# Variability of Nitrogen and Carbon Contents in the Needles of Canadian Douglas-fir Provenances on Two Soil Types in Serbia

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*Abstract*

Seed transfer and introduction risks can be reduced by setting up provenance tests and studying the elements of genotypic, phenotypic, physiological, and anatomical structure. Forest tree increment and productivity depend on photosynthesis and the concentrations of nitrogen and carbon in the needles. With the aim of introducing Douglas-fir into Serbia, the Institute of Forestry in Belgrade established several experimental provenance tests to assess the genetic ability of Douglas-fir to adapt to new environmental conditions in Serbia. The provenance tests included fourteen different Douglas-fir provenances originating from Canada. All the trees of the study provenances were of the same age and grown in the same conditions, but on two different soil types: eutric cambisol and vertisol. There was considerable variability of nitrogen and carbon contents in the needles of all tested provenances on both locations. This variability was used as the basis for the study of the intensity and dynamics of the physiological processes of Douglas-fir mineral nutrition as indicators of its capacity to adapt to the sites in Serbia. All Douglas-fir provenances planted on eutric cambisol had higher contents of nitrogen and lower contents of carbon, i.e. narrower C/N ratios than the provenances planted on vertisol. The differences resulted from different conditions for Douglas-fir physiological activity and nutrition in the two types of soil.

**Key words:** Douglas-fir, provenance, nitrogen, carbon, eutric cambisol, vertisol

## Introduction

High potential growth rate and good wood quality together with an extensive distribution range in the western USA and Canada have made Douglas-fir (*Pseudotsuga menziesii* Mirb/Franco) the most introduced conifer species in the forestry worldwide. Douglas-fir is one of the most widely studied exotic species (Ebell 1972, Larsen 1981, Hermann and Lavender 1990, Li and Adams 1993, Miller and Knowles 1994 Balduman et al. 1999, Fu et al. 1999, Forrest et al. 2001, Amarasinghe and Carlson 2002, Johnson 2002, Woods et al. 2002, Howe at al. 2006, Wilczyński and Feliksik 2007, Slesak 2008, Harrison at al. 2009, Mcfarlane et al. 2009, Jorge et al. 2011, Slesak et al. 2010, Yan, C-F. et al. 2012, Wang et al. 2013, Popov 2014).

The Programme of Douglas-fir provenance introduction and selection was initiated by the Institute of Forestry with the establishment of provenance tests at several locations in Serbia. Long-term research included all dendrometric characteristics, anatomy, physiology, mechanical wood properties and tree essential oils in order to select genetically most adaptive provenance habitat in Serbia (Popović and Lavadinović 2003, Lavadinović et al. 2008, Tešević et al. 2009, Lavadinović et al. 2010, Lavadinović et al. 2011d).

Douglas-fir trees are easy to establish, fast growing, and most often free from major insect and disease agents, which all make them an ideal forest crop (Glenn et al. 2006). Therefore, Douglas-fir is a species that can be introduced to diverse sites in Serbia. Its monoculture plantations have been established as substitutes for natural forests from submontane to subalpine vegetation belts. The substitution of Douglas-fir plantations for autochthonous coppice forests were mostly performed in oak and beech belts.

Litterfall represents the most important source of organic carbon (C) input to the forest floor (Norden 1994), and consequently the main source of organic matter. The replacement of native forests with Douglas-fir plantations causes changes in the chemical properties of dead organic matter that reaches the soil. It can subsequently change soil properties and its production potential.

The aim of this study is to examine the possibility of Douglas-fir introduction to forest sites in Serbia, and based on provenance tests to assess which of the study provenances can attain better nitrogen (N) uptake on eutric cambisol, vs. vertisol. Based on the C/N ratio in the needles of the study Douglas-fir provenances, it can be figured out which of the study provenances can cause adverse effects on the above-stated soil types.

## Material and Methods

Two provenance tests, each containing 14 Douglas-fir provenances originating from Canada, were established in nursery conditions at two locations: the Faculty of Forestry Arboretum, and the Nursery at Sremčica near Belgrade. The seeds originated from the locations presented in Table 1.

The nurseries, where Douglas-fir seedlings were produced, are 20 km away, which makes their macroclimate conditions approximately the same. According to FAO soil classification (Škorić et al 1985) the soil of the Faculty of Forestry Arboretum belongs to the type of eutric cambisol and the soil type of Sremčica nursery is vertisol. The seedlings were raised in containers, with 60 plants of each provenance being transplanted to sample plots at the age of three (3+0). The planting spacing was 2 × 2 m.

The soil analyses included:

• Active and substitution acidity (pH in $H_2O$ and pH in KCl), by potentiometry;

• Total humus content, by Tyurin method (Škorić and Racz 1966);

**Table 1.** Geographical characteristics of the tested Douglas-fir provenances

| Provenance No. | Provenance Code | Seed zone | Location | Latitude | Longitude | Altitude |
|---|---|---|---|---|---|---|
| 1 | 03333 | East Kootenay | Cranbrook | $49^0$ 25' | $115^0$ 20' | 1050 m |
| 2 | 00848 | West Kootenay | Inonoaklin | $49^0$ 50' | $118^0$ 10' | 671 m |
| 3 | 30667 | Shuswap Adams | Mann Creek | $51^0$ 35' | $120^0$ 10' | 600 m |
| 4 | 05227 | East Kootenay | Gavia Lake tfl 14 | $50^0$ 56' | $116^0$ 35' | 1070 m |
| 5 | 05226 | East Kootenay | Nine Bay TFL 14 | $50^0$ 58' | $116^0$ 32' | 975 m |
| 6 | 03356 | Thompson Okanagan Arid | Trout Cr | $49^0$ 40' | $119^0$ 52' | 884 m |
| 7 | 03360 | Thompson Okanagan Arid | Michell Cr | $49^0$ 54' | $119^0$ 37' | 1035 m |
| 8 | 01198 | West Kootenay | Salmo | $49^0$ 15' | $117^0$ 30' | 793 m |
| 9 | 30460 | Shuswap Adams | Mara LK | $50^0$ 48' | $119^0$ 00' | 488 m |
| 10 | 00278 | Thompson Okanagan Arid | Monte Crk | $50^0$ 37' | $119^0$ 52' | 701 m |
| 11 | 03383 | West Kootenay | Sheep Creek | $49^0$ 10' | $117^0$ 15' | 1000 m |
| 12 | 30461 | Shuswap Adams | Cooke Creek | $50^0$38' | $118^0$ 49' | 900 m |
| 13 | 03389 | West Kootenay | Benton Creek | $49^0$ 12' | $117^0$ 25' | 933 m |
| 14 | 05092 | East Kootenay | Sun Creek | $50^0$ 08' | $115^0$ 52' | 1000 m |

• Total nitrogen content, by Kjeldahl method (Džamić et al 1996);

• Soil texture was determined by sedimentation method using Na-pyrophosphate as a peptisation agent (Racz 1971).

The samples for foliar analysis were taken from both locations at the seedling age of eleven. The needles for laboratory analysis were taken from the upper third of the crown during the dormancy period.

The study material was collected in four replications for each provenance on both localities. Each replicate combined needle samples collected on twelve trees.

The collected needles were air-dried and milled. The moisture content of the needles was determined after they had been dried at 105°C to absolute dry state. The carbon content and the nitrogen content in the needles were determined in the air-dry state, and the obtained values were recalculated to absolute dry state.

The contents of total carbon and nitrogen were determined as follows: carbon percentage was determined by wet combustion in the mixture of $CrO_3$ and sulphuric acid (using the Anstet method, modified by Ponomareva and Plotnikova 1975), and nitrogen content was determined using the Kjeldahl method.

Needle age affects physiological activity and plays an important role in the determination of carbon and nitrogen physiology. Therefore great care must be taken to select needle samples of the same age and from similar crown positions (Yan et al. 2012). To avoid the impact of needle age on nitrogen and carbon contents, the present analysis was focused on one-year-old needles.

The content of nitrogen, carbon and C/N ratio in the needles of the seedlings grown on eutric cambisol and on vertisols were compared using *t*-test. The deviation of the studied parameters of each provenance from the mean value was calculated by the following formula:

$$Z = \frac{X - \overline{X}}{S},$$

where: X is the value of each provenance; $\overline{X}$ is the average value at a given locality; S is the standard deviation.

## Results

The soil type in the nursery Sremčica is vertisol, while it is eutric cambisol in the arboretum of the Faculty of Forestry. Both soil types are characterized by a high content of clay, with vertisol having significantly higher clay content than eutric cambisol (Table 2). Due to the high content of clay in the texture of ver-

**Table 2.** Particle size composition

| Soil type | Depth | Coarse sand | Fine sand | Silt | Clay | Total sand | Total clay | |
|---|---|---|---|---|---|---|---|---|
| | cm | % | % | % | % | % | % | Soil texture class |
| Eutric cambisol | 0-20 | 0.62 | 37.98 | 34.03 | 27.37 | 38.60 | 61.40 | Loam |
| | 20-40 | 0.63 | 33.07 | 36.73 | 29.57 | 33.70 | 66.30 | Clay loam |
| Vertisol | 0-20 | 1.29 | 22.69 | 35.96 | 40.06 | 23.98 | 76.02 | Clay |
| | 20-40 | 0.73 | 24.49 | 33.95 | 40.83 | 25.22 | 74.78 | Clay |

tisols, they are generally less water permeable and less aerated than eutric cambisols. This means that the physical properties of eutric cambisol provide more favourable conditions for the absorption of water and nutrients from the soil compared to vertisol. Vertisol is a heavy, compacted soil, which is coherent in dry state, but plastic, poorly drained and poorly aerated in wet conditions, whereas eutric cambisol, which is clay loam by textural class, is much more permeable than vertisol, better aerated, and with more favourable oxidative conditions. This means that eutric cambisol does not provide conditions that can cause the retardation of physiological processes.

Eutric cambisol has a slightly higher organic carbon content and total nitrogen content in comparison to vertisol (Table 3). The ratio of carbon to nitrogen is narrow in both tested soil types, but it is slightly narrower in eutric cambisol than in vertisol. The narrower C/N ratio and more favourable physical characteristics of eutric cambisol provide better mineralization of organic nitrogen and its conversion into plant available forms.

**Table 3.** Content of carbon and nitrogen in soil

| Soil type | Depth (cm) | pH $H_2O$ | pH KCl | C (%) | N (%) | C/N |
|---|---|---|---|---|---|---|
| Vertisol | 0-20 | 6.06 | 4.76 | 1.33 | 0.13 | 10.23 |
| | 20-40 | 6.36 | 4.84 | 1.20 | 0.12 | 10.00 |
| Eutric cambisol | 0-20 | 6.94 | 5.71 | 1.64 | 0.17 | 9.65 |
| | 20-40 | 7.06 | 5.88 | 1.47 | 0.16 | 9.19 |

According to the tested soil properties, the two sites have different conditions for the absorption of nitrogen by Douglas-fir. Eutric cambisol provides more favourable conditions than vertisol. However, the conditions for the nitrogen nutrition of Douglas-fir at each individual locality are more or less uniform. Even so, the test results indicate high variability of nitrogen and carbon in the needles of Douglas-fir grown under the same site conditions (Table 4). The highest percentage of nitrogen in Douglas-fir needles grown on vertisol was found in provenance 1 (03333 seed zone East Kootenay) accounting for 0.62 %, and the lowest percentage of 0.17 % was found in provenance 11 (03383 seed zone West Kootenay). The seedlings grown on eutric cambisol had the highest percentage of nitrogen in the needles of provenance 12 (30461 seed zone

**Table 4.** Nitrogen and carbon contents in the needles of Douglas-firs of different provenances

| Provenance | | Vertisol | | | | Eutric cambisol | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Code | C (%) | Z | N (%) | Z | C (%) | Z | N (%) | Z |
| 1 | 03333 | 41.2 | -0.912 | 0.62 | 1.349 | 40.99 | 0.268 | 0.88 | 0.363 |
| 2 | 00848 | 40.9 | -1.076 | 0.58 | 1.074 | 40.75 | 0.233 | 0.93 | 0.818 |
| 3 | 30667 | 43.8 | 0.434 | 0.42 | 0.086 | 45.03 | 0.869 | 0.99 | 1.394 |
| 4 | 05227 | 44.1 | 0.634 | 0.39 | -0.080 | 45.64 | 0.960 | 0.71 | -1.071 |
| 5 | 05226 | 41.5 | -0.749 | 0.44 | 0.201 | 43.32 | 0.615 | 0.83 | -0.033 |
| 6 | 03356 | 43.8 | 0.437 | 0.25 | -0.960 | 47.00 | 1.162 | 0.73 | -0.960 |
| 7 | 03360 | 42.1 | -0.439 | 0.31 | -0.582 | 43.14 | 0.588 | 0.91 | 0.641 |
| 8 | 01198 | 40.2 | -1.475 | 0.62 | 1.326 | 46.83 | 1.136 | 0.77 | -0.535 |
| 9 | 30460 | 44.6 | 0.851 | 0.32 | -0.517 | 40.19 | 0.150 | 0.76 | -0.635 |
| 10 | 00278 | 40.5 | -1.307 | 0.19 | -1.327 | 32.37 | -1.014 | 0.59 | -2.169 |
| 11 | 03383 | 45.4 | 1.312 | 0.17 | -1.443 | 34.62 | -0.680 | 0.86 | 0.231 |
| 12 | 30461 | 44.0 | 0.562 | 0.24 | -1.002 | 31.77 | -1.103 | 1.01 | 1.489 |
| 13 | 03389 | 46.1 | 1.682 | 0.59 | 1.140 | 29.32 | -1.467 | 0.86 | 0.240 |
| 14 | 05092 | 43.0 | 0.044 | 0.52 | 0.735 | 27.64 | -1.717 | 0.86 | 0.228 |

C – Nitrogen, N – Carbon, Z – the deviation of the studied parameters of each provenance from the mean value

Shuswap Adams), and the lowest percentage was found in provenance 10 (00278 (seed zone Thompson Okanagan Rid).

Due to more favourable conditions for nitrogen nutrition on eutric cambisol compared to vertisol all provenances cultivated on vertisol are characterized by significantly lower concentrations of the total nitrogen in the needles compared to the provenances cultivated on eutric cambisol (Table 5).

Douglas-fir seedlings produced on vertisol had the highest percentage of carbon in provenance 13 (03389 seed zone West Kootenay), and the lowest in provenance 8 (01198 seed zone West Kootenay). Eutric cambisol seedlings attained the highest percentage of carbon in the needles in provenance 6 (03356 seed zone Thompson Okanagan Arid), and the lowest percentage was measured in provenance 14 (05092 seed zone East Kootenay). The content of total carbon in the needles of Douglas-fir cultivated on vertisol was somewhat higher in comparison to that on eutric cambisol, but this difference was not statistically significant (Table 5). Unlike nitrogen, carbon is a nutrient absorbed from the air. For this reason, there were no statistically significant differences in its contents in the needles of the provenances cultivated on cambisol and on vertisol.

**Table 5.** The result of *t*-test

| | | Average % | Std. Dev | n | *t* | *P* |
|---|---|---|---|---|---|---|
| Content of N in needles | Vertisol | 0.41 | 0.1615 | 14 | - | <0.000 |
| | Eutric cambisol | 0.83 | 0.1140 | 14 | 8.1183 | |
| Content of C in needles | Vertisol | 42.95 | 1.8859 | 14 | 2.0178 | 0.054 |
| | Cambisol | 39.13 | 6.72435 | 14 | | |
| C/N ratio in needles | Vertisol | 126.93 | 60.7326 | 14 | 4.7887 | <0.000 |
| | Eutric cambisol | 47.93 | 11.0575 | 14 | | |

The needles of Douglas-fir of all provenances grown on vertisol had a very wide C/N ratio. Eight provenances had a C/N ratio wider than 100. The widest C/N ratio (263.2) was found in provenance 03383 of the West Wootenay seed zone. This value deviates from the mean C/N value of the provenances grown on vertisols by 2.244 standard deviations. A very wide C/N ratio was also found in provenance 00278 from the Thompson Okanagan Arid seed zone. The narrowest C/N ratio was found in provenance 01198 from the West Kootenay seed zone and in provenance 03333 from the East Kootenay seed zone. The value of the C/N ratio in both provenances deviates from the mean value of the provenances grown on vertisols by more than one standard deviation (Table 4).

Douglas-fir needles of all provenances grown on eutric cambisol were characterized by a significantly narrower C/N ratio compared to the provenances grown on vertisol (Table 5, Figure 1), so that the widest C/N ratio in the provenances grown on eutric cambisol was approximately the same as the narrowest C/N ratio in the provenances grown on vertisol.

The widest C/N ratio in the provenances grown on eutric cambisol was attained in provenance 03356 from the Thompson Okanagan Arid seed zone, followed by provenances 05227 from the East Kootenay seed zone and 01198 from the West Kootenay seed zone. In these three provenances the C/N ratio was higher than the mean of the Douglas-fir needles produced on eutric cambisol by more than one standard deviation. The narrowest C/N ratio on vertisol was in provenance 30461 from the Shuswap Adams seed zone, followed by provenance 03389 from the West Kootenay and 05092 from the East Kootenay seed zone.

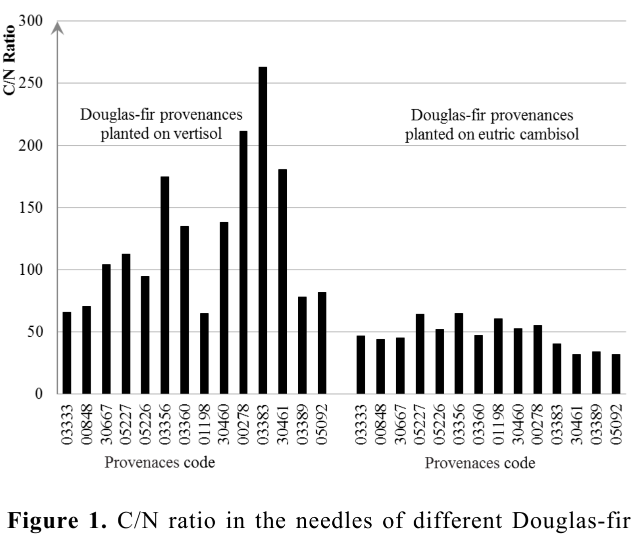


**Figure 1.** C/N ratio in the needles of different Douglas-fir provenances planted on eutric cambisol and on vertisol

## Discussion

C/N ratio is a very important characteristic of the organic matter freshly deposited on the soil surface, as it affects the rate of decomposition and humification of organic matter (Ohta and Kumada 1978). In the case of a wide C/N ratio of needle litterfall, the processes of biochemical decomposition can be expected to produce organic acids, which cause soil acidification.

The concentration of nitrogen in the needles depends on the conditions of nitrogen uptake, i.e. on overall site conditions. Different Douglas-fir provenances originating from the USA, although of the same age and cultivated in identical site and stand conditions on dystric cambisol, accumulated quite different concentrations of macronutrients in the needles (Lavadinović et al. 2011a, Lavadinović et al. 2011b, Lavadinović et al. 2011c). Douglas-fir capacity to use the site potential and attain adequate levels of macronutrients on allochthonous sites in Serbia depended on the provenances, i.e. on their genetic adaptation to local site conditions.

In the silvicultural conditions of Serbia, nitrogen uptake of Douglas-firs originating from the USA depended largely on the geographical features of the study provenances in this country. Under the same site conditions, the provenances originating from higher latitudes and at the same time from higher altitudes absorbed lower quantities of nitrogen from the soil (Lavadinović et al. 2011).

The differences in the carbon content in the needles of Douglas-firs cultivated on the same soil type point to the variability in the intensity of physiological processes in the genotypes of different provenances. This also indicates that some of the provenances express certain genetic specificities. Our research showed that the C/N ratio in the needles of Douglas-fir strongly depended on soil characteristics. However, on the same soil and under the same site conditions, C/N ratio depends on the provenance. This suggests that the plantations of Douglas-fir used as substitutes for native forests will not have the same effect on the soil properties.

The changes in soil characteristics caused by the substitution of Douglas-fir plantations for autochthonous coppice forests were researched at different sites in Serbia. Previous investigations on the effects of Douglas-fir on the change in the soil characteristics after its substitution for autochthonous forests provided different results for different sites in Serbia. At the site of submontane beech on acid brown soil on Maljen in central Serbia (Kostić 2008), a Douglas-fir plantation lowered the soil pH compared to the native forest soil.

On the site of submontane beech forest (*Fagetum submontanum*) on acid brown soil on Mt. Juhor, Douglas-fir had not produced any significant effect on the soil, not even after 40 years of its existence. There were no big differences when compared to the soil under natural forests (Miletić et al. 2003, 2005). The substitution of Douglas-fir plantations for submontane beech forest had not had any significant impact on the properties affecting the soil resistance to erosion agents. On the site of Hungarian oak and Turkey oak with hornbeam (*Carpino betuli-Quercetum farnetto-cerris* (Rud. 1949) Jov. 1979), Douglas-fir litterfall and the products of organic matter decomposition led to increased soil erodibility in comparison to the situation with natural forests (Miletić et al. 2010). In the reclamation of mine soils of REIK "Kolubara", Douglas-fir monocultures improved the soil properties when compared to freshly deposited mine waste. The soil reclamation effects of Douglas-fir plantations on the characteristics of the reclaimed spoil heaps exceeded the effects of other coniferous plantations (Japanese larch (*Larix leptolepis* Gord.), Austrian pine (*Pinus nigra* Arn.) and Scots pine (*Pinus sylvestris* L.), or European black alder (*Alnus glutinosa* Gaertn), which is a deciduous tree species (Miletić 2004, Miletić et al. 2011, 2012). In most cases, the changes observed in the soil of Douglas-fir plantations compared to autochthonous forests mainly relate to the changes in the humus content and its group-fraction composition. The changes in the content of total humus in the soil are the result of the changed quantities and chemical characteristics of dead organic residues transformed into humus (Knežević 1992).

## Conclusions

Based on the research on the contents of carbon and nitrogen in the needles of 14 provenances of eleven-year-old Douglas-firs cultivated on eutric cambisol and vertisol, it can be concluded as follows:

The level of nitrogen in Douglas-fir needles depends primarily on the soil type and its characteristics. All 14 Douglas-fir provenances growing on eutric cambisol were characterized by considerably higher nitrogen percentages in the needles compared to those growing on vertisol. This is due to more favourable nutrient conditions in eutric cambisol.

There were no major differences in carbon contents in the needles between the provenances growing on eutric cambisol and the provenances growing on vertisol.

As the consequence of the lower nitrogen content, all provenances cultivated on vertisol were characterized by a considerably wider C/N ratio.

This proves that Douglas fir is not suitable for afforestation of vertisol sites since the majority of the studied provenances originating from Canada and growing on vertisol would initiate the process of acidification and thus reduce the production potential of the site. The weakest negative effect on vertisol was made by provenance 01198 of the West Kootenay seed zone and by provenance 03333 of the East Kootenay seed zone.

Douglas-fir leaf litter on the sites with the eutric cambisol soil type would not significantly affect soil acidification, because the C/N ratio is comparatively narrower. The following provenances should be avoided on the sites with eutric cambisol: provenance 03356 of the Thompson Okanagan Arid seed zone, provenance 05227 from the East Kootenay seed zone and provenance 01198 from the West Kootenay seed zone.

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