Foliar $\delta^{13}$C and $\delta^{15}$N Response of Lodgepole Pine and Douglas-Fir Seedlings to Soil Compaction and Forest Floor Removal

Woo-Jung Choi, Scott X. Chang, Michael P. Curran, Hee-Myong Ro, Mohammed Kamaluddin,† and Janusz J. Zwiazek

Abstract: The effects of soil compaction, forest floor (FF) removal, and rehabilitation treatments on foliar $\delta^{13}$C and $\delta^{15}$N of lodgepole pine (Pinus contorta) and Douglas-fir (Pseudotsuga menziesii) were studied on a calcareous soil. Regardless of soil compaction, FF removal (which reduces soil water potential) resulted in less negative foliar $\delta^{13}$C values of lodgepole pine (from $-25.9$ to $-23.4\%$), whereas soil compaction effects on foliar $\delta^{13}$C were observed only within the FF intact treatment. This result and the more negative foliar $\delta^{13}$C with increasing seedling growth most likely reflected limitation on $\text{CO}_2$ diffusion due to water stress caused by those treatments. However, foliar $\delta^{13}$C of Douglas-fir (range $-25.0$ to $-24.5\%$) were not affected by the treatments, indicating less susceptibility to water stress. Soil compaction reduced $\text{NH}_4^+$-$\text{N}$ concentrations in the FF (from 48.5 to 28.0) and $\text{NO}_3^-$-$\text{N}$ concentrations in the FF (from 13.8 to 6.4) and mineral soil (from 4.3 to 2.1 mg kg$^{-1}$), and FF removal tended to decrease $\text{NH}_4^+$-$\text{N}$ concentrations in the mineral soil. Foliar $\delta^{15}$N of both species were not affected by soil compaction but were increased by the FF removal and rehabilitation treatments, indicating that the latter two treatments dramatically altered soil $\text{N}$ dynamics. FOR. SCI. 51(6):546–555.

Key Words: Water stress, C and N stable isotope compositions, nitrogen dynamics, disturbance, long-term soil productivity (LTSP).

Concerns about forest productivity and sustainability have been increasing because intensive forest management practices frequently lead to soil compaction and displacement of the forest floor (FF). Such disturbances may adversely alter soil physical, chemical, and biological processes and thus impair tree growth (Kozlowski 1999, Powers 1999). Soil compaction may deteriorate soil physical conditions, such as decreased water permeability, restricted rooting space, and reduced aeration. These factors may affect water uptake by roots (Greacen and Sands 1980, Wolkowski 1990). Soil compaction may also decrease mineralization rates and reduce nutrient availability (Tan et al. 2005, Zabowski et al. 1994). Removal of FF may result in decreased soil nutrient availability because the FF is the principal reservoir of nutrients in forests (Van Cleve et al. 1983), and the elimination of the mulching effect by FF removal may change soil water availability (Zabowski et al. 1994).

Despite the potential impact of soil compaction and FF removal on tree growth, there have been few attempts to integrate whole tree responses to such disturbances (Wronski and Murphy 1994). Previous studies suggested that although soil compaction and FF removal could change many physical and biological properties of soils, their effects on tree growth seem to be related to reduced water and nutrient availabilities (Whalley et al. 1995). During photosynthesis, $\text{CO}_2$ diffusion rates and carboxylation efficiency are largely controlled by soil water and nutrient status, which affect stomatal conductance and photosynthetic enzyme activities, respectively (Högberg et al. 1995, Korol et al. 1999). Therefore, forest site disturbances may lead to changes in the carbon isotope composition ($\delta^{13}$C) of plant tissues due to their effects on carbon isotope discrimination, which can be described for $\text{C}_3$ plants by the model of Farquhar et al. (1989).

$$\delta^{13}C_{\text{plant}} = \delta^{13}C_{\text{air}} - a - (b - a)C/C_{\text{a}},$$

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where $\delta^{15}C_{\text{plant}}$ and $\delta^{15}C_{\text{air}}$ are the carbon isotope abundances of plant and atmospheric CO$_2$, respectively; $C/C_0$ is the ratio of intercellular to atmospheric CO$_2$ concentration, $a$ and $b$ are discrimination against $^{13}C$ during CO$_2$ diffusion through stomata (normally $\sim 4.4\%$) and during CO$_2$ assimilation by RuBP carboxylase (normally $\sim 27\%$), respectively. Although discrimination during CO$_2$ dissolution (diffusion in the liquid phase) and (photo) respiration may also affect $\delta^{15}C_{\text{plant}}$, this simple equation is widely used to represent the relationship between $\delta^{15}C_{\text{plant}}$ and $C/C_0$. This model indicates that decreased CO$_2$ diffusion rates result in less negative $\delta^{15}C$ of plants by reducing the $C/C_0$ ratio, while decreased carboxylation efficiency leads to more negative $\delta^{15}C$ of plants by increasing the $C/C_0$ ratio (O’Leary 1981).

Based on the Farquhar model, Gomez et al. (2002b) reported that impairment of root growth induced by soil compaction resulted in less negative foliar $\delta^{13}C$ of ponderosa pine ($Pinus ponderosa$) in two soils with a loam or clay texture, reflecting water stress; however, the effects of forest floor removal on foliar $\delta^{13}C$ were not studied, although that treatment was available in their study. Forest floor removal may also cause such $\delta^{13}C$ variation because of its impact on soil water availability (Zabowski et al. 1994). Few have examined the effects of FF removal or both compaction and FF removal on $\delta^{13}C$ variation, despite the fact that these disturbances usually occur concurrently. In addition, since plants differ widely in their stomatal sensitivity to water stress, species with different susceptibility to drought stress may have different $\delta^{13}C$ patterns under water stress (Picon et al. 1996, Zhang et al. 1997, Martı́nez-Vilalta et al. 2004).

Nitrogen isotope abundance ($\delta^{15}N$) of tree tissues may also be affected by compaction and FF removal, which influence soil N dynamics, including N source-sink relationships and N transformations (Tan et al. 2005, Gomez et al. 2002a). Since $\delta^{15}N$ of plants is an integrator of the nitrogen isotopic composition of external N sources and $^{15}N/^{14}N$ fractionations during N transformation, assimilation, loss, and internal retranslocation (Högberg 1997, Chang and Handley 2000, Choi et al. 2003), the $\delta^{15}N$ of tree tissues under such disturbance regimes would provide insights into the effects of soil compaction and FF removal on soil N dynamics. Therefore, we hypothesized that (1) FF removal and soil compaction would cause plant $\delta^{13}C$ to become less negative as a result of water stress caused by those disturbances; (2) the $\delta^{13}C$ response to such disturbances may vary depending on the susceptibility to water stress of different tree species; and (3) changed soil N dynamics caused by site disturbances may leave a plant $\delta^{13}N$ pattern different from that in undisturbed sites. To test these hypotheses, we investigated variations of foliar $\delta^{13}C$ and $\delta^{15}N$ of lodgepole pine ($Pinus contorta$ Dougl. ex Loud. var. latifolia Engelm) and Douglas-fir ($Pseudotsuga menziesii$ var. glauca [Baisn.] Franco) as affected by soil compaction and FF removal on a long-term soil productivity (LTSP) experiment established on a site that has calcareous soil horizons appearing within the first 25 cm of the soil profile. Douglas-fir has been shown to be less susceptible to water limitation as compared with lodgepole pine (Piñol and Sala 2000, Martı́nez-Vilalta et al. 2004). This research was conducted in conjunction with another study (Kamaluddin et al. 2005), which examined the effects of soil compaction and FF removal on seedling growth and physiology including photosynthesis, transpiration, and shoot water potential.

**Materials and Methods**

**Study Site**

This study was conducted in the LTSP installation at Mud Creek (50°08’08”N 115°44’44”W), about 40 km southwest of Invermere in southeastern British Columbia, Canada. Details of the study site have been described in Kamaluddin et al. (2005). The soils of the study site are Orthic Eutric Brunisols (Soil Classification Working Group 1998) developed on glacial tills and soil texture in the surface 30 cm ranges from fine sandy to silty clay loam. The mean annual precipitation and temperature between 1971 and 2000 recorded at the Cranbrook weather station (49°36’N 115°46’W), the closest station to the study site, are 400 mm and 6.0°C, respectively.

**Experiment Design**

The LTSP studies have been designed to evaluate the long-term impacts of soil compaction and FF removal on soil processes and stand productivity (Kranabetter and Chapman 1999). This study site contains a 3 × 3 factorial design of treatments, consisting of three levels of organic matter removal (stem-only harvest, whole-tree harvest, and whole-tree harvest plus FF removal) and three levels of soil compaction (no compaction, light compaction, and heavy compaction). Each plot (40 × 70 m) was conventionally harvested in the winter of 1999 by Tembec Forest Industries (Canal Flats, BC) using directional hand-felling techniques. After harvest, the treatments were randomly applied to those plots. In the FF removal plots, the complete FF averaging about 4 cm thick was removed. The soil compaction treatments were created using a hydraulic “hoe-pack” compactor head on an excavator, with a 75 × 75 cm tamping plate. The effectiveness of the soil compaction treatment in changing soil bulk density can be seen in Table 1. In the spring of 2000, a rehabilitation treatment (the reverse of soil compaction) was also established by fluffing a heavily compacted plot (with FF) with an excavator bucket to examine whether it was possible to remediate compacted soils. One-year-old containerized seedlings (410, with 4 cm plug diameter and 10 cm plug length) of lodgepole pine and Douglas-fir were planted out in spring 2000, with each half-plot randomly assigned to one of these two species.

The following five treatments were studied: (a) no compaction with intact FF (NcFi), (b) heavy compaction with intact FF (HcFi), (c) no compaction with FF removal (NcFr), (d) heavy compaction with FF removal (HcFr), and (e) rehabilitation (Rehab). In this study the treatment plots were not replicated, but each treatment plot was divided into.
four subplots. Three randomly located seedlings from each subplot were selected for measurement. A cylindrical gypsum block (223 Delmhorst, Campbell Scientific, Inc., Logan, UT) was buried at 10 cm below mineral soil surface to measure soil moisture status, and the results were presented in Kamaluddin et al. (2005). Annual precipitation in 2000 and 2001 was around 260 mm, much less than the long-term annual precipitation of 400 mm.

**Determination of FF and Mineral Soil Characteristics**

In June 2000 after the treatments were applied, the chemical and physical properties of the FF and mineral soil were measured. Bulk density of the FF and mineral soil was determined by a gravitational method. FF samples were collected from an area of 400 cm² and a depth up to 4 cm from the surface (n = 6) and mineral soil samples were collected using a soil core that is 10 cm in diameter and 10 cm deep (n = 12). Bulk density was calculated as the dry mass (FF or mineral soil) per unit volume of soil. For the mineral soil, total porosity was also determined using the bulk density and particle density values (n = 12). The standard particle density of 2.65 Mg m⁻³ was used in the calculations. Chemical properties of FF and mineral soils were measured on composite samples collected from each treatment plot; total C and N by the combustion method, pH using the Broadley James “Soil Probe” pH electrode after addition of distilled water at 1:2 (sample/water) for FF and 1:1 for mineral soil samples, and mineral N (NH₄⁺ and NO₃⁻) using an auto-analyzer (Technicon Autoanalyzer II) after extraction with 2 M KCl at 1:1 (sample/KCl) ratio for mineral soils and 1:2.5 ratio for FF. The ¹⁵N composition of FF and mineral soil samples were determined in the same way as that of foliar samples described below.

**Sampling and Chemical Analysis of Needle Samples**

Toward the end of the second growing season (between Sept. 5 and 8, 2001) when the treatment effects were expected to be large as the site received less precipitation (103.4 mm) than normal (165.7 mm) in the summer (between May and Aug.), seedling growth and physiological parameters (including rates of photosynthesis and transpiration, shoot water potential, and foliar N concentration) were measured on three randomly located seedlings within each of the four subplots, and the results were reported in Kamaluddin et al. (2005). A detailed description of the methods for the physiological measurement was provided in Kamaluddin et al. (2005). From those data we calculated the instantaneous water use efficiency by dividing the rate of photosynthesis by the rate of transpiration. After the above measurements, current-year needles in all orientation around the tree crown were collected from the same three randomly located seedlings in each subplot as in the seedling size and physiology measurements (total number of samples were 5 treatments × 4 subplots = 20). Needle samples were oven-dried at 60°C and ground to fine powder. Nitrogen concentration and ¹³C and ¹⁵N compositions were determined using a continuous-flow stable isotope ratio mass spectrometer (IsoPrime-EA, Micromass, Manchester, UK) linked to a CN analyzer (NA Series 2, CE Instruments, Milano, Italy). In our study, isotope compositions of whole foliar samples were measured because whole tissue had been shown to retain the strongest C isotope signal resulting from environmental stress; the isotope compositions of whole tissues were closest to that of leaf sugars through a study comparing whole tissue, cellulose, and lignin (Loader et al. 2003). Korol et al. (1999) and Warren et al. (2001) also found strong correlations for ¹³C compositions between whole tissue and cellulose.

Carbon and nitrogen isotope compositions were calculated as

\[
\delta(\%e) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1000, \quad (2)
\]

where \( R \) is the ratio of \(^{13}C/^{12}C\) or \(^{15}N/^{14}N\), and the standards are the Pee Dee Belemnite (PDB) standard for carbon and atmospheric N₂ for nitrogen. Pure CO₂ (\( \delta^{13}C = -28.2 \pm 0.1\%e \)) and N₂ (\( \delta^{15}N = -2.1 \pm 0.1\%e \)) gases calibrated against standard CO₂ (−10.38‰) and N₂ (−0.22‰) gases (Oztech Trading Company, Dallas, TX) using a dual-inlet stable isotope ratio mass spectrometer (Optima, Micromass, UK) served as reference gases for \( \delta^{13}C \) and \( \delta^{15}N \), respectively.

**Table 1. Effects of soil compaction, forest floor (FF) removal, and rehabilitation treatments on bulk density and total porosity of the FF and mineral soil**

<table>
<thead>
<tr>
<th>Treatment⁴</th>
<th>Forest floor</th>
<th>Mineral soil</th>
<th>Total soil porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bulk density (Mg m⁻³)</td>
<td></td>
<td>(m⁻³)</td>
</tr>
<tr>
<td>NcFi</td>
<td>0.11 (0.03)</td>
<td>1.05 (0.15)</td>
<td>0.63 (0.09)</td>
</tr>
<tr>
<td>HcFi</td>
<td>0.19 (0.01)</td>
<td>1.18 (0.16)</td>
<td>0.53 (0.03)</td>
</tr>
<tr>
<td>NcFr</td>
<td>NA b</td>
<td>1.17 (0.10)</td>
<td>0.56 (0.03)</td>
</tr>
<tr>
<td>HcFr</td>
<td>NA b</td>
<td>1.29 (0.13)</td>
<td>0.51 (0.04)</td>
</tr>
<tr>
<td>Rehab</td>
<td>1.04 (0.20)</td>
<td>0.61 (0.05)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means with standard errors in parentheses.

¹ NcFi, no compaction with FF intact; HcFi, heavy compaction with FF intact; NcFr, no compaction with FF removal; HcFr, heavy compaction with FF removal; Rehab, rehabilitation (deep ripping following heavy compaction).

b NA, Not applicable.
Statistical Analyses

Statistical analysis was carried out with SPSS 11.5 (SPSS Inc., Chicago, IL). Since we did not have any true replication in the study design, only means and standard errors are reported, and no statistically based mean comparisons were made. Pearson correlation analysis was used to examine relationships between foliar $\delta^{13}$C and seedling physiology (rate of net photosynthesis and transpiration, instantaneous water use efficiency, and shoot water potential), growth (height and diameter) parameters, and foliar N concentration using subplot mean values.

Results

Physical and Chemical Characteristics of FF and Mineral Soil

Soil compaction increased bulk density of mineral soil from 1.05 (NcFi) to 1.18 Mg m$^{-3}$ (HcFi) for the FF intact and from 1.17 (NcFr) to 1.29 Mg m$^{-3}$ (HcFr) for the FF removal treatments (Table 1). However, within the no compaction treatment the higher bulk density in the FF removal than those in the FF intact treatments indicates that the FF removal practice also caused the soil to compact. Within the FF intact treatment, the bulk density of FF was increased by soil compaction from 0.11 (NcFi) to 0.19 Mg m$^{-3}$ (HcFi) (Table 1). Total soil porosity followed a reversed trend (Table 1) as the higher the bulk density, the lower the total soil porosity.

Whether the study plot was compacted or not, the FF had much higher mineral N concentrations and lower pH as compared to the mineral soil (Table 2). Soil compaction tended to reduce ammonium N concentration in the FF but not in the mineral soil. Ammonium N concentration in the mineral soil appeared to be higher in the plots with FF intact than in those with FF removed. Soil compaction tended to reduce nitrate concentration in the FF. Nitrate concentration in the mineral soil tended to be higher in the Rehab than in the other treatments and appeared to be reduced by soil compaction, but was not affected by FF removal (Table 2). The $\delta^{15}$N values of FF (range $-1.6 \sim -1.0%$) were much lower than those of mineral soil ($4.4 \sim 6.7%$). Overall, foliar N concentrations were higher in the Rehab than in the other treatments regardless of the species (Table 3). For lodgepole pine, foliar N concentration in the Rehab treatment tended to be higher than those in the HcFi, NcFr, and HcFr treatments (Table 3). For Douglas-fir, foliar N concentrations tended to be higher in the Rehab than in the HcFr treatment.

For Douglas-fir, foliar $\delta^{13}$C ranges between $-25.0$ and $-24.5%$ did not appear to be affected by soil compaction, FF removal, or the Rehab treatment (Table 3). For lodgepole pine, the NcFi and the Rehab treatments had virtually the same foliar $\delta^{13}$C, which were more negative than that in the soil compaction and/or FF removal treatments. Regardless of soil compaction, FF removal caused foliar $\delta^{13}$C of lodgepole pine to become less negative (from $-25.9$ to $-23.4%$ for the noncompacted and from $-24.8$ to $-23.5$ for the compacted treatments). However, the change in $\delta^{13}$C resulting from FF removal was much greater in the non-compacted (NcFi versus NcFr) than that in the compacted treatments (HcFi versus HcFr). Within the FF intact treatment, soil compaction tended to result in less negative foliar $\delta^{13}$C values in HcFi ($-24.8%$) than that in NcFi ($-25.9%$), while such an effect was not observed within the FF removal treatment.

Regardless of the species, foliar $\delta^{15}$N was lower in the FF intact (range $0.2 \sim 0.9%$) than in the FF removal treatment ($1.6 \sim 2.8%$) (Table 3). However, the overall effect of compaction on foliar $\delta^{15}$N was not evident.

Correlation between $\delta^{13}$C and Seedling Parameters

Regardless of the species, foliar $\delta^{13}$C of seedlings was not correlated with seedling physiology measurements such as rates of net photosynthesis and transpiration, or shoot water potential (Figure 1). For lodgepole pine, a negative correlation ($r^2 = 0.59$, $P < 0.001$) between foliar $\delta^{13}$C and instantaneous water use efficiency calculated as the ratio of

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Table 2. Effects of soil compaction, forest floor removal, and rehabilitation treatments on chemical properties of the FF and mineral soil

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample type</th>
<th>Total C (g kg$^{-1}$)</th>
<th>Total N (g kg$^{-1}$)</th>
<th>$d^{15}$N (%o)</th>
<th>pH$_{H2O}$</th>
<th>NH$_4^+$-N (mg kg$^{-1}$)</th>
<th>NO$_3^-$-N (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NcFi</td>
<td>Forest floor</td>
<td>490.9 (20.0)</td>
<td>16.5 (1.2)</td>
<td>$-1.0$ (0.1)</td>
<td>5.4 (0.2)</td>
<td>48.5 (25.8)</td>
<td>13.8 (8.3)</td>
</tr>
<tr>
<td></td>
<td>Mineral soil</td>
<td>27.0 (8.9)</td>
<td>1.3 (0.2)</td>
<td>$4.4$ (0.1)</td>
<td>6.9 (0.4)</td>
<td>46.1 (1.9)</td>
<td>4.5 (6.2)</td>
</tr>
<tr>
<td>HcFi</td>
<td>Forest floor</td>
<td>452.0 (36.1)</td>
<td>9.0 (2.3)</td>
<td>$-1.6$ (0.3)</td>
<td>5.2 (0.3)</td>
<td>28.0 (9.1)</td>
<td>6.4 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Mineral soil</td>
<td>23.9 (8.1)</td>
<td>1.1 (0.2)</td>
<td>$4.6$ (1.9)</td>
<td>7.2 (0.4)</td>
<td>4.9 (2.6)</td>
<td>2.4 (1.9)</td>
</tr>
<tr>
<td>NcFr</td>
<td>Forest floor</td>
<td>NA$^b$</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Mineral soil</td>
<td>14.4 (3.1)</td>
<td>0.8 (0.1)</td>
<td>6.7 (0.8)</td>
<td>7.3 (0.4)</td>
<td>2.7 (2.0)</td>
<td>4.2 (4.6)</td>
</tr>
<tr>
<td>HcFr</td>
<td>Forest floor</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Mineral soil</td>
<td>20.8 (6.9)</td>
<td>1.1 (0.2)</td>
<td>4.8 (0.7)</td>
<td>7.2 (0.4)</td>
<td>3.0 (1.1)</td>
<td>1.8 (2.5)</td>
</tr>
<tr>
<td>Rehab</td>
<td>Forest floor</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Mineral soil</td>
<td>21.2 (10.1)</td>
<td>1.2 (0.4)</td>
<td>5.2 (1.3)</td>
<td>7.1 (0.6)</td>
<td>3.1 (2.7)</td>
<td>25.7 (28.8)</td>
</tr>
</tbody>
</table>

Values are means with standard errors in parentheses.

$^a$ Treatment codes are described in the footnote to Table 1.

$^b$ NA, Not applicable.
net photosynthesis to transpiration rates was observed (Figure 1c). Negative correlations between foliar $^{13}C$ and seedling height ($r^2 = 0.43$, $P = 0.002$) and diameter ($r^2 = 0.29$, $P = 0.014$) of lodgepole pine were observed, while the $^{13}C$ of Douglas-fir was not correlated with seedling growth (Figure 2). Foliar $^{13}C$ was not correlated with the corresponding N concentrations for Douglas-fir, but was correlated for lodgepole pine (Figure 3).

### Discussion

#### Foliar $^{13}C$ in Relation to Soil Water and Nutrient Limitation

According to the carbon isotope discrimination model of Farquhar et al. (1989), as water becomes limiting, decreases in CO$_2$ diffusion rate (because of stomatal closure) limit CO$_2$ exchange, which leads to enrichment of $^{13}C$ in the CO$_2$
retained in the mesophyll. Negative relationships between plant $^{13}C$ and soil water availability have been reported for several tree species (Picon et al. 1996, Zhang et al. 1997, Korol et al. 1999, Livingston et al. 1999, Gomez et al. 2002b). In our study, the less negative foliar $^{13}C$ of lodgepole pine in the FF removal than those in the FF intact treatments (Table 3) most likely reflected decreased water availability resulting from the elimination of the mulching effect of the FF as reported by Kamaluddin et al. (2005). According to their data, average soil water potentials were decreased by FF removal from $-0.07$ (NcFi) to $-0.41$ MPa (NcFr) within the no compaction treatment and from $-0.16$ (HcFi) to $-0.35$ MPa (HcFr) within the heavy compaction treatment during the second growing season (May to Sept. 2001). The number of days when water potentials were below the permanent wilting point ($<-1.5$ MPa) was also increased by FF removal from 0 (NcFi) to 6 (NcFr) days within the no compaction treatment and from 3 (HcFi) to 7 days (HcFr) within the heavy compaction treatment. The soil water potential of rehabilitation treatment averaged $-0.06$ MPa and was consistently above $-1.5$ MPa. The almost identical foliar $^{13}C$ for the Rehab ($-25.8\%$) and NcFi ($-25.9\%$) treatment could also be primarily attributed to the similar soil water availabilities in those treatments.

The effects of soil compaction on foliar $^{13}C$ have been shown to vary with soil texture. For example, Gomez et al. (2002b) reported that foliar $^{13}C$ of ponderosa pine grown in two fine-textured soils (a loam and a clay) became less negative by soil compaction, and they attributed the $^{13}C$ enrichment to restricted water uptake due to impaired root growth. However, in a sandy soil they found that foliar $^{13}C$ was decreased by compaction (Gomez et al. 2002b), likely resulting from increased water uptake through improved root-soil contact and greater unsaturated hydraulic conductivity (Taylor and Brar 1991, Arvidsson 1999). In our study, different responses of foliar $^{13}C$ of lodgepole pine to soil compaction between the FF removal and intact treatments suggest that the effect of soil compaction on foliar $^{13}C$ is dependent on soil water content (i.e., such effects may not show up when seedling growth is already being affected by soil water limitation, such as that caused by FF removal). Thus results from this study expand the patterns that we would normally expect to find or that have been reported in the literature. The negligible effect of soil compaction on foliar $^{13}C$ within the FF removal treatment may also be attributable to the increased soil bulk density of the NcFr ($1.17$ Mg$\cdot$m$^{-3}$, a side effect of the FF removal practice) treatment as compared to that of the NcFi ($1.05$ Mg$\cdot$m$^{-3}$) treatment (Table 1).

We did not observe any significant correlation of foliar $^{13}C$ with seedling physiology measurements except with instantaneous water use efficiency (Figure 1), indicating that short-term measurements of such parameters do not always corroborate with stable isotope signals that integrate the effects of physiological response to treatments over a growing season or longer (Picon et al. 1996). The negative
correlation between foliar $\delta^{13}C$ and instantaneous water use efficiency of lodgepole pine was unexpected because improvements of water use efficiency tend to decrease carbon isotope discrimination, leading to a less negative $\delta^{13}C$ (Choi et al. 2005). However, a negative correlation between foliar $\delta^{13}C$ and seedling growth of lodgepole pine (Figure 2) supports that growth of the species was affected by limitation on CO$_2$ diffusion caused by water deficiency rather than by limitation on carboxylation efficiency resulting from nutrient deficiency. Foliar $\delta^{13}C$ is usually negatively correlated with tree growth when the variation in foliar $\delta^{13}C$ is primarily due to the changes in CO$_2$ diffusion rate, but positively correlated when the variation in foliar $\delta^{13}C$ is related to changes in carboxylation efficiency (Flanagan and Johnsen 1995, Pita et al. 2001). The negative correlation between foliar $\delta^{13}C$ and seedling growth observed in our study is consistent with those reported for other tree species, both coniferous and deciduous (Le Roux et al. 1996, Pita et al. 2001, Ferrio et al. 2003).

Several authors (e.g., Livingston et al. 1999, Ripullone et al. 2004) reported positive correlations between foliar $\delta^{13}C$ and N concentration. Hence, negative correlation between foliar $\delta^{13}C$ and the corresponding N concentration observed in our study (Figure 3) suggests that either photosynthesis was not restricted by N limitation or water limitation (decreasing $^{13}C$ discrimination) prevailed over N limitation (increasing $^{13}C$ discrimination) effects on foliar $\delta^{13}C$. Such contrasting patterns could be attributed to the different experimental conditions of the above-mentioned studies from that of ours in terms of N gradients, i.e., these studies investigated foliar $\delta^{13}C$ of seedlings with or without N addition, which resulted in a wider range of foliar N concentrations and thus such positive correlation could be exhibited.

Compared to lodgepole pine, the negligible treatment effect on foliar $\delta^{13}C$ in Douglas-fir (Table 3) suggested that the response of this species to water limitation differed from that of lodgepole pine. Tree species that are susceptible to water limitation such as lodgepole pine (Piñol and Sala 2000, Oliet et al. 2002, Wang et al. 2003) can use water and accumulate biomass quickly when water is available, but close their stomata to maintain leaf water potential at a constant level when water stress is experienced (Picon et al. 1996, Zhang et al. 1997). In contrast, tree species that are less susceptible to water limitation such as Douglas-fir (Livingston and Black 1987, Fleming et al. 1996, Piñol and Sala 2000) can maintain a relatively high stomatal conductance even during drought periods as compared with species more susceptible to drought stress, resulting in less sensitive $\delta^{13}C$ responses to water limitation (Picon et al. 1996, Martinez-Vilalta 2004). The different shoot water potential between lodgepole pine ($-1.7$ to $-1.1$ MPa) and Douglas-fir ($-3.0$ to $-2.8$ MPa) observed at this study site (Figure 1; Kamaluddin et al. 2005) illustrates that response to drought between these two species was different. The shoot water potential values for the species are comparable with those of Piñol and Sala (2000), who observed that shoot water potential causing a 50% loss of stem conductivity for Douglas-fir ($-5.19$ MPa) was much lower than that ($-3.03$ MPa) for lodgepole pine in the Northern Rocky Mountains, Montana.

**Foliar $\delta^{15}N$ in Relation to N Source and Loss**

Foliar $\delta^{15}N$ values (range $0.5 \sim 2.8\%e$, Table 3) in this study were not different between lodgepole pine and Douglas-fir, and the values were within the range ($-6.0 \sim 3.0\%e$) normally observed in forest ecosystems (Garten 1993, Nadelhoffer and Fry 1994, Chang and Handley 2000). Foliar $\delta^{15}N$ of trees depends on the $\delta^{15}N$ of N sources and N isotopic fractionation accompanying N fluxes in soil-plant systems (Nadelhoffer et al. 1996, Högberg 1997, Robinson 2001, Kolb and Evans 2002).

In our study, the higher foliar $\delta^{15}N$ in the FF removal (range $2.1 \sim 2.7\%e$) than in the FF intact ($0.2 \sim 0.9\%e$) treatment (Table 3) reflected altered N source and dynamics caused by the FF removal treatment. Removal of the FF, which is the principal reservoir of nutrients in boreal forests (Table 2; Van Cleve et al. 1983), may directly influence foliar $\delta^{15}N$ by altering the isotope composition of N available for plant uptake because of the different $\delta^{15}N$ signature of the FF (range $-1.6 \sim -1.0\%e$) and mineral soil ($4.4 \sim 6.7\%e$) (Table 2). It is well documented that FF nitrogen, which came from $^{15}N$-depleted plant N through litterfall, has much lower $\delta^{15}N$ values (range $-6.0 \sim 2.0\%e$) than the mineral soil ($2.0 \sim 8.0\%e$) (Nadelhoffer and Fry 1994, Emmett et al. 1998, Vervaet et al. 2002). A decrease in the contribution of N$_2$ fixation and mycorrhizal N to plant N caused by site disturbance (Wei and Kimmens 1998, Byrd et al. 2000) would be another plausible reason for higher foliar $\delta^{15}N$ in the FF removal than in the FF intact treatment. The $\delta^{15}N$ of biologically fixed N is close to $0\%e$, which is lower than that of mineral N (range $2.0 \sim 8.0\%e$) in the soil (Nadelhoffer and Fry 1994, Boddey et al. 2000). Transfer of N from mycorrhizal fungi to plants leads to the depletion of $^{15}N$ in plant biomass because of large isotopic fractionation during N translocation (Högberg 1997, Hobbie et al. 2000).

Since N losses through processes such as ammonia volatilization, denitrification, and leaching of $^{15}N$-depleted NO$_3^-$ result in $^{15}N$ enrichment of the N remaining in the soil (Högberg 1997), thus foliar $\delta^{15}N$ has often been related to the openness of an ecosystem against N loss (Högberg and Johannisson 1993, Högb erg et al. 1995, Austin and Vitousek 1998). In our study, the higher foliar $\delta^{15}N$ in the Rehab than in the FF intact treatments (Table 3) may have reflected the effects of both the removal of $^{15}N$-depleted N sources and a flush of soil N mineralization followed by N loss resulting from the reclamation of the compacted soil with an excavator (Bulmer 2000, Sainju et al. 2002). Soil disturbances, such as those caused by tillage, enhance decomposition of soil organic matter by incorporating organic residue into the mineral soil, exposing protected organic matter to microbial decomposition, and increasing aeration (Bulmer 2000, Sainju et al. 2002). Among the treatments
applied in this study, soil mineral N concentration analyzed in June 2000, 1 year before the seeding δ¹⁵N measurements, was highest in the Rehab treatment (Table 2), indicating the highest potential for N loss. Shearer and Kohl (1989) and Boddey et al. (2000) also reported that intensively cultivated soils tend to be more enriched in δ¹⁵N than pasture or forest soils because of increased N loss in the cultivated soil. In addition, the mixing of FF (pH ≈ 5.0) with the mineral soil (pH ≈ 7.0) in the Rehab treatment might have also enhanced N loss through NH₃ volatilization.

Conclusions

Forest floor removal resulted in less negative foliar δ¹³C of lodgepole pine in response to decreased soil water potential, whereas soil compaction effects on foliar δ¹³C were observed only within the FF intact treatment. Foliar δ¹³C of Douglas-fir was not affected by site disturbance, reflecting a response strategy to drought different from that of lodgepole pine. For lodgepole pine, limitation on foliar CO₂ diffusion via stomatal or mesophyll cells (caused by that of lodgepole pine. For lodgepole pine, limitation on foliar CO₂ diffusion via stomatal or mesophyll cells (caused by source and changed N dynamics. Therefore, this study suggests that foliar δ¹³C reflects water limitation on relatively dry sites/seasons and foliar δ¹⁵N indicates altered N dynamics caused by soil compaction, FF removal, and Rehab treatments. However, stable isotope responses of trees may vary depending on other factors affecting soil water and nutrient availability, such as site characteristics (precipitation, groundwater level, soil texture, and nutrient content) and tree variables (species, age, and rooting depth). Therefore, site- and species-specific δ¹³C and δ¹⁵N ranges need to be examined and established for using foliar δ¹³C and δ¹⁵N as indicators of forest disturbance.

Literature Cited


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