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Improvement of nutritional site quality 13 years after single application of fertiliser N and P on regenerating cedar-hemlock cutovers on northern Vancouver Island, B.C.

Robert L. Bradley[1,*], Brian D. Titus[2], Caroline M. Preston[2] and Jennifer Bennett[3]
[1]*Département de biologie, faculté des sciences, université de Sherbrooke, Sherbrooke, Québec, Canada, J1K 2R1;* [2]*Pacific Forestry Centre, 506 West Burnside Road, Victoria, British Columbia, Canada, V8Z 1M5 and* [3]*Department of Forest Sciences, Faculty of Forestry, Forest Science Centre - 3rd floor, 2424 Main Mall, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4*

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Abstract

Post-clearcut silvicultural treatments, to improve tree growth and reduce salal (*Gaultheria shallon*) competition, were established in five different forest blocks on northern Vancouver Island, in 1984. Plots were either left untreated, brushed of competing salal vegetation, fertilized [(250 kg N + 100 kg P) ha^{-1}], or brushed + fertilized. Three of these blocks were revisited 13 years later, in the summer of 1997, and various chemical, biochemical and microbial parameters were measured in forest floor humus samples to determine long-term effects of treatments on nutritional site quality. Brushing resulted in lower humus pH and extractable base cations, whereas fertilization increased Bray-extractable P. Over a 20-week aerobic incubation, significantly more N was mineralised in humus from fertilized plots than from brushed plots. Over a 14-d anaerobic incubation, significantly more N was mineralised in humus from the fertilized treatment than other treatments. Similarly, gross transformation rates of $NH_4^+$and NO_3^-, measured by ^{15}N-dilution, were higher in humus from the fertilized treatment than other treatments. Ecophysiological indices of microbial communities (basal respiration, specific death rate, metabolic quotient, and energy deficiency index), derived by humus respirometry, suggested that there was higher available C in fertilized and brushed + fertilized treatments than in the brushed and control treatments. Total microbial biomass was equal to C-limited microbial biomass, which further confirmed that available C was the growth-limiting factor for microbial communities in all treatments. The prokaryotic fractions of microbial biomass in all treatments were approximately equal ($\approx$ 65%). PCA ordination of microbial communities, based on C source utilisation patterns, showed a distinct clustering of humus samples taken from one of the sites. Within the cluster of samples taken from the other two sites, samples from fertilized plots scored separately from those from control plots. In salal foliage, concentrations of condensed tannins were higher in brushed and control plots than in fertilized and brushed + fertilized plots. In spite of other studies that have reported increased tree height following fertilization and/or removal of salal, results of the present study suggest improvement in nutritional site quality occurs only with fertilization, whereas brushing may in fact be detrimental. The long-term growth of hemlock observed in fertilized plots may be the result of changes to key ecosystem structures and processes brought about by increased speed of succession and accelerated canopy closure.

* FAX No: 819 821 8049.
E-mail: robert.bradley@courrier.usherb.ca

Introduction

Current forest management practices, such as fertilization or the brushing of minor vegetation, are designed to artificially alter resource availability during early succession to favour the growth of crop tree species. This, in turn, may establish new resource supply trajectories leading to favourable changes in long-term 'site quality' if resultant successional changes within the stand lead to improved humus properties and enhanced nutrient cycling processes.

Site quality is defined as the combination of site factors that determine the potential growth of a given tree species. Two approaches are commonly used to estimate site quality. The first defines 'site index' based on average height (Gracia and Retana, 1996) or height increment (Wang et al., 1994) of dominant trees at a specific reference age. The second approach uses ecological variables, such as climate, geography, soil class and floristic composition, to predict site quality (Chen et al., 1998; Espeleta and Mize, 1993; Wang and Klinka, 1996). The mensurational approach is favoured by foresters because it is practical, but it could also be misleading. For example, Wang (1998) found poor correlation between height of dominant trees and site productivity across 68 white spruce stands in central British Columbia. Stem analysis data do not make explicit reference to causal factors such as nutrient supply.

In the coastal western hemlock wet maritime (*CWHvm*) biogeoclimatic zone of British Columbia, growth stagnation or 'growth check' of conifer seedlings on cutovers of former old-growth western red cedar (*Thuja plicata* Donn.) – western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) stands is attributed to low nutritional site quality. Availability of nutrients (mainly N and P) to tree seedlings is particularly low in thick forest floors dominated by root systems of the ericaceous shrub salal (*Gaultheria shallon* Pursh.) (Messier, 1993). Salal can quickly re-establish on clearcuts and can comprise up to 12 t ha^{-1} of live biomass, or 70% of total competing vegetation, within 8 years of harvesting (Fraser et al., 1993; Messier and Kimmins, 1991). Salal root systems are rhizomatous, extensive, have a high specific area and are ericoid mycorrhizal which presumably increases their efficiency in absorbing organic nutrients (Bending and Read, 1996). Besides being a strong competitor for soil nutrients, salal produces litter which reduces the decomposition rate of companion litters (Fyles and Fyles, 1992). Availability of litter N released in salal rich environments could be further reduced through tanning reactions (Keenan et al., 1994, 1996).

Foresters are faced with several options for treating regenerating sites dominated by salal. Reducing the abundance and growth of salal should remove understory competition and, more importantly, could lead to improvement of nutrient cycling characteristics in the forest floor. Another option is the application of mineral nutrient fertilizers which presumably increase the competitive ability of conifers relative to ericaceous shrubs (Prescott et al., 1995) and thus accelerate canopy closure. Although salal is shade-tolerant, its growth is expected to diminish under low light conditions that prevail after full canopy closure (Fraser et al., 1993).

In 1984, a site preparation trial was established in the *CWHvm* to compare factorial treatment combinations of brushing (as manual grubbing + herbicide applications) and fertilization, for reforesting cedar – hemlock cutovers (Weetman et al., 1989). Thirteen years later, mensurational data suggested that site quality had improved in both the brushed plots and the fertilized plots. It was not understood, however, whether nutritional site quality had improved on these plots, or whether treatment affects had only been temporary. To test the hypothesis that treatments could have increased nutritional site quality over 13 years, we sampled forest floors from these research plots and compared gross and net N mineralisation rates, basal and substrate induced respiration rates, and C source utilisation patterns by microbial communities. We also sampled and analysed salal foliage for condensed tannin concentrations. The overall aim of the study was to better understand temporal shifts in resource availability in response to brushing and/or fertilization to help foresters attain long-term management objectives.

Material and methods

Study sites and treatments

The study was conducted on a silvicultural trial established in the *CWHvm*, located within Tree Farm License 25 near Port McNeill on northern Vancouver Island, British Columbia. Mean annual temperature is 7.8 °C, and mean annual precipitation is 1700 mm, of which only 27% falls between 1 April and 30 September. The topography gently undulates and elevations vary by up to 300 m. Organic horizons are well developed (114 t ha^{-1}) and are overlain by 530 t ha^{-1}

of coarse woody debris (Keenan et al., 1993). Soils consist of humus-enriched Bf mineral horizons originating from morainal-glacial and glacio-fluvial outwash till and marine deposits (Lewis, 1982). Further information on this ecosystem may be found in Prescott and Weetman (1994).

The silvicultural trial consisted of five replicate blocks located on five old-growth cedar – hemlock sites that had been clearcut and burned between 1971 and 1973. The sites were located several km apart and were chosen because of similar species composition, stand structure and age class prior to clearcutting. For the present study, three of the five blocks were randomly chosen for sampling. All three sites were planted with Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings the year after cutting and burning, however, the plantations quickly became dominated by naturally regenerated western hemlock and western red cedar. Understory vegetation consisted mainly of salal.

Each replicate block consisted of 12 randomised plots (400 m^2 – 625 m^2) each assigned one of 12 post-clearcut site preparation treatments in the summer 1984. In the present study, only four of these treatments were sampled: (1) untreated control plots, (2) brushed plots, in which salal was manually grubbed and left on site, (3) fertilized plots, consisting of a single broadcast application of 250 kg N ha^{-1} (ammonium nitrate) + 100 kg P ha^{-1} (triple superphosphate), and (4) brushed + fertilized plots, in which both factors were combined. Garlon 4E herbicide (i.e. triclopyr ester) was applied in 1985 and 1989 on brushed and brushed + fertilized plots to minimise salal resprouting. The eight unsampled treatment plots consisted of factorial combinations of brushed and fertilizer treatments with P alone and with N as urea. Three-year and 10-year height responses and foliar nutrient concentrations of each treatment may be found in Weetman et al. (1989) and Bennett (1996), respectively.

Humus and salal foliage sampling in the field

Three bulked samples of F-layer humus (ca. 1 kg fresh wt.) as well as three bulked samples of green salal leaves (ca. 20 leaves) were collected from each plot in May 1997. All samples were placed in a cooler over ice and immediately returned to the Pacific Forestry Centre for processing. Coarse roots and fragments were carefully removed from humus by hand and remaining material was coarse sieved (6 mm). Gravimetric moisture content was determined by weight loss of subsamples (ca. 20 g fresh wt.) after 48 h in a draft oven (101 °C). Humus subsamples were analysed for pH (soil:water = 1:10), total organic C (LECO combustion), total-N (Kjeldahl digestion), Bray-extractable P, and NH_4-acetate extractable cations (Ca^{2+}, Mg^{2+}, K^+).

Humus N dynamics

Net rates of NH_4^+–N and NO_3^-–N accumulation were measured in each bulked humus sample over a 20 wk aerobic incubation. Six subsamples (ca. 30 G fresh wt.) per bulked sample were weighed into 500 ml Mason jars, the openings were sealed with polyethylene film to prevent moisture loss, and jars were kept at 25 °C. Sequential destructive sampling was performed at T = 0, 2, 4, 8, 14 and 20 weeks. On each of these dates, one of the six subsamples was extracted in 200 ml of 2 *N* KCl solution, shaken for 1 h and suction filtered (Whatman no. 5 filter paper). Filtrates were analysed colorimetrically for NH_4^+–N and NO_3^-–N concentrations using a Technicon auto-analyzer.

Anaerobic N mineralisation rates (Waring and Bremner, 1964) were determined for each bulked humus sample from each plot. Two subsamples (ca. 3–4 g fresh wt.) per bulked sample were weighed into 45 ml snap-cap bottles, mixed with 40 ml deionized water, sealed and incubated (25 °C for 14 d). Bottles were then shaken and the contents transferred to 250 ml Erlenmeyer flasks. Each bottle was rinsed with 40 ml of 2 *N* KCl solution to bring the final concentration of extractant in each flask to 1 *N* KCl. Flasks were shaken for 1 h and solutions were filtered and analysed for NH_4^+–N using a Technicon auto-analyser.

Gross mineral N transformation rates were measured by isotope dilution (Hart et al., 1994). Four subsamples (ca. 8 g dry wt) of each bulked humus sample were placed in four separate 500 ml Mason jars and gently tamped. Exactly 3 ml of aqueous $(^{15}NH_4)_2SO_4$ solution (282.9 mg l^{-1} at 99 atom% ^{15}N) were uniformly distributed through humus in each of two jars by making numerous small injections with a hypodermic needle. Similarly, 3 ml of $K^{15}NO_3$ solution (210.5 mg l^{-1} at 50 atom% ^{15}N) were distributed through humus in each of the other two jars. One jar of $^{15}NH_4$-enriched humus and one jar of $^{15}NO_3$-enriched humus were extracted exactly 15 min after injection in 200 ml of 2 *N* KCl solution, shaken for 1 h and suction filtered (Whatman no. 5 filter paper). Filtrates were stored at 2 °C. The two

remaining jars were sealed, kept at 22 °C for exactly 24 h, and extracted in the same manner. All filtered extracts were analysed colorimetrically for NH_4^+–N concentration, whereas only extracts from samples injected with $K^{15}NO3$ solution were further analysed for NO_3^-–N concentration. Based on these mineral N concentrations, aliquots of each extract ranging from 5 to 25 ml (± 0.1 ml) were diffused onto acidified glass micro-fibre disks (Bradley and Fyles, 1996) and encapsulated in Sn sleeves (Europa Scientific, Franklin, OH). Extracts of samples injected with $K^{15}NO_3$ were further amended with micro-spatula amounts of Devarda's alloy and diffused a second time. Diffused samples were analysed for atom% ^{15}N analysis by continuous flow mass spectrometry. Gross production and consumption rates of NH_4^+–N and NO_3^-–N were calculated using zero-order equations derived by Kirkham and Bartholemew (1954). It was assumed that isotope addition would not bias ammonification and nitrification rates in the short-term, but might cause an overestimation of gross consumption rates of both ions due to enrichment of reactant pools (Hart et al., 1994).

Humus respirometry

Various microbial biomass fractions (C-limited, prokaryotic and total) as well as various indices of available C (basal respiration rate, specific death rate, metabolic quotient and energy deficiency index) were derived respirometrically on humus samples from each study plot. Before measurement, humus samples were adjusted to optimal gravimetric water content (i.e. 250–300%) by spreading the wetter samples over paper towel and drying at 35 °C for 1 h (Bradley and Fyles, 1995a). All humus samples were covered and left for 2 weeks at 22 °C prior to measurements to remove any minor C flush and to ensure homogeneity of moisture content.

C-limited, prokaryotic and total microbial biomass were determined on each humus sample by substrate-induced respirometry (SIR). Three subsamples (ca. 8 g dry wt) were weighed into separate 500 ml plastic containers, the first was amended with ground (65 μm) glucose (1000 μg C g^{-1}), the second with ground glucose + cycloheximide (80 mg g^{-1}), and the third with ground glucose + nutrient broth powder (1000 μg g^{-1}) + yeast extract (500 μg g^{-1}). The eukaryotic inhibitor cycloheximide was delivered 20 h prior to glucose addition in a 4 ml aliquot of 160 g l^{-1} aqueous solution (Beare et al., 1990) whereas humus subsamples not receiving cycloheximide were amended with 4 ml deionized water. Glucose, nutrient broth and yeast extracts were applied as 250 mg talc mixtures and dispersed into humus using a kitchen handmixer with one beater (Bradley and Fyles, 1995b). Following amendments, humus subsamples were transferred into 190 ml gas sampling jars and left uncovered for 100 min to reach optimum SIR rates (Anderson and Domsch, 1978). Subsamples were flushed (5 min) with ambient air, sealed for 30 min and headspace air was analysed for CO_2 concentration using a Hewlett-Packard model 5890 GC (Hewlett-Packard, Avondale, PA) equipped with an FID and methanizer, and using nitrogen as the carrier gas. SIR rates were converted into microbial biomass (Beare et al., 1990).

Basal respiration rate was measured by placing humus subsamples (ca. 8 g dry wt) in 190 ml gas sampling jars, flushing the headspace with ambient air (5 min), sealing jars with air-tight lids equipped with rubber septa, and sampling aliquots of air in the headspace with a needle and syringe after 2 h. Air samples were analyzed for CO_2 concentration using a GC.

Specific death rate (qD) was determined by measuring total microbial biomass (as described above) of additional humus subsamples that had been kept in the dark at 22 °C for 4 additional weeks. The qD, representing relative loss in microbial C over time, was then calculated (Anderson and Domsch, 1990):

$$qD = [(C_{mic})_{T=0} - (C_{mic})_{T=4wk})/(C_{mic})_{T=0}] \div (4wk) \quad (1)$$

The metabolic quotient (qCO_2), or rate of CO_2 evolution per unit microbial biomass, was calculated as the quotient of basal respiration rate and total microbial biomass.

The energy deficiency index (*EDI*), or the relative increase in microbial respiration due to addition of glucose, was calculated as:

$$EDI = [(G - B) \div GN] \times 100\% \quad (2)$$

where B is basal respiration rate, G is glucose-induced respiration rate, and GN is (glucose + nutrient)-induced respiration rate.

Room temperature and atmospheric pressure were noted during each respirometry measurement, and ambient CO_2 concentration was measured several times each day. For each sample, ambient CO_2 concentration was subtracted from sampled CO_2 concentration and the difference was adjusted according to Ideal Gas Laws and centered at 22 °C using Q_{10} = 2.

Utilisation patterns of C sources

The functional diversity of microbial communities was characterised in five duplicate humus subsamples from each plot using the Biolog GN microplating system (Biolog Inc., Hayward, CA). Duplicate subsamples (ca. 10 g $\pm$ 1 mg) were transferred to polyethylene bottles containing glass beads (3 mm dia.), mixed with 100 ml of 0.1% Na-pyrophosphate solution and shaken 15 min. Suspensions were diluted 100 $\times$ in deionized water as initial tests had shown optimal color development occurring at this dilution. Aliquots (150 μL) of each diluted suspension were added to each of 96 wells in a Biolog GN plate. Ninety-five wells contained redox-sensitive tetrazolium dye and a unique C source, whereas one well contained dye only (i.e. control). Biolog plates were incubated at 25 °C and color formation in each well was read as light absorbance (590 nm) after 20, 24, 28, 44, 48, 52, 68, 72 and 76 h using an automated plate-reader (Biolog Microstation and software; Biolog Inc., Hayward, CA). Light absorbance values of each control well at each reading interval were subtracted from light absorbance values in the corresponding 95 wells containing substrates. Average well color development (AWCD) of each plate was calculated at each reading interval to determine the incubation time ($T_{0.75}$) corresponding to AWCD = 0.75 absorbance units.

Salal leaf tannins

Condensed tannins in salal foliar samples from each plot were analysed colorimetrically after hydrolysis with butanol/HCl using the proanthocyanidin assay with purified salal tannins as a standard (Preston, 1999).

Statistical analyses

The effects of silvicultural treatments on mineralisable N (aerobic incubation) on each of six sampling dates, on anaerobically mineralisable N, on gross mineral N transformation rates, on indices of available C, on microbial biomass fractions, and on condensed tannin concentrations were tested statistically using one-way ANOVA tests (n=3). Significant ($P<0.05$) differences among treatment means were explored using Duncan's multiple range test.

Absorbance values of each Biolog wells at $T_{0.75}$ were centered and normalised [i.e. (Abs.- AWCD)/σ], and principal component analysis (PCA) was performed on transformed data to explore effects of treatments on C source utilisation patterns by microbial communities.

Results

It was observed at the time of field sampling that salal cover on the open canopied control and brushed plots was abundant, and that therefore grubbing and repeated triclopyr application was not successful in controlling salal in the long-term. In contrast, salal distribution was patchy and taller, and canopy closure was more advanced, on fertilized and brushed + fertilized plots.

Humus pH as well as extractable base cation concentrations (Ca^{2+}, Mg^{2+} and K^{+}) were generally lower in brushed plots (Table 1). Average concentration of Bray extractable P was significantly higher in humus from fertilized and brushed + fertilized plots.

Average NH_4^+–N pools increased geometrically in all treatment plots over the 20 wk aerobic incubation (Figure 1). On the final sampling date, humus from fertilized plots had significantly ($P < 0.05$) higher concentrations of NH_4^+–N (464 μg N g^{-1}) than humus from brushed plots (135 μg N g^{-1}). Average NO_3^-–N accreted in humus from all plots was less than 1 μg N g^{-1} during the entire 20 week incubation, and no treatment effects were observed.

Anaerobic N mineralisation rate was significantly higher in humus from fertilized plots than from other treatment plots, as were gross NH_4^+–N production and consumption rates (Table 2). Similar patterns were observed for gross NO_3^-–N production and consumption rates, but coefficients of variation associated with these measurements were high and, therefore, treatment effects were not statistically significant.

Basal respiration rate (B) was significantly higher in humus from fertilized plots than in humus from control and brushed treatment plots (Table 2). B was significantly higher in humus from brushed + fertilized plots than from brushed plots. Specific death rate (qD) was highest in humus from fertilized plots and lowest in humus from brushed plots, although differences in qD among treatments were not significantly different. Treatment effects on metabolic quotient (qCO_2) were not statistically significant although values in fertilized and brushed + fertilized treatments were about 30% higher than those in control and brushed treatments. Similarly, energy deficiency index (EDI) was not significantly different among treatments although values

Table 1. Effect of post-clearcut treatments on forest floor chemical properties; means and standard errors are for $n = 3$

	Control	Brushed	Fertilized	Brushed + fertilized
pH (1:10 water)	4.43 (0.07)	4.17 (0.07)	4.50 (0.00)	4.33 (0.22)
Total C (%)	50.7 (1.6)	52.4 (1.2)	54.9 (1.0)	53.1 (2.0)
Total N (%)	0.95 (0.03)	0.92 (0.11)	1.09 (0.05)	1.03 (0.02)
Bray-extractable P (μg g^{-1})	27.0 (7.2)	22.8 (5.6)	74.9 (17.7)	52.9 (7.2)
Exchangeable Ca (me · 100 g^{-1})	26.9 (3.8)	19.0 (2.9)	32.3 (3.0)	26.5 (8.7)
Exchangeable Mg (me · 100 g^{-1})	6.7 (1.1)	3.9 (0.4)	7.2 (0.2)	3.7 (1.0)
Exchangeable K (me · 100 g^{-1})	2.3 (0.4)	1.6 (0.2)	3.1 (0.3)	1.7 (0.4)

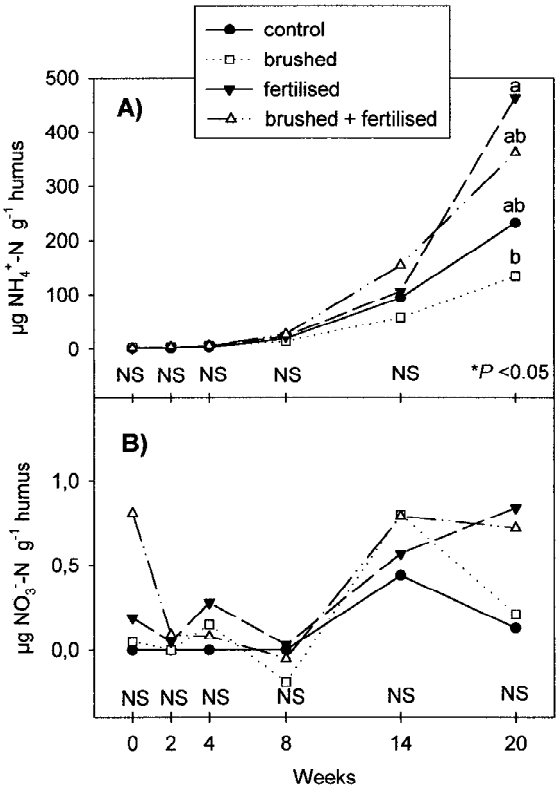

Figure 1. Cumulative (A) NH_4^+–N and (B) NO_3^-–N mineralised over a 20 week aerobic incubation in humus gathered from experimental plots; means within the same sampling date assigned different lower case letters differ significantly according to Duncan's Multiple Range Test ($P < 0.05$, $n = 3$); NS = means that are not significantly different on the given sampling date.

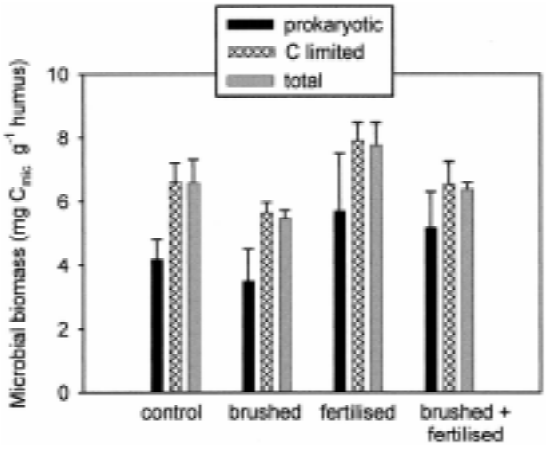

Figure 2. Effect of post-clearcut site preparation treatments on various microbial biomass fractions in the forest floor; vertical lines represent 1 S.E. ($n = 3$).

in fertilized and brushed + fertilized treatments were lower than those in control and brushed treatments.

There was no statistical difference between C-limited (i.e. SIR with glucose) and total (i.e. SIR with glucose + nutrients) microbial biomass fractions within any of the treatments (Figure 2). Both C-limited and total microbial biomass in humus from fertilized plots were statistically higher than in humus from brushed plots. The average prokaryotic (i.e. SIR with glucose + cyclohexamide) microbial biomass ranged between 61% (brushed) and 78% (brushed + fertilized) of total microbial biomass, but there were no significant treatment effects due to relatively high experimental errors associated with this measurement.

PCA ordination of microbial communities based on C source utilisation patterns showed a distinct clustering of humus samples from Sites 1 and 2, compared to those from Site 3 (Figure 3a). Within the cluster of samples taken from Sites 1 and 2, all samples from fertilized plots scored separately from all those from control plots (Figure 3b). PCA did not reveal distinctive patterns in substrate use among different

Table 2. Effects of post-clearcut silvicultural treatments on (A) anaerobic N mineralisation rates (2 weeks), (B) gross mineral-N transformation rates, (C) four indices of humus available-C derived by respirometry measurements, and (D) concentrations of condensed tannins in salal foliage; treatments were applied in 1984 and measurements performed in 1997; mean values within same line followed by different lowercase letters differ significantly according to Duncan's Multiple Range Test ($P < 0.05$, $n = 3$)

	control	brushed	fertilized	brushed + fertilized
A) Anaerobic N mineralisation rates				
(μg mineralized-N g^{-1} humus)	0.79 b	0.78 b	1.49 a	1.00 b
B)Gross mineral-N transformation rates				
(ug N g^{-1} humus d^{-1})				
NH_4^+ production	7.5 b	13.8 b	32.4 a	8.9 b
NH_4^+ consumption	20.8 b	26.1 b	47.8 a	23.0 b
NO_3^- production	21.5 a	12.5 a	26.1 a	17.4 a
NO_3^- consumption	30.2 ab	20.3 b	36.5 a	25.7 ab
C) Indices of available C				
Basal respiration rate				
(μg CO_2-C g^{-1} humus h^{-1})	16.6 bc	14.4 c	25.3 a	21.6 ab
Specific death rate				
(mg C_{mic}-loss mg^{-1} C_{mic} $week^{-1}$) $\times 10^{-3}$	58.5 a	55.8 a	63.3 a	58.0 a
Metabolic quotient				
(μg CO_2–C mg^{-1} C_{mic} h^{-1})	2.50 a	2.66 a	3.32 a	3.40 a
Energy deficiency index				
(%)	81 a	82 a	76 a	75 a
D) Salal leaf tannin concentrations				
(mg condensed tannins g^{-1})	163.3 a	168.0 a	137.4 ab	120.3 b

treatments in Site 3 (Figure 3b). PCA axes 1 and 2 explained 26% and 6%, respectively, of the variance in the data set.

Salal leaf tannin concentrations were significantly higher in control treatment and brushed treatment plots than in brushed + fertilized treatment plots (Table 2).

Discussion

Treatment effects on nutritional site quality

Useful assessments of nutritional site quality should integrate measurements of soil chemical and biochemical variables and of soil microbial dynamics (Halvorson et al., 1996). In other words, it can be concluded that nutritional site quality of cedar – hemlock cutovers is increasing if fundamental improvements in humus nutrient cycling and energy fluxes are observed. Mineralisable N measured by aerobic and anaerobic laboratory incubations indicated that humus from fertilized plots supply more mineral N to plants than humus from other plots. The aerobic assay allowed comparison of treatments based solely on the chemical quality of humus and removed possible confounding effects of temperature, moisture, throughfall, plant uptake and leaching. The anaerobic assay was indicative of actively cycling microbial N pools (Myrold, 1987) and of forest site productivity (Powers, 1980). ^{15}N pool dilution assays provided a means for exploring underlying processes controlling mineral N availability, by estimating the rates at which NH_4^+ and NO_3^- were simultaneously produced and consumed. Gross cycling rates of these ions were higher in humus from fertilized plots, providing further evidence that the single application of fertilizer N + P to these ericaceous-dominated sites had gradually improved nutritional site quality over the subsequent 13 years. Although care is warranted in interpreting such dynamic measurements from field samples taken on a single date, it is likely that these treatment differences would nonetheless vary synchronously through time. For example, Tietima and Wessel (1992) measured

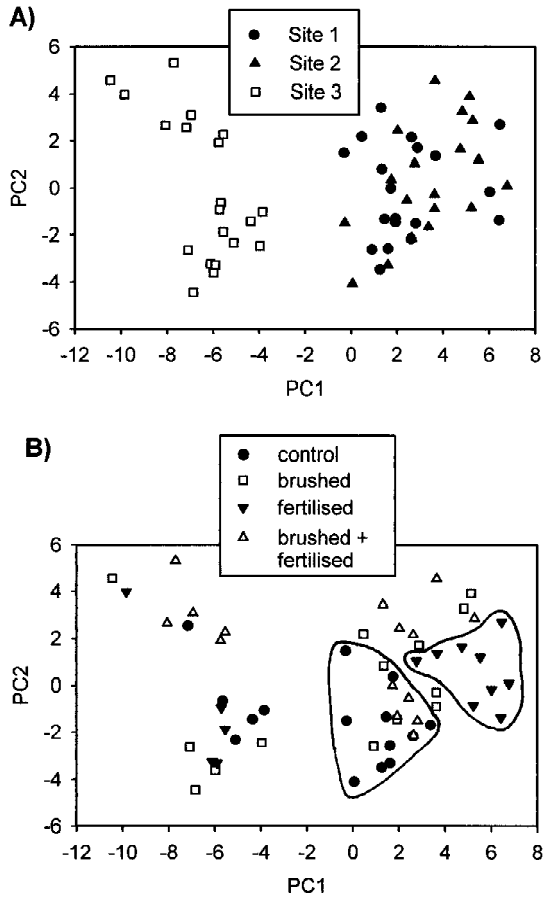

Figure 3. Principal component analysis of C source utilisation patterns by microbial communities in humus gathered from experimental plots; PCA axes 1 plus 2 accounted for 32% of the variance in the data set; (A) samples sorted by Site, and (B) samples sorted by Treatment; hand-drawn boundaries denote the separate clustering of humus samples gathered from fertilized and control plots on Sites 1 and 2.

gross N transformation rates in organic layers of three acid forest ecosystems on four dates, between February and November, and found that values fluctuated by as much as 40% between dates, although differences between sites remained constant throughout the year. They concluded that higher gross N transformation rates resulted from higher substrate quality as reflected by lower lignin:N ratios and by higher pH values.

In our study, we defined substrate quality based on respirometry measurements that reflected C availability in forest floors. Respirometry measurements are dynamic and integrate the effects of above- and below-ground litter quality, including throughfall and rhizodeposition, on the biomass and the physiological state of microbial communities. High basal respiration rates in fertilized plots reflected high available C supply at the time of sampling. High *q*D reflected a higher proportion of 'r-selected' microbial biomass dependent on high C supply, which declines during incubation (Bradley et al., in press). High qCO_2 indicated low C use efficiency and the presence of energetically wasteful microbial communities that would be expected under conditions of higher substrate quality (Insam and Haselwandter, 1989). Finally, low *EDI* indicated low microbial demand (i.e. higher satiation) for C substrates (Bradley et al., 1997). Thus, all respiration-based indices of available C indicated higher substrate quality in fertilized plots, even though treatment differences for some of these measurements were not statistically significant (i.e. $P > 0.05$). Microbial biomass, which is related to historical C supply in humus (Bradley and Fyles, 1995a), was also higher in fertilized plots. The C_{mic}:C_{total} ratio (calculated from data in Table 1 and Figure 2) is a sensitive indicator of substrate quality (Sparling, 1992), and was 1.1% in both control and brushed treatments, 1.4% in the fertilized treatment and 1.2% in the brushed + fertilized treatment.

There was no difference between C-limited and total microbial biomass, suggesting that C was still the principal factor limiting microbial activity in all treatments, in spite of higher indices of C availability in fertilized plots. For this reason, glucose only (i.e. without nutrient broth or yeast extracts) was added in combination with cyclohexamide to humus to estimate the total proportion of eukaryotic micro-organisms. Beare et al. (1990) reported that fungal hyphae accounted for 60–70% of total microbial biomass in fresh plant residues. By contrast, our results showed a 1:2 ratio of fungal:bacterial biomass based on respiration rates. The difference between our results and those of Beare et al. (1990) may be due to the more advanced humified state of our forest floor humus compared to their fresh plant residues, or perhaps to inefficient inhibition of eukaryotic metabolism by cyclohexamide. Regardless of this uncertainty, the fact that fungal:bacterial ratios remained approximately equal in all treatments suggests that microbial community structure was not greatly affected by changes in forest floor nutrient or C supply.

The stability of microbial communities was further illustrated by PCA ordination of humus samples based on C source utilisation patterns. These patterns reflected the functional diversity within microbial communities and, hence, population diversity (i.e. microbial community structure). Microbial communities in

fertilized and control treatments clustered independently from each other on Sites 1 and 2, which is consistent with fertilizer effects observed on other humus properties. However, the other treatments on Sites 1 and 2 did not have discernible effects on microbial community structure, and humus samples from Site 3 clustered distinctly by site rather than by treatment. Therefore, microbial community structure withstood significant changes in nutrient availability, energy fluxes, as well as in its own biomass and physiological state, brought about by contrasting silvicultural treatments, and remained strongly related to historical site properties. Expectations of distinct treatment effects on microbial community structure could arise from generalizations concerning the dynamic nature of individual microbial populations studied under axenic conditions. Replacement of microbial communities appears, however, to be a slow process, and we conclude that Biolog assays are not sensitive indicators of site improvement as were measurements of nutrient cycling and C availability.

The build-up of condensed tannins and other phenolic compounds released by salal in the forest floor could be a contributing factor to low nutritional site quality in the *CWHvm*, on Northern Vancouver Island (Preston, 1999). In our study, concentrations of condensed tannins in green salal foliage were significantly lower in fertilized and brushed + fertilized plots than in control and brushed plots. This physiological feedback, which has been observed elsewhere for conifer species in fertilization studies (Gebauer et al., 1998; Tiarks et al., 1989), supports the carbon-nutrient balance hypothesis (Bryant et al., 1983) whereby higher N availability results in plants shunting less non-structural carbon towards the synthesis of secondary compounds such as condensed tannins.

Discrepancy between nutritional site quality and site index

In general, our data showed that fertilization increased nutritional site quality, whereas the effects of brushing were either negative or non significant. Nutritional site quality in the brushed + fertilized treatment was generally higher than in the control treatment but lower than in the fertilized treatment, suggesting that the positive effects of fertilization were partially offset by brushing. These conclusions are not corroborated, however, by mensurational data taken from these sites. For example, Weetman et al. (1989) found that the effect of treatments on 3-year growth response of cedar was of the order [brushed + fertilized] $>$ [brushed] $\approx$ [fertilized] $>$ [control], whereas for hemlock the order was [brushed + fertilized] $\approx$ [fertilized] $>$ [brushed] $\approx$ [control]. In a follow-up study, 13-years after treatment, they again found that average tree height was significantly higher on both the brushed and the fertilized plots, than on the control plots. We conclude that mensurational data cannot be used to infer the effects of silvicultural treatments on nutritional site quality.

Contrasting effects of brushing and fertilization

To understand the contrasting long term effects of brushing and fertilization on nutritional site quality, we need to consider a combination of ecological factors.

Firstly, needle N and P concentrations of both hemlock and cedar were much higher in fertilized plots than in brushed plots (Weetman et al., 1989), which suggests that brushing simply removed conifer growth inhibition mechanisms whereas fertilization increased actively cycling nutrient capital. A prolonged increase in N and P availability suggests efficient recycling of fertilizer nutrients through vegetation and forest floor strata. The fact that *CWHvm* forest floors are very thick is an important contributing factor to the retention of nutrients. This is especially noteworthy for P which normally precipitates into stable recalcitrant forms once it has eluviated to mineral soil horizons.

Brushing favoured cedar growth whereas fertilization favoured hemlock growth, therefore, the cedar:hemlock litterfall ratio increased with brushing and decreased with fertilization. Western red cedar produces the most recalcitrant of all litter types in the *CWHvm* (Keenan et al., 1996) and is not expected to improve nutritional site quality. Conversely, the increase in needle N concentration due to fertilization was higher in hemlock than in cedar foliage (Weetman et al., 1989), and this would lead to higher N cycling in the forest floor.

The effect of brushing was apt to be only temporary. Although Barker (1988) found that Garlon herbicide removed 78% of salal cover, d'Anjou (cited in Haeussler et al., 1990) showed that salal roots continue to survive long after salal shoots have been controlled by triclopyr. Chang et al. (1996a) also showed persistence of salal roots after 6 years of aboveground control. Since salal can reproduce rapidly through vegetative spread of rhizomes (Fraser et al., 1993), the brushed treatment probably did not control re-invasion by salal.

Litter production on ericaceous-dominated sites can increase soil acidity to the extent that forest productivity decreases (De Montigny and Weetman, 1990). The brushed treatment consisted of grubbing the aboveground salal biomass which was then left *in situ* to decompose. This represented an episodic input of up to 12 t ha^{-1} of fresh salal material (Messier and Kimmins, 1991), or approximately 10% of forest floor mass (Keenan et al., 1993). This may explain why forest floors in brushed plots had lower pH values and lower exchangeable base cation concentrations.

Fertilization could also have direct effects on salal growth. For example, Prescott et al. (1995) found lower *Kalmia angustifolia* cover in jack pine stands that had been fertilized 14 years prior to measurement, and proposed that inorganic nutrients directly reduced the abundance of this ericaceous shrub. Although the notion of preferential organic nutrition by an ericaceous shrub is appealing, Chang et al. (1996b) showed that salal can compete strongly against trees for mineral nutrients applied as fertilizer. A more plausible direct effect of fertilization on salal, which could improve nutritional site quality, is the lower condensed tannin response found in salal foliage on fertilized plots.

Maintaining salal (i.e. no brushing) results in less N absorbed by trees but more total N taken up by vegetation (Chang et al., 1996b). Brushing may therefore encourage rapid N loss and reduce retention of fertilizer nutrients on site. Proposing that salal improves N and P cycling in the vegetation and forest floor strata challenges current thinking that salal cover can only be detrimental to nutritional site quality. However, until proof is made of the contrary, an undisturbed salal cover may in fact be more beneficial than the absence of understory growth altogether.

Finally, it was observed at the time of sampling that fertilization had accelerated canopy closure relative to other treatments. Although salal is shade tolerant, several studies (Fraser et al., 1993; Messier, 1993) have shown that vigour, growth rate and flowering of salal decreases with canopy closure. Salal leaves growing in shade may be larger but have only half the mass of those growing in full sunlight (Messier et al., 1989). Similarly, canopy closure reduced growth or condensed tannin concentrations of other ericaceous shrubs (Hester et al., 1991; Iason and Hester, 1993). Canopy closure should, therefore, coincide with a reduction in salal growth (Fraser et al., 1993), salal litter input and possibly salal tannin concentrations, and the time required to achieve this successional change appears to be reduced, especially for hemlock, through application of fertilizer.

Implications for stand management

Past economic models developed to assess operational fertilization of cedar – hemlock cutovers have been based on four hypothetical scenarios (Thompson and Weetman, 1992). Two of these assume fertilization accelerates canopy closure without changing site quality, and two assume a permanent improvement in site quality resulting in additional merchantable volume. Our results indicate that site quality, as defined by the integration of chemical, biochemical and microbial forest floor parameters and by salal foliar tannin concentrations, had indeed improved on salal-dominated cedar-hemlock regenerating sites 13 years after a single application of N + P fertilizer. Mensurational data presently being gathered on the study plots will allow this additional volume to be quantified in the future.

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