i.e., mineral N content was less in the pots planted with seedlings than in the control (Fig. 2(a) and (c)). Because the western hemlock

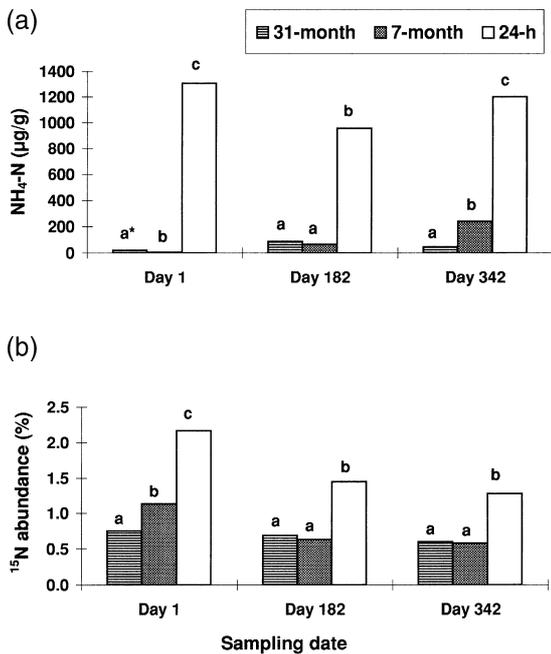


Fig. 1. Changes of  $\text{NH}_4^+\text{-N}$  ( $\mu\text{g/g}$ ) and  $^{15}\text{N}$  abundance (%) in the  $\text{NH}_4^+\text{-N}$  pool in the no-plant pots during the greenhouse incubation. \*: The same lowercase letters across the treatments on each sampling date means that there was no treatment effect. The lowercase letters were used here to indicate significant treatment by sampling date interaction for the variable concerned.

seedlings were grown for only 242 days compared to 342 days for western redcedar, the amount of mineral N left in the soil at the final harvesting was generally less in the redcedar than in the hemlock pots. The majority of mineral N was  $\text{NH}_4^+\text{-N}$  (Fig. 2(a) and (c)), but  $\text{NO}_3^-\text{-N}$  was more than half of the total mineral N in the 31-month treatment in the control at the final harvesting. The  $\text{NO}_3^-\text{-N}$  contents in pots planted with seedlings were low relative to the control pots. The  $^{15}\text{N}$  abundance values for  $\text{NO}_3^-\text{-N}$  were fairly similar to that of  $\text{NH}_4^+\text{-N}$  in the 31- and seven-month treatments but were lower than those for  $\text{NH}_4^+\text{-N}$  in the 24-h treatment (Fig. 2(b) and (d)).

In terms of  $^{15}\text{N}$  abundances in the extracted  $\text{NH}_4^+\text{-N}$  fraction at the final sampling, they followed the same trend in the planted and control pots (Fig. 2(b) and (d)). The  $^{15}\text{N}$  abundance in the respective treatments was not reduced by the planted seedlings which means that the seedlings did not usually discriminate between  $^{15}\text{N}$  and the lighter  $^{14}\text{N}$  isotope.

### 3.2. Plant growth

At the end of the pot experiment, shoot mass (g/plant) was significantly greater in the 24 h than in the other two treatments. The interaction between treatment and species was not significant although the magnitude of difference between the 24 h and other treatments was different for the two species planted (Fig. 3(a)). Overall, species effect on shoot mass was not significant. Although the whole plant mass (shoot plus root mass per plant, Fig. 3(c)) was shown to be much greater in the 24 h than in the other two treatments, there was no significant overall treatment or species effect on whole plant biomass production, probably affected by the non-significant treatment and species effects on the root biomass (Fig. 3(b)) and by the increased variability when the root mass was added to the shoot mass.

The greater availability of nitrogen in the 24-h treatment seemed to reduce the root/shoot ratio of western redcedar (Fig. 3(d)), even though the difference in root/shoot ratios was only significant between the 24 h and seven-month treatments. These relationships were not changed by the species used in the pot experiment.

### 3.3. Plant N uptake and soil N mineralisation

The trends of nitrogen content differences among the treatments in shoot, root and whole plant biomass followed very closely those of plant mass (Fig. 4(a), (b) and (c)); however, the magnitude of difference between the 24 h and other two treatments, in shoot, root and whole plant N contents (mg/plant) was much greater than plant biomass in the respective comparisons. This result was brought about by a significant difference between the 24 h and other treatments in N concentration in the shoot, root and the whole plant (data not shown). Both shoot and whole plant N contents were significantly higher in the 24 h than in the other two treatments.

The  $^{15}\text{N}$  abundances in shoots, roots and whole plants also had very similar trends with regard to species planted and  $^{15}\text{N}$  residence time in the humus (Fig. 5). The  $^{15}\text{N}$  abundance values in shoots, roots, and thus the whole plant were almost identical for the respective treatments, with the value in the 24-h

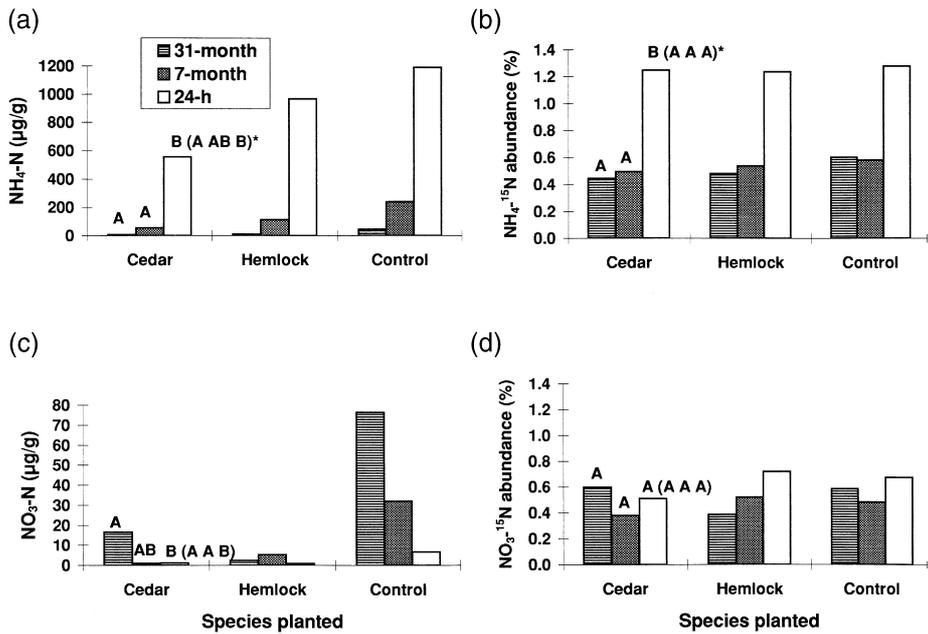


Fig. 2.  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  pool sizes ( $\mu\text{g/g}$ ) and their  $^{15}\text{N}$  abundance (%) in the planted and control (no-plant) pots at the end of the greenhouse incubation. \*: The same uppercase letters indicate that there was no overall treatment effect. The uppercase letters were used here to indicate that there was no significant treatment by species interaction. The same upper case letters in parenthesis indicate that there was no significant difference among the species (and the control) studied.

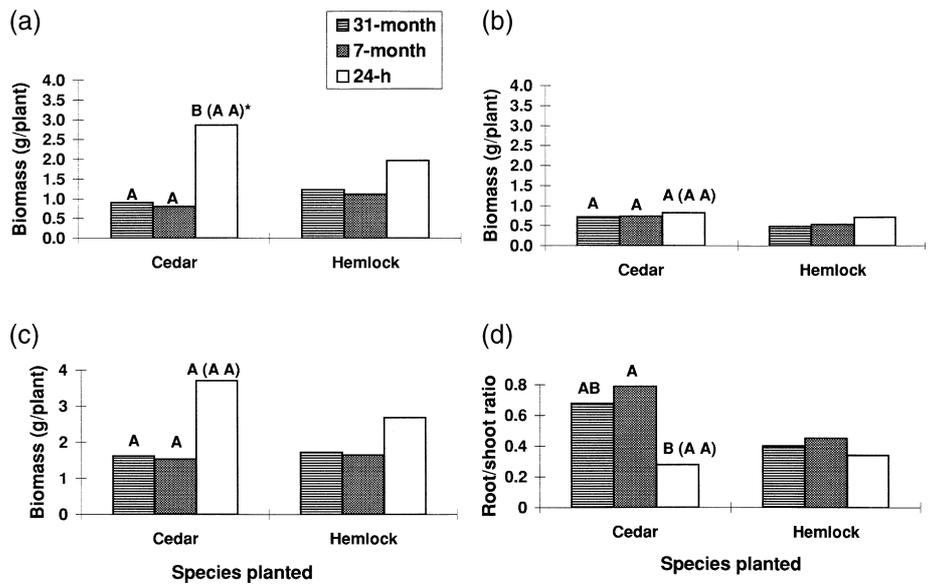


Fig. 3. Shoot, root and total plant mass (g/plant) and root/shoot ratios for western redcedar and western hemlock. \*: See notes for Fig. 2.

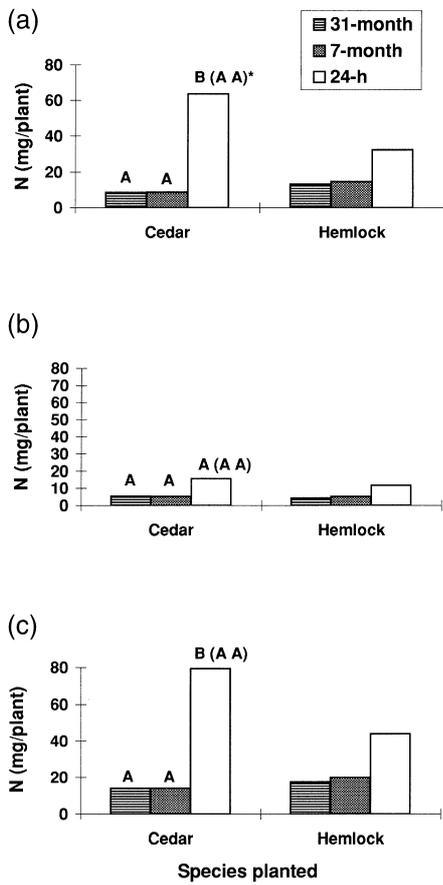


Fig. 4. Nitrogen content in shoot, root and whole plant (mg/plant) in western redcedar and western hemlock. \*: See notes for Fig. 2.

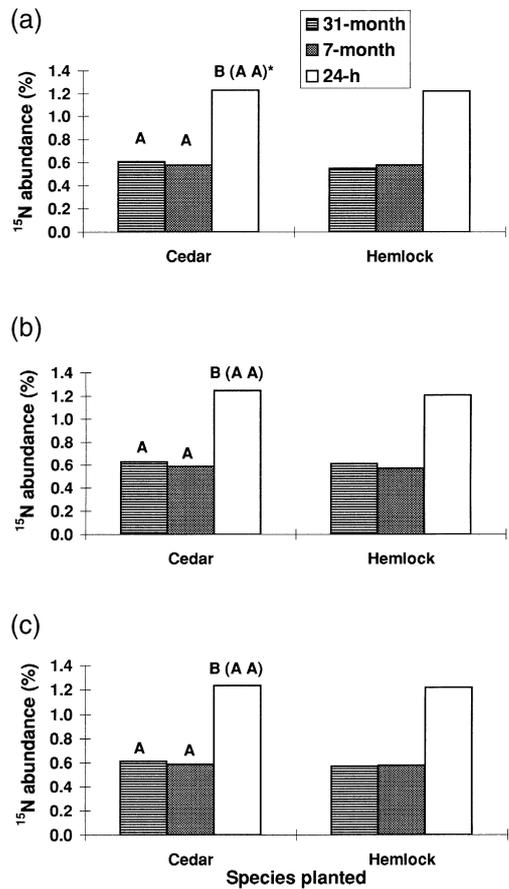


Fig. 5. Nitrogen-15 abundance (%) in shoot, root and whole plant in western redcedar and western hemlock. \*: See notes for Fig. 2.

treatment significantly greater than that in the other two treatments. The values were very similar to the <sup>15</sup>N abundance values in NH<sub>4</sub><sup>+</sup>-N and were higher than the <sup>15</sup>N abundance in the bulk soil.

The availability ratio (<sup>15</sup>N abundance % in the plant divided by that in the humus material) showed that the N taken up by the plants was more enriched with <sup>15</sup>N than the bulk soil (Fig. 6). Total N (the sum of native and applied N) mineralised was positive except in the control and hemlock pots where the <sup>15</sup>N had been labelled for 24 h (Fig. 7(a)). Calculation of N mineralised during the pot trial clearly showed that there was net N mineralisation, except for the 24-h treatment for which there was net immobilisation of resi-

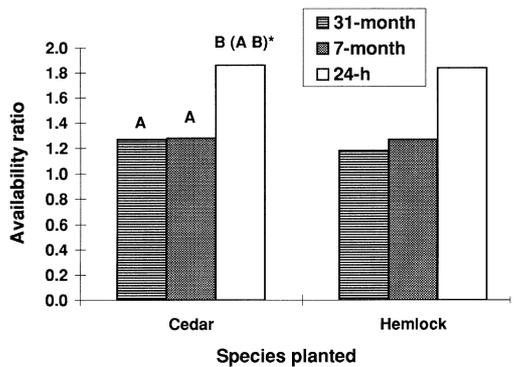


Fig. 6. Availability ratio of residual <sup>15</sup>N in the two species studied. \*: See notes for Fig. 2.

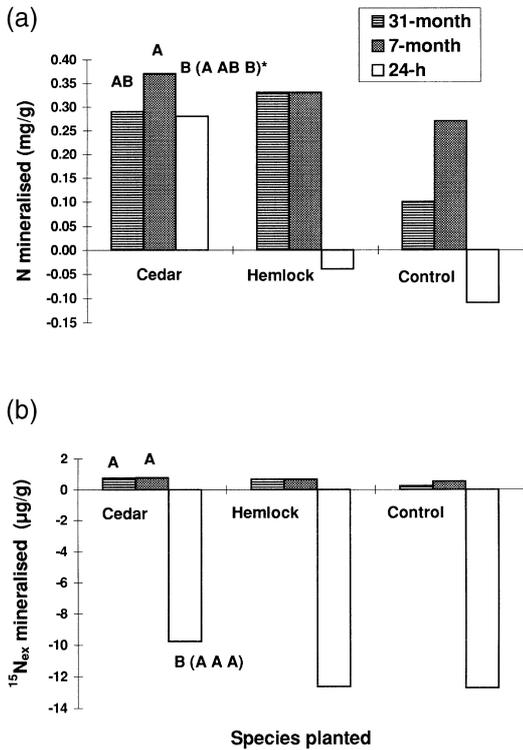


Fig. 7. Total N and  $^{15}\text{N}$  in excess of natural abundance ( $^{15}\text{N}_{\text{ex}}$ ) mineralised during the pot experiment. \*: See notes for Fig. 2.

dual  $^{15}\text{N}$  from the fertiliser applied in the field (Fig. 7(b)).

### 3.4. Comparison with chemical extractions

Preliminary analysis showed that shoot N concentration (g/kg), shoot  $^{15}\text{N}$  abundance (%),  $^{15}\text{N}$  mineralisation ( $\mu\text{g/g}$ ), root  $^{15}\text{N}$  abundance (%) and plant biomass (g/plant) represent the best mix of parameters for evaluating the uptake of N by the plants and plant biomass production, as they were significantly correlated with many others, such as root N concentration (g/kg) and shoot biomass. These parameters were analysed for correlation with chemical indices in previously reported extraction studies on the same samples. Results showed that shoot  $^{15}\text{N}$  abundance and total  $^{15}\text{N}$  mineralised during the pot experiment were most significantly correlated with a range of chemical extraction indices studied (Table 1), for both the redcedar and hemlock pots, except that correlations with  $^{15}\text{N}$  contents in the humic acid and humin

fractions were non-significant. Shoot N concentration, root  $^{15}\text{N}$  abundance and plant biomass (except for redcedar pots) also had significant but weaker correlations with the chemical extraction indices.

## 4. Discussion

One of the findings from this pot experiment was that the immobilisation process of fertiliser N was delayed (at least when only the total N – the sum of native and applied N – was evaluated) if the disturbance (sampling) and incubation of the collected samples in the greenhouse (higher temperature than in the field) happens immediately after fertiliser application. Fig. 1(a) shows that for the 24-h treatment, the mineral N content was maintained at a high level even after 342 days of incubation, but mineral N content in the seven-month treatment was low at day 1 (after being labelled with  $^{15}\text{N}$  for seven months in the field). This statement is true whether or not there were plants in the pots (Fig. 2a). This phenomenon could probably be explained, in part, by the ‘added nitrogen interaction’ (ANI) proposed by Jenkinson et al. (1985) and by the effect from handling (sampling and sieving) (Binkley and Hart, 1989) and changed environmental conditions in the greenhouse incubation.

In the 31- and seven-month treatments, a slight decrease in  $^{15}\text{N}$  abundance in the  $\text{NH}_4^+\text{-N}$  fraction at the end of the incubation period in the no-plant pots indicated a dilution effect by the greater amounts of native N mineralised during the incubation (Fig. 1(a) and (b)). In the 24-h treatment, a significant decrease in  $^{15}\text{N}$  abundance in the  $\text{NH}_4^+\text{-N}$  fraction and only a slight decrease in the  $\text{NH}_4^+\text{-N}$  pool size from day 1 to day 342 indicated that fertiliser  $^{15}\text{N}$  in the  $\text{NH}_4^+\text{-N}$  pool was exchanged for the native soil N during incubation for this particular treatment. This is better illustrated by the N mineralisation data discussed below.

The higher total amounts of N mineralised (or lower amounts of N immobilised) in the planted pots (Fig. 7(a)) confirmed that the presence of plants can increase N mineralisation (Haider et al., 1989; Wheatley et al., 1990). However, the presence of plants seemed to have little effect on mineralisation or immobilisation of the  $^{15}\text{N}$  (Fig. 7(b)). This was the case especially in the 24-h treatment where there was

strong immobilisation of the applied  $^{15}\text{N}$  whether plants were present or not (Fig. 7(b)). This showed that although the  $^{15}\text{N}$  immobilisation process was slowed in the greenhouse incubation experiment when compared to field situation,  $^{15}\text{N}$  immobilisation in the 24-h treatment was still significant. The mineralisation/immobilisation processes during the 342-day incubation seemed to resemble these during an anaerobic incubation (two weeks at  $30^\circ\text{C}$ ) on the same samples (Chang et al., 1997).

Nitrification was negligible when determined on day 1 and 182. The  $\text{NO}_3^-$ -N found at the final sampling date was accumulated after day 182 in the incubation. The significantly lower amounts of  $\text{NO}_3^-$ -N accumulated in the pots planted to redcedar and hemlock (Fig. 2(c)) might have been caused by the uptake of  $\text{NO}_3^-$ -N by the plants or inhibition of nitrification by the presence of the plants (Donaldson and Henderson, 1990a, b; Norton and Firestone, 1996). Di Stefano and Gholz (1989) reported that  $\text{NH}_4^+$ -N availability had a controlling effect on nitrification; therefore, the reason for the lower nitrate level in the 24 h than in the other treatments in the no-plant pots is not clear. Christ et al. (1995) also encountered little nitrification in plots with high N input either in the field or under laboratory incubation. Others (Adams and Attiwill, 1986; Munson and Timmer, 1991) found that the size of N pools was not correlated with nitrification potentials. In general, nitrification in forest soils was found to be extremely variable (Robertson and Klemmedtsson, 1996; Deboer et al., 1996). The significantly lower  $^{15}\text{N}$  abundance in the 24-h treatment in the  $\text{NO}_3^-$ -N than in the  $\text{NH}_4^+$ -N fraction may mean that there was strong discrimination against the heavier N isotope during the nitrification process (Garten, 1993; Koopmans et al., 1997). However, this seemed not to be the case when  $^{15}\text{N}$  enrichment in the source  $\text{NH}_4^+$ -N was not very high, as there was little difference in  $^{15}\text{N}$  abundances between the  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N fractions in the seven- and 31-month treatments (Fig. 2(b) and (d)).

The overall species effect on plant biomass production, plant N uptake and  $^{15}\text{N}$  abundance in biomass was not significant, reflecting three points: (1) N content in the 24-h treatment had been maintained at high levels throughout the incubation; (2) there was nitrogen mineralisation and accumulation in the other two treatments and thus providing some of the needs

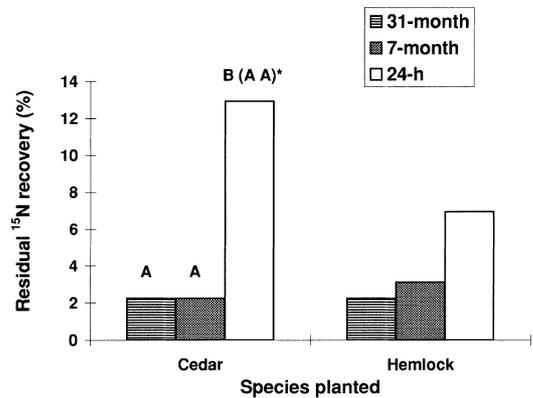


Fig. 8. Residual  $^{15}\text{N}$  recovery rate (%). \*: See notes for Fig. 2.

of the plants for nutrients; and (3) the two species have very similar capacities to take up N from the soil. The higher N availability level in the 24 h than in the 31- and seven-month treatments was reflected in the higher shoot mass and higher shoot N in the former than in the latter; and a reduction in root/shoot ratios in the treatment with higher N availability was consistent with other studies (Vogt et al., 1986).

On a per plant basis, a significantly greater percentage of the residual  $^{15}\text{N}$  was recovered by the plants in the 24 h than in the other two treatments (Fig. 8). The lower recovery rate by hemlock than by redcedar in the 24-h treatment seemed to be an effect of the immobilisation of  $^{15}\text{N}$  by the soil microbial population from day 1 to day 100, at which point the hemlocks were replanted.

The higher  $^{15}\text{N}$  abundance values in the shoot, root and whole plant in the 24 h than in the 31- and seven-month treatments reflected the higher  $^{15}\text{N}$  abundance in mineral N in the 24-h treatment. The higher ( $p < 0.05$ ) availability ratio in the sample with recent  $^{15}\text{N}$  labelling than in the others and the overall ratios of greater than one showed good agreement with the laboratory extraction methods (Chang and Preston, 1998).

Significant correlations between shoot N, shoot  $^{15}\text{N}$  abundance,  $^{15}\text{N}$  mineralised, root  $^{15}\text{N}$  abundance and chemical N availability indices mean that the chemical indices can be reliably used as N availability indicators. Among the chemical indices, the  $^{15}\text{N}$  released during a two-week anaerobic incubation,  $^{15}\text{N}$  mineralised in a 42-day aerobic incubation,  $^{15}\text{N}$  extracted by 0.01 M  $\text{KMnO}_4$  or 2 M  $\text{KCl}$ ,  $^{15}\text{N}$  released by auto-

claving or fumigation (-extraction), and even  $^{15}\text{N}$  abundance in the fulvic acid fraction of the organic matter all seemed to reflect soil residual N availability well. These results agree well with similar work of others (Carlyle and Malcolm, 1986b; Kraske and Fernandez, 1990). Kraske and Fernandez (1990) also found that the two-week anaerobic incubation and the KCl extraction were best correlated with seedling growth in an greenhouse experiment. This study also showed that  $^{15}\text{N}$  abundance in the more stable organic matter fractions (humic acid and humin) was poor indicators of N availability.

## 5. Conclusions

In evaluating residual fertiliser N availabilities in forestry, field studies should be used as the primary source of information. Results from this study strongly suggest that sample handling and growth condition manipulation could substantially change the dynamics of residual fertiliser N and thus cause discrepancies between laboratory/greenhouse and field studies such as the one reported by Preston and Mead (1994b). Good correlation between N obtained through extraction and plant N uptake in the greenhouse shows that chemical indices can be reliably used to predict soil N availability and fertiliser N dynamics although to exactly mimic the field situation is impossible. Chemical extraction methods and other quick and inexpensive laboratory-based methods can provide a good indication of soil N availability, although the reliability of the assessment can be affected by a range of factors.

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