Impacts of repeated fertilization on components of the soil biota under a young lodgepole pine stand in the interior of British Columbia

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Abstract: We studied elements of the soil biota in a 24-year-old lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) stand in interior British Columbia 10 years after initiation of annual fertilizer treatments. The treatments included an unfertilized control, ON1 (650 kg nitrogen (N), 400 kg phosphorus (P), 400 kg potassium (K)), and ON2 (1350 kg N, 400 kg P, 400 kg K). In the forest floor, the C/N ratio was lower in ON1 and ON2 than in the unfertilized control, while available P and exchangeable magnesium were higher; NO_3 was higher only in ON2. In the upper mineral soil, available P was higher in ON1 and ON2, while NO_3 was higher only in ON2. In both the forest floor and upper mineral soil, microbial activity was higher in ON1 than in the unfertilized control or ON2. In the forest floor and mineral soil, Acari density, especially Oribatida and Prostigmata, was higher in ON2 than in ON1 and the unfertilized control. In contrast, Collembola density, especially Hypogastruridae, increased in ON2 relative to that in other treatments. ON2 had less lodgepole pine fine-root length, fewer ectomycorrhizal roots, fewer active fine roots, more nonmycorrhizal fine roots, and a different ectomycorrhizal community structure than ON1 and the unfertilized control. These dynamic changes to the soil biota appear to reflect changes to the plant community in response to fertilization.

Résumé : Les auteurs ont étudié les éléments de la biocénose du sol dans un peuplement de pin tordu (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) âgé de 24 ans et situé dans le centre de la Colombie-Britannique 10 ans après le début des traitements annuels de fertilisation : témoin non fertilisé, ON1 (650 kg azote (N), 400 kg phosphore (P), 400 kg potassium (K)) et ON2 (1350 kg N, 400 kg P, 400 kg K). Dans la couverture morte, le rapport C/N était plus faible dans les traitements ON1 et ON2 que dans le témoin non fertilisé tandis que P disponible et Mg échangeable étaient plus élevés; NO_3 était plus élevé seulement dans le traitement ON2. Dans la partie supérieure du sol minéral, P disponible était plus élevé dans les traitements ON1 et ON2 tandis que NO_3 était plus élevé seulement dans le traitement ON2. Tant dans la couverture morte que dans la partie supérieure du sol minéral, l'activité microbienne était plus élevée dans le traitement ON1 que dans le témoin non fertilisé ou le traitement ON2. Dans la couverture morte et le sol minéral, la densité des acariens, particulièrement les oribates et les prostigmates, était plus élevée dans le traitement ON2 que dans le traitement ON1 et le témoin non fertilisé. À l'inverse, la densité des collemboles, particulièrement les Hypogastruridae, a augmenté dans le traitement ON2 relativement aux autres traitements. Dans le traitement ON2, il y avait moins de longueur de racines fines de pin tordu, moins de racines colonisées par des ectomycorhizes, moins de racines fines actives, plus de racines fines non mycorhizées et une structure de la communauté ectomycorhizienne différente comparativement au traitement ON1 et au témoin non fertilisé. Ces changements dynamiques dans la biocénose du sol semblent refléter les changements dans la communauté végétale en réaction à la fertilisation.

[Traduit par la Rédaction]

Introduction

Extensive forest fertilization research in the interior of British Columbia has confirmed that nitrogen (N) deficiencies are widespread throughout the region. A single N application, applied alone or combined with sulphur (S) or boron (B), usually has a substantial positive effect on the growth of young lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) and interior spruce (*Picea glauca* (Moench) Voss and *Picea engelmannii* Parry ex Engelm. or naturally

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occurring hybrids of these species) forests (Weetman et al. 1988; Brockley 1992, 1995, 2000, 2003, 2004; Swift and Brockley 1994). As such, fertilization is widely viewed by forest planners and practitioners as a potentially viable strategy for addressing future timber supply challenges such as improving the amount and timing of wood harvested from interior forests.

A single fertilizer application typically produces only a temporary increase in tree and stand growth (usually 6–9 years). However, fertilization research with *Pinus* and *Picea* species in boreal forest regions has indicated that sustained growth responses, and large increases in harvest volume (or shortened rotation), can be achieved by frequent nutrient additions (Malkonen and Kukkola 1991; Tamm 1991; Weetman et al. 1995; Bergh et al. 1999; Tamm et al. 1999). Beginning in 1992, a small network of "maximum productivity" field installations was established by the British Columbia Ministry of Forests to document the potential growth and yield benefits of permanently removing nutritional constraints in young lodgepole pine and interior spruce stands in the interior of British Columbia (Brockley and Simpson 2004). Eight installations (five pine, three spruce) were established in 9- to 15-year-old plantations and juvenile-spaced, harvest-origin stands between 1992 and 1999 on zonal sites within three major biogeoclimatic zones. By documenting the volume gains potentially achievable by different rates and frequencies of fertilization, these research installations are providing the long-term growth and yield information that is needed to rationalize future fertilization investment decisions and timber supply mitigation strategies for interior forests.

As well as increasing aboveground productivity, forest fertilization may also affect belowground biological components of forest ecosystems (Marshall 1977). A single fertilizer application generally has only a small and temporary effect on belowground resources. However, large and persistent changes in soil microbial populations and fine-root development and in the activity, diversity, and structure of mycorrhizal and mesofaunal communities have been reported from long-term fertilization and N deposition studies in European forests (Tamm 1991; Arnebrant and Söderström 1992; Clemensson-Lindell and Persson 1995; Arnebrant et al. 1996; Tamm et al. 1999; Lindberg and Persson 2004). Although the long-term functional implications of these changes are poorly understood, they may potentially affect processes such as nutrient cycling, nutrient uptake, and drought tolerance in intensively fertilized stands.

Because of concerns about the effect of this fertilization treatment on the belowground ecosystem, we initiated research into the soil biota. To fully understand how the young lodgepole pine soil ecosystem responds to this fertilizer treatment, we would clearly need to study many elements of the soil food web and many sites with different native fertility. Available resources limited the extent of our sampling to an exploration of three distinct elements of the soil biota and one of the available sites. In this paper, we discuss the effects of 9 years of annual nutrient additions on fine-root length, ectomycorrhizal colonization, soil microbial activity and diversity, and mesofauna abundance and community structure at one lodgepole pine "maximum productivity" study site in the interior of British Columbia.

Materials and methods

Location and site description

The Sheridan Creek study site is located approximately 30 km north of Williams Lake, British Columbia (52°25′N, 122°11′W), within the Blackwater variant of the dry warm subzone of the Sub-Boreal Spruce biogeoclimatic zone (SBSdw2) at an elevation of 900 m. Soil and vegetation description indicates that the site belongs to the zonal SxwFd – Pinegrass (01) site series (Steen and Coupé 1997). The soil moisture regime is mesic and the soil nutrient regime is medium. It occurs on a moderately well-drained, gently undulating morainal blanket. The rooting zone has a loamy texture with about 25% volume of gravel and cobbles of acidic, igneous intrusive lithology. There is a root-restricting layer at 35 cm, below which the texture is more clay rich with more coarse fragments. The soil is classified as a Brunisolic Gray Luvisol (Soil Classification Working Group 1998). The site is occupied by a naturally regenerated lodgepole pine stand that originated from a 1978 clearcut and subsequent drag scarification. At the time of installation establishment in 1992, the 13-year-old stand had an average density of 20 000 stems/ha. All treatment plots were thinned to a uniform density of 1100 stems/ha during plot establishment. In the fall of 1992, the average height of the crop trees within treatment plots was 4.2 m.

Treatment and experimental design

Each of six treatments is replicated three times for a total of eighteen 0.164 ha treatment plots, arranged in a randomized complete block design. Each treatment plot contains approximately 180 crop trees, equivalent to a stand density of approximately 1100 stems/ha. Growth analyses for each plot are based on periodic measurement of 64 permanently tagged trees within the inner assessment plot. There are six treatments, but for this study only three of them were used:

(1) Control: not fertilized
(2) ON1: yearly fertilization to maintain foliar N concentration at 1.3% and other nutrients in balance with foliar N
(3) ON2: yearly fertilization to maintain foliar N concentration at 1.6% and other nutrients in balance with N

The ON1 and ON2 treatments are patterned after Scots pine (*Pinus sylvestris* L.) "optimum nutrition" experiments in Sweden (Tamm et al. 1999), in which N is added frequently to approximate steady-state nutrition. Yearly fertilizer prescriptions for ON1 and ON2 treatments are developed following foliar sampling and nutrient analysis each fall. These two treatments typically receive 50–100 and 100–200 kg N/ha, respectively, each spring (no fertilizer was added in 1997). From the time of initial fertilization in 1993 through 2002, the ON1 and ON2 plots received a total of 700 and 1350 kg N/ha, respectively. Other nutrients (e.g., phosphorus (P), potassium (K), magnesium (Mg), S, B) were added periodically to provide an appropriate foliar nutrient balance and to minimize growth limitations resulting from secondary deficiencies. Urea (46–0–0; N–P–K) is the major source of N for all fertilizer treatments. Additional sources of N are monoammonium phosphate (11–52–0; N–P–K) and ammonium nitrate (34–0–0; N–P–K). Phosphorus is always added as monoammonium phosphate. Sulphate potash magnesia

Table 1. Fertilization regimes by treatment and year for sampled plots at Sheridan Creek optimum fertilization trial.

	Treatment[a]	
Year	ON_1	ON_2
1993	100N, 100P, 100K, 50S, 25Mg, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B
1994	100N, 100P, 100K, 50S, 25Mg	200N, 100P, 100K, 50S, 25Mg
1995	100N, 100P, 100K, 50S, 25Mg	200N, 100P, 100K, 50S, 25Mg
1996	100N, 100Mg, 17S	200N, 100Mg, 17S
1997	None	None
1998	50N, 50P, 50K, 50S, 50Mg, 1.5B	100N, 50P, 50K, 50S, 50Mg, 1.5B
1999	50N	100N
2000	100N, 50P, 50K, 63S, 32Mg	150N, 50P, 50K, 63S, 32Mg
2001	50N	100N
2002	50N, 1.5B	100N, 1.5B

[a]N, nitrogen; P, phosphorus; K, potassium; S, sulphur; Mg, magnesium; B, boron. Values preceding the nutrients indicate the amount of nutrient applied in kilograms per hectare.

(0–0–21–21–11; N–P–K–S–Mg) is used extensively as a source of K, S, and Mg. Potassium chloride (0–0–60; N–P–K), ammonium sulphate (21–0–0–24; N–P–K–S), and ProMag 36 (36% Mg, 6% S) are also used to supply additional K, S, and Mg, respectively. Boron is supplied as granular borate (15% B). Total quantities of nutrients added for each of the treatments are shown in Table 1.

Soil sampling

In mid-September 2002, samples were collected from three replicate installations of the trial: unfertilized control, ON1, and ON2. For soil mesofauna and soil microbial community, 10 separate forest floor and upper mineral soil (0–3 cm) samples were randomly collected from each plot using a 4.5 cm internal diameter coring device. For ectomycorrhizas and fine roots, ten 10 cm × 10 cm × 10 cm combined forest floor and mineral soil samples were randomly collected. Larger samples were used to ensure that enough fine roots of lodgepole pine were found. All samples were kept cool until processed.

Soil mesofauna community

Within 48 h of collection, samples were placed in a modified high-gradient extractor for 1 week (Lussenhop 1971), with dataloggers monitoring the temperature gradient during the extraction process. Mesofauna were collected into an ethylene glycol solution and then transferred into glass vials with 70% ethanol by washing the contents of the collecting dishes with distilled water through a 50 μm sieve until no ethylene glycol remained. All samples were sorted and counted under a dissecting microscope. Collembola were identified to family and Acari to suborder. All mesofauna samples are stored with the British Columbia Ministry of Forests and Range, Research Branch Laboratory, P.O. Box 9536, Victoria, BC V8W 9C4.

Soil microbial community

A serial dilution (1:1000) of each soil sample was prepared in one-quarter strength Ringers solution. The carbon (C) substrate utilization profile was assessed using Biolog EcoPlates™ (Biolog Inc., Hayward, California). Each plate includes 31 substrates replicated three times within the plate, each replicate with its own substrate-free control. Aliquots (150 μL) of the 10^{-3} dilutions were added to each well of a plate for each of the 180 soil dilutions. Plates were incubated at 20–24 °C for approximately 60 h. Following incubation, the plates were read for absorbance at 492 nm using a Titertek Multiscan plate reader, and the absorbance readings were stored as an Excel worksheet file. Approximately 10 g samples of each soil were oven-dried and used to determine a correction for soil moisture content of the original samples.

Fine roots and ectomycorrhizal community

Lodgepole pine fine roots (<2 mm diameter) from each soil core were extracted by gently washing the soil from the roots over a 1 mm sieve. Using a stereomicroscope, all fine pine roots in the sample were selected from among the remaining coarse woody debris and other plant material and set aside for root length estimation. Root length was estimated by the line intersect method (Tennant 1975). The ectomycorrhizal community was then quantified and characterized by cutting the root systems into 2 cm sections, which were randomly selected until 200 active mycorrhizal roots had been encountered (in addition to inactive and non-mycorrhizal tips). Ectomycorrhizas were observed under 400× or 1000× magnification, either as the whole root tip or fungus only (done by peeling the mantle from the root tip), and subsequently classified according to the detailed procedure described by Goodman et al. (1996). Representative tips of each morphotype were frozen for future reference.

Soil chemistry

After extraction of soil mesofauna, the soil cores were used for chemical analysis. Because of budget restrictions, we combined five randomly selected samples to create two composite samples per treatment plot and horizon for chemical analysis. Although the soil cores were relatively small in volume, they were the same samples from which the soil mesofauna were extracted. Analyses were carried out by the Research Branch Laboratory of the British Columbia Ministry of Forests and Range, using the following methods: pH in H_2O, total carbon (C) (Tiessen and Moir 1993), total N (McGill and Figueiredo 1993), mineralizable N (Keeney

Table 2. Forest floor and mineral soil chemical properties by fertilizer treatment in a young lodgepole pine stand.

	Forest floor			Mineral soil		
	Control	ON1	ON2	Control	ON1	ON2
Total C (%)	29.85	32.69	33.45	5.76	9.64	9.16
Total N (%)	0.8	1.12	1.11	0.17	0.29	0.31
C/N ratio	37.99a	29.22b	30.84b	33.15	33.52	28.04
Mineralizable N (ppm)	332.65	537.17	414.06	23.89	53.05	75.56
NO_3-N (ppm)	0.57a	7.77a	92.79b	0a	0a	8.67b
NH_4-N (ppm)	23.96	41.12	72.66	4.84	7.85	15.27
Available P (ppm)	78.78a	235.34b	221.35b	42.89a	181.77b	156.37b
Exchangeable Mg (cmol+/kg)	5.57a	14.36c	9.79b	1.71	4.35	3.64
CEC (Ba) (cmol+/kg)	—	—	—	9.53	13.68	13.38
pH (H_2O)	4.78	5	4.56	4.73	4.64	4.59

Note: Means followed by the same letter in each row are not significantly different at $P < 0.05$. C, carbon; N, nitrogen; P, phosphorus; Mg, magnesium; CEC, cation exchange capacity; Ba, barium.

1982), NO_3 and NH_4 (Bremner 1965), available P (Kalra and Maynard 1991), and exchangeable cations and CEC (Kalra and Maynard 1991; Hendershot et al. 1993).

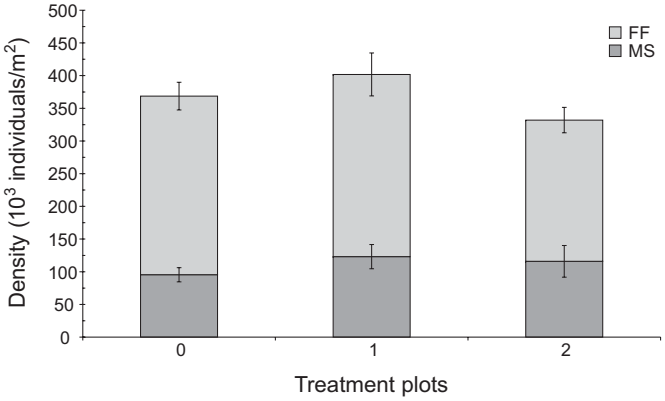
Data analyses

For soil chemistry, statistical analyses were performed with JMP IN version 5.1 (SAS Institute Inc. 2003). Analysis of variance (ANOVA) was used to compare values among the three treatments with forest floor and mineral soil samples analyzed separately and $\alpha = 0.05$. If differences were found with ANOVA, then Tukey's pairwise comparison was used.

For soil mesofauna, density (number of individuals per sample) was used in analyses and reported as mean ± standard error, unless stated otherwise. Only Collembola and Acari were considered for analysis, since the remaining fauna were represented by very few individuals and further analysis of these taxa had limited value. Data were transformed to meet assumptions of normality before analyses; density data were log transformed [$\log_{10}(X + 1)$ where X is the actual count of individuals for a taxon. ANOVA was used to compare values among the three treatments. Forest floor and mineral soil samples were analyzed separately. Minitab version 9.5 (Minitab 1996) was used for statistical procedures with $\alpha = 0.05$. If differences were found with ANOVA, then Tukey's pairwise comparison was used.

For the soil microbial community, the EcoPlate™ data were corrected for the nonsubstrate control background and soil moisture content. The following measures were calculated using the corrected data matrix: (1) total absorption, the sum of all positive absorbance values normalized by dividing cell values by the average well colour development, according to the methodology described by Garland and Mills (1991) and Heuer and Smalla (1997); (2) average well colour development, the total absorbance divided by the total number of positive substrate wells; (3) species richness, the number of substrates showing a positive absorbance value that exceeded an arbitrary threshold value of 0.3; and (4) substrate diversity, calculated as the Shannon diversity index: $H = -\Sigma pI \ln(pI)$, where pI is the ratio of the activity on a particular substrate to the sum of activities on all substrates (Magurran 1988). The computed microbial community parameters were analysed by ANOVA using Statistix 7 (Analytical Software, Talahassee, Florida). The data were analysed separately for each soil horizon to account for the effects of subsampling within plots. Mean separation of treatment differences was determined using Tukey's honestly significant difference test.

Fig. 1. Comparison of mean density (±SE) of total soil mesofauna in forest floor (FF) and mineral soil (MS) and combined (FF + MS) among treatments.



For fine roots and ectomycorrhizas, statistical analyses were performed with JMP version 5.1 (SAS Institute Inc. 2003). Despite transformation, root response variables (individual ectomycorrhizal types, total combined ectomycorrhizas, active fine roots, inactive fine roots, nonmycorrhizal roots, and estimated fine root length) failed to meet assumptions of homogeneity of variances and normality and therefore the nonparametric Kruskal–Wallis test was used to compare (nontransformed) data between treatments in a randomized block design ($n = 3$). Significant results ($\alpha < 0.05$) were further evaluated using the Tukey–Kramer honestly significant difference pairwise comparison test to identify differences among means. The relative abundance of each ectomycorrhizal type was calculated as a percentage of the total number of active ectomycorrhizal roots.

Results

Soil chemistry

In general, both forest floor and mineral soil fertility were increased by fertilization, though the differences were not

Table 3. Comparison of mean density of mesofauna (SE in parentheses) in forest floor under fertilized and unfertilized lodgepole pine plots.

	Mean density (10^3 individuals/m^2)				
	Control	ON1	ON2	$F_{[2,87]}$	p
Acari					
Oribatida	70.3 (8.40)ab	78.0 (6.85)a	49.9 (5.65)b	5.18	0.007
Mesostigmata	5.8 (0.84)b	8.4 (0.92)a	7.4 (0.94)ab	4.47	0.014
Prostigmata	161.2 (11.76)a	162.5 (30.06)a	102.8 (14.16)b	10.05	0.0005
Astigmata	9.3 (1.80)	9.0 (1.70)	14.6 (3.18)	1.94	1.49
Total	246.6 (18.51)a	257.9 (32.67)a	174.7 (18.16)b	6.88	0.002
Collembola					
Entomobryidae	0.8 (0.28)	0.8 (0.18)	0.8 (0.27)	0.14	0.89
Hypogastruridae	3.3 (0.81)	3.2 (1.38)	18.4 (10.17)	2.01	0.14
Isotomidae	8.3 (1.73)	6.1 (1.26)	10.8 (4.43)	0.3	0.74
Neelidae	0.0 (0.02)	0.0 (0.02)	0.5 (0.43)	1.36	0.26
Onychiuridae	11.4 (4.03)	7.8 (1.43)	7.9 (1.49)	0.22	0.8
Sminthuridae	0.3 (0.12)	0.4 (0.12)	0.2 (0.06)	1.25	0.29
Tomoceridae	0.3 (0.09)	0.1 (0.05)	0.5 (0.24)	2.23	0.11
Total	24.3 (4.94)	18.4 (2.41)	39.2 (10.81)	0.9	0.41

Note: Means followed by the same letter in each row are not significantly different at $p < 0.05$.

Table 4. Comparison of mean density of mesofauna (SE in parentheses) in mineral soil under fertilized and unfertilized lodgepole pine plots.

	Mean density (10^3 individuals/m^2)				
	Control	ON1	ON2	$F_{[2,87]}$	p
Acari					
Oribatida	29.5 (4.12)a	29.1 (4.40)a	12.9 (2.91)b	11.61	0.001
Mesostigmata	3.0 (0.62)	2.4 (0.48)	2.4 (0.42)	0.08	0.93
Prostigmata	51.3 (6.57)ab	82.1 (14.04)a	50.5 (8.10)b	4.74	0.011
Astigmata	3.4 (0.88)	3.0 (0.84)	3.0 (0.83)	0.17	0.85
Total	87.1 (10.13)ab	116.7 (18.14)a	68.8 (10.42)b	5.2	0.007
Collembola					
Entomobryidae	0.0 (0.02)	0.0 (0.02)	0.1 (0.05)	1.84	0.165
Hypogastruridae	0.4b (0.23)	0.9ab (0.39)	40.0a (22.00)	5.27	0.007
Isotomidae	1.9 (0.50)	1.4 (0.33)	1.4 (0.36)	0.62	0.54
Neelidae	0.0 (0.02)	0.0 (0.02)	0.0 (0.02)	0	1
Onychiuridae	4.7 (0.97)	2.7 (0.74)	4.5 (0.76)	2.43	0.09
Sminthuridae	0	0	0	—	—
Tomoceridae	0.0b	0.0b	0.1a (0.05)	4.22	0.018
Total	7.0ab (1.36)	5.1b (0.97)	46.2a (22.17)	3.45	0.036

Note: Means followed by the same letter in each row are not significantly different at $p < 0.05$.

always statistically significant (Table 2). There was significantly more NO_3-N in ON2 forest floor and mineral soil than in either the unfertilized control or ON1. Available P was higher in the forest floor and mineral soil of both fertilizer treatments (ON1 and ON2) than in the unfertilized control. Forest floor C/N ratios and exchangeable Mg were significantly lower and higher, respectively, in fertilized treatments than in the control. Total C, total N, mineralizable N, and NH_4-N were not significantly changed by fertilization. In all cases, however, mean values were highest in fertilized treatments. Treatment effects on forest floor and mineral soil pH were not statistically significant, though both forest floor and mineral soil pH means were lowest in the ON2 treatment.

Soil mesofauna community

Overall, the density of mesofauna in the forest floor and mineral soil combined was greater in ON1 than in the unfertilized control or ON2 (Fig. 1). In the forest floor, there was a significant decline in oribatid and prostigmatid mite densities in ON2 compared to that in ON1 and, because of the dominance of these two groups, in the density of total Acari (Table 3). There was a statistically insignificant increase in forest floor hypogastrurids and total Collembola in ON2.

In the mineral soil, as in the forest floor, oribatids declined in density in ON2 (Table 4). In contrast to the forest floor, prostigmatid density was significantly higher in ON1 mineral soil relative to that in the control and ON2. Similarly to the trend in the forest floor, hypogastrurid and, because of their

Table 5. Community-level carbon substrate utilization (mean ± SE) in forest floor under fertilized and unfertilized lodgepole pine plots.

	Carbon substrate utilization				
	Control	ON1	ON2	$F_{[2,87]}$	p
Average well colour development	0.36±0.21b	0.47±0.17a	0.29±0.09c	31.65	0.0000
Total absorbance	11.15±6.59b	14.44±5.40a	8.84±2.76b	7.37	0.0000
Substrate diversity	2.88±0.15b	2.97±0.08a	2.82±0.23b	4.48	0.0146
Richness	12.40±5.06b	15.73±4.53a	12.10±3.50b	9.35	0.0002

Note: Means followed by the same letter in each row are not significantly different at $p < 0.05$.

Table 6. Community-level carbon substrate utilization (mean ± SE) in mineral soil under fertilized and unfertilized lodgepole pine plots.

	Carbon substrate utilization				
	Control	ON1	ON2	$F_{[2,87]}$	p
Average well colour development	0.32±0.16b	0.44±0.14a	0.25±0.09c	45.40	0.0000
Total absorbance	9.87±5.05b	13.76±4.47a	7.88±2.64b	16.47	0.0000
Substrate diversity	2.77±0.14b	2.92±0.11a	2.77±0.20b	9.57	0.0002
Richness	10.83±2.97b	13.97±3.45a	10.53±3.15b	10.3	0.0000

Note: Means followed by the same letter in each row are not significantly different at $p < 0.05$.

Table 7. Mean abundance (SE in parentheses) of the dominant ectomycorrhizal types (>5% of the overall community) under fertilized and unfertilized lodgepole pine plots.

	No. of colonized fine roots/soil core			
Ectomycorrhizal type	Control	ON1	ON2	p
Cenococcum sp.	61.9 (8.6)a	31.0 (7.4)b	3.4 (1.7)c	<0.0001
Piloderma sp. (white)	27.3 (6.5)a	29.5 (6.7)a	0.0 (0.0)b	<0.0001
Wilcoxina sp.	0.8 (0.8)b	18.0 (6.5)ab	24.8 (8.6)a	0.0050
Russula-type I	26.4 (11.7)a	10.8 (5.5)ab	0.0 (0.0)b	0.0416
Suillus-like	26.7 (8.0)a	0.0 (0.0)b	0.0 (0.0)b	<0.0001
Amphinema sp.	2.3 (1.8)a	18.3 (7.8)a	5.5 (5.5)a	0.0749
Mycelium radicis atrovirens	10.3 (5.1)a	4.5 (3.0)a	3.7 (2.0)a	0.3445

Note: For descriptions of morphotypes, see Hagerman et al. (2001). Means followed by the same letter in each row are not significantly different at $p < 0.05$ (Kruskal–Wallis test).

dominance, total Collembola densities were significantly higher in ON2. The density of mineral soil Onychiuridae was lower in ON1 than in the control or ON2. As in the forest floor, the density of mineral soil Acari decreased and Collembola increased in ON2.

Soil microbial community

For both the forest floor (Table 5) and mineral soil (Table 6) there were significant treatment differences in total absorbance, average well colour development, substrate diversity, and richness. The soil microbial community in the moderately fertilized treatment (ON1) had the greatest activity (average well colour development and total absorption) and community diversity (richness and diversity) of the three treatments. Average well colour development treatment differences were ranked ON1 > control > ON2, while total absorbance, substrate diversity, and richness showed ON1 > ON2 = control.

Ectomycorrhizas and fine roots

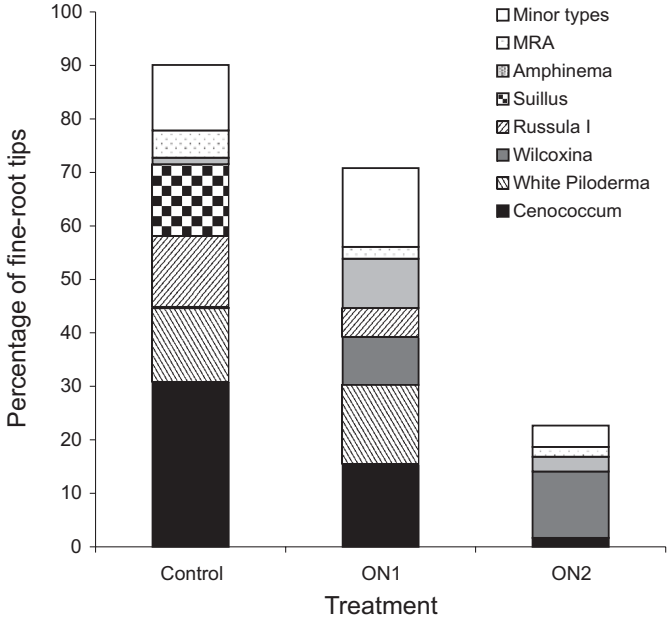
Thirteen distinct ectomycorrhizal morphotypes were described over the three treatments, with seven dominant types (>5% overall relative abundance) constituting 84.4% of the total community (Table 7). Increased N fertilization reduced ectomycorrhizal richness and altered the community structure among treatments (Fig. 2). Plots subjected to high N additions (ON2) had 40% fewer types than did control plots (10 types in control versus 6 in ON2). Within this declining trend, some ectomycorrhizal types were significantly more sensitive to N addition than others (Table 7). Specifically, high levels of N fertilization eliminated or greatly reduced the abundance of *Russula* sp., *Suillus* sp., *Piloderma* sp. (white), and *Cenococcum* sp. ectomycorrhizas. In contrast, other types either increased with high N fertilization (*Wilcoxina* sp.) or remained unaffected (*Mycelium radicis atrovirens* and *Amphinema* sp.).

Overall, these findings are reflective of the fine-root analysis, which showed that estimated root length, the number of live fine roots, and total ectomycorrhizas all declined significantly with increasing fertilization, with the total number of nonmycorrhizal roots increasing at highly fertilized relative to control sites (Fig. 3).

Discussion

We found that the repeated fertilization of lodgepole pine

Fig. 2. Community structure of the ectomycorrhizal types sampled from combined forest floor and mineral soil cores occurring under different fertilization regimes. Ectomycorrhizal types with an overall relative abundance <5% are grouped into one "minor types" category.



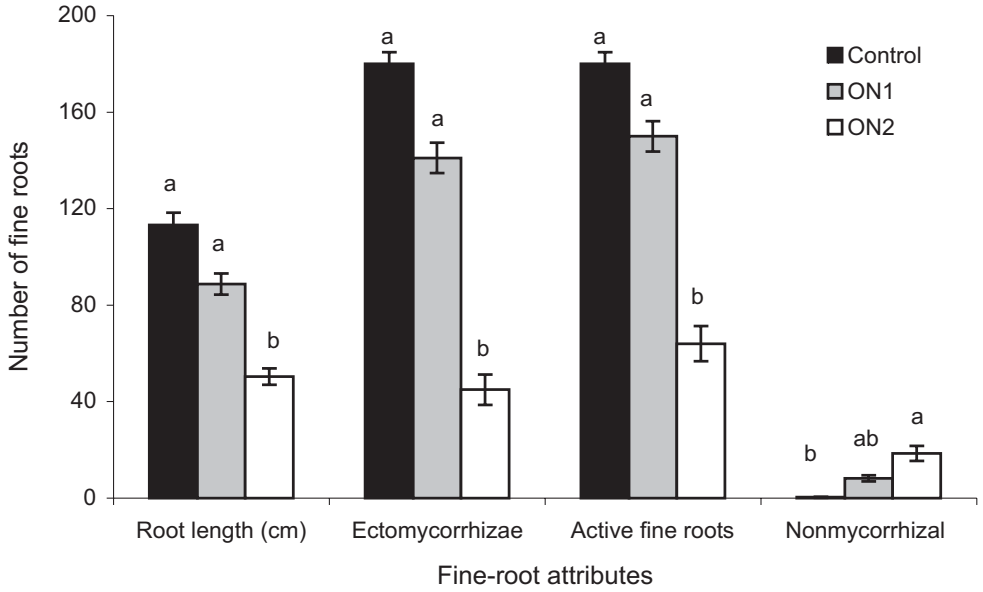
with moderate rates of N (ON1), in combination with frequent addition of other nutrients, generally had either no effect or a small positive effect on soil mesofauna and microbial populations. However, we found a significant depression of components of the mesofauna and microbial communities at the higher N application rate (ON2). Ectomycorrhizal colonization and fine-root biomass and vigour were also negatively affected by fertilization, and shifts in ectomycorrhizal community structure were observed. The trend that we observed of increasing NO_3 and NH_4 levels and decreasing C/N ratios with increasing fertilization intensity in both the upper mineral soil and humus layers is consistent with findings from repeatedly fertilized "optimum nutrition" trials with Scots pine and Norway spruce (*Picea abies*) in Sweden (Tamm 1991; Tamm et al. 1999). The presence of higher levels of NO_3-N in fertilized treatments compared to that in the control indicates that significant nitrification of the applied N has occurred in these repeatedly fertilized acidic forest soils.

Others have suggested that fertilization may affect mycorrhizal colonization and species composition by reducing C allocation to the roots and altering the balance of competition between fungi that tolerate high levels of inorganic N and those that do not (Arnebrant and Söderström 1992) or by causing accumulation of free amino acids in the foliage, thus diverting N away from photosynthesis and C assimilation (Bauer et al. 2004). Although several studies have documented neutral or negative impacts on Acari and Collembola of single N additions (Axelsson et al. 1973; Marshall 1974; Lohm et al. 1977; Vilkamaa and Huhta 1986; Jandl et al. 2003; Lindberg and Persson 2004; Minor and Norton 2004), few studies have determined the long-term effects of repeated fertilization. We found that the ON1 treatment (50–100 kg $N{\cdot}ha^{-1}{\cdot}year^{-1}$) had no effect on forest floor Oribatida and Prostigmata densities and a positive effect on Mesostigmata density relative to the control, while Lindberg and Persson (2004) in a study of 13 years of intensive fertilization (75–100 kg $N{\cdot}ha^{-1}{\cdot}year^{-1}$) of Norway spruce found decreases in oribatid and mesostigmatid densities, and Axelsson et al. (1973) reported declines in Oribatida and Collembola in N3 (120–180 kg $N{\cdot}ha^{-1}{\cdot}year^{-1}$) in an "optimum nutrition" experiment with Scots pine. Interestingly, we found lower forest floor Oribatida and Prostigmata densities and higher Collembola densities at ON2 (100–200 kg $N{\cdot}ha^{-1}{\cdot}year^{-1}$) relative to that at ON1. Some previous studies have reported increased microbial activity, at least over the short term, following N fertilization (Roberge and Knowles 1967; Salonius and Mahendrappa 1975; Allen and Schlesinger 2004), while others have reported declines in soil microbial activity and biomass (on a soil mass basis) after fertilization, with the size of the decrease being proportional to the amount of N applied (Söderström et al. 1983; Nohrstedt et al. 1989; Arnebrant et al. 1996; Berg and Matzner 1997). Our results were mixed for both forest floor and mineral soil: we found that annual fertilizer additions of 50–100 kg $N{\cdot}ha^{-1}{\cdot}year^{-1}$ resulted in higher microbial activity and diversity relative to that in the unfertilized treatment, but that higher N rates reduced activity and diversity to levels that were similar to those in the control.

Given that high fertilizer application in our study resulted in a decrease in ectomycorrhizal fungi, it is not surprising that mycophagous mites (oribatids and some prostigmatids) also declined. Schneider et al. (2005) have shown that three different oribatid mite species prefer ectomycorrhizal fungi to saprophytic fungi. Higher density of predaceous mites (Mesostigmatids) with moderate fertilization (ON1) suggest a higher density of their prey, but we did not observe an increase in other mites or Collembola that would support this, nor did we study how other potential prey soil fauna, such as nematodes, responded to the fertilizer treatments. The work of Forge and Simard (2001) would suggest that the short-term response of soil nematodes to fertilizer application is mixed, with more bacterivorous and fewer omnivorous nematodes. We did, however, observe a greater density of Collembola with high fertilizer application (ON2), especially the hypogastrurids, which could have resulted from the decline in competition or predation from mites. High fertilizer application (ON2) decreased the bacterial and ectomycorrhizal communities, suggesting that changes in those food sources do not explain the greater hypogastrurid density.

The lack of response of the soil microbial community to higher fertility (ON2) may have resulted from induced C limitations or from competitive interactions between populations of the microbial communities. Since plants exude more sugars from their fine roots when nutrients are limiting (Marschner 1995), the expected decline in sugars exuded into the rhizosphere when nutrients are abundant could partly explain the decline in microbial activity. Higher levels of synthetic N fertilizer use have been correlated with reduced microbial populations and community composition. Arnebrant et al. (1996) found that microbial activity (respiration rate) and biomass (ATP content, substrate-induced respiration) were reduced in 45- to 130-year-old Scots pine forests by fertilization with 150 to >500 kg N/ha applied 1–11 years previously except on sites with high native fertility.

Fig. 3. Lodgepole pine fine-root attributes sampled from combined forest floor and mineral soil cores under different nitrogen fertilization levels. Within column groups, means (±SE) with the same letter are not significantly different ($\alpha = 0.05$) as tested by Tukey–Kramer honestly significant difference pairwise comparison tests.



We are not able to fully explain the pattern of microbial community activity response to fertilizer application that we detected because of the limitations of the technique we used and the complexity of the entire biotic community's response to fertilizer application. It is clear that many factors are involved. In a grassland microcosm study, Kennedy et al. (2004) determined that plant rhizosphere effects were less important to bacterial community structure than the addition of lime and N. Others have found that plant species strongly influence microbial activity (e.g., in a tropical pasture with remnant trees; Galicia and Garcia-Oliva 2004). Because they determined that responses of the soil microbial community in 15-year-old temperate conifer stands were dependent on tree species and site, Leckie et al. (2004) suggested that the effects were indirect and mediated by differences in plant growth and litter inputs. We speculate that fertilizer-induced changes in the understory plant community in our study might help to explain the pattern of response in the microbial community.

Changes in soil chemistry may affect the biomass and composition of the aboveground plant community (Persson 1981; Tamm et al. 1999) and cause shifts in the C allocation pattern from below- to above-ground components (Albaugh et al. 1998). These changes may, in turn, influence soil biota through effects on root exudation, fine-root turnover, and litter quality and quantity. Quantitative sampling of understory vegetation at our study site in August 1997, 5 years after the initiation of fertilization treatments, detected differences in total biomass and species distribution (R.P. Brockley, unpublished data). All vegetation within each of twenty 1 m^2 subplots within each treatment plot was clipped at ground level and sorted by species and life form. Total plant biomass was 37% and 75% higher in ON1 and ON2 treatments, respectively, relative to that in the unfertilized treatment. The absolute amounts and relative proportions of the moss and lichen layer decreased sharply in both fertilizer treatments. The herbaceous and shrub layers in ON1 and ON2 plots were dominated by nitrophilic species such as *Epilobium* and *Rosa*. Interestingly, the amount and relative abundance of grasses (mainly *Calamagrostis*) were greater in ON1 plots than in control or ON2 treatments. In this study, we found that microbial activity followed the same pattern, which suggests that a relationship may exist between microbial activity and the abundant and relatively easily decomposed grass roots and shoots. Using techniques far more sophisticated than ours (i.e., community-level physiological profiling (CLPP), polar lipid fatty acid (PLFA) analysis, and community DNA techniques), Grayston et al. (2004) determined that plant community structure correlated with microbial community structure and that the microbial community structure shifted with grassland improvement. We also found that ectomycorrhizal fine roots of pine were less abundant in ON2 than in the unfertilized control or ON1. Although we did not assess the roots of the understory vegetation, we know that the aboveground biomass of shrubs and herbs increased with fertilizer application. This may mean that the community of fine roots (both species composition and biomass) differed between treatments. In addition, most of the shrubs, herbs, and grasses in these stands are known to form arbuscular mycorrhiza rather than ectomycorrhiza. Langley and Hungate (2003) hypothesized that mycorrhizal status influences fine-root decomposition, with ectomycorrhizas and their hyphae being more recalcitrant than arbuscular mycorrhizas and their hyphae. Furthermore, specific ectomycorrhizal fungi (including *Suillus variegatus*) are known to influence soil bacterial biomass and activity (Olsson and Wallander 1998), so as their populations change with treatment, it is likely that the populations of bacteria they affect will also change.

Of the soil biotic components we studied, only the ectomycorrhizal fungi were assessed at the species or morphospecies level. The decline in ectomycorrhiza density and

richness in response to increased N fertilization reported here is consistent with the theory that N deposition can negatively impact many species of ectomycorrhizal fungi over time (reviewed by Wallenda and Kottke 1998; Eaton 2004). Our results are supported by recent studies conducted in both coniferous and deciduous forest ecosystems. Specifically, Lilleskov et al. (2002) found that increasing N fertilization reduced ectomycorrhizal richness and altered community structure of mature white spruce stands in southern Alaska, as did Avis et al. (2003) in a deciduous temperate forest in eastern Minnesota. Yet others have reported more subtle responses of ectomycorrhizas to increased N, with shifts in belowground community structure occurring without any overall loss of diversity (Peter et al. 2001). Still other studies (e.g., Nilsen et al. 1998; Jonsson et al. 2000) have reported no detectable change in belowground ectomycorrhizal communities with N addition. However, the 5-year study of Nilsen et al. (1998) of Norway spruce grown in outdoor pot lysimeters made only a cursory attempt to distinguish among ectomycorrhizal types, thereby decreasing the possibility of detecting community shifts had they occurred. Jonsson et al. (2000) acknowledged that both treated and control communities at their Norway spruce study site in Sweden had been influenced by gradual deposition of N for decades and that community shifts may already have occurred. In our case, all sampled ectomycorrhizas were identified to morphotype (Hagerman et al. 2001) and the study site was not affected by N deposition.

In naturally regenerated lodgepole pine stands in Alberta, Bradbury et al. (1998) determined that *Mycelium radicis atrovirens* was the most abundant ectomycorrhiza, followed by species of *Piloderma*, *Cenococcum*, *Russula*, *Suillus*, and *Lactarius*. We found a very similar assemblage of fungi, and it is well known that these fungi respond differently to N additions. *Russula* species in particular have been described as "nitrophobic" (Lilleskov et al. 2002), and our study and those of others conducted in a variety of temperate forest ecosystems have shown these species to be negatively affected by N fertilization (Peter et al. 2001; Lilleskov et al. 2002; Edwards et al. 2004). Other dominant types, which were eliminated or greatly reduced by N additions in this study, include *Suillus*-like, *Piloderma* (white), and *Cenococcum*. In the case of *Piloderma*, this result echoes the findings of Lilleskov et al. (2002), who similarly observed this taxon to be associated with low but not high-N sites. However, the response of *Cenococcum* to increased N is more variable, decreasing with fertilization in some cases (Gill and Lavender 1983) and remaining relatively constant in others (Jonsson et al. 2000; Peter et al. 2001; Lilleskov et al. 2002). *Suillus* and the related *Rhizopogon* form tuberculate mycorrhizas that associate with nitrogen-fixing bacteria (Li et al. 1992; Paul 2003) and are known to be sensitive to N fertility (Arnebrant 1994; Trudell and Edmonds 2004). By contrast, *Wilcoxina* and *Thelephora* ectomycorrhizas, two types common in nurseries and young stands, were affiliated with high-N sites in this study. *Thelephora* in particular has been shown repeatedly to increase in response to N fertilization (Lilleskov et al. 2002; Edwards et al. 2004).

Many studies have reported increased fine-root biomass following N fertilization (Ahlstrom et al. 1988; Helmisaari and Hallbacken 1999; Majdi 2001). However, the reduction in lodgepole pine fine-root biomass in response to increased N observed in this study is consistent with the findings of Alexander and Fairley (1983), Clemensson-Lindell and Persson (1995), and Majdi and Kangas (1997).

The direct and indirect effects of nutrient changes and pH shifts on the soil biota and fine roots are undoubtedly complex. In our study, the pH of the forest floor was not significantly affected by repeated fertilization. These results differ somewhat from those of several other studies that have reported a trend of increasing pH of humus layers with increasing fertilization intensity (Nohrstedt 1990, 1992; Tamm et al. 1999). More intensive soil sampling of our plots may have detected differences. However, the small (but statistically insignificant) decline in mineral soil pH with increasing fertilization intensity in our study is consistent with the small fertilization-induced changes in the pH of upper mineral soils (0–5 cm) reported by Nohrstedt (1992) and Tamm et al. (1999). The effects of fertilization on the pH of deeper mineral soil horizons were not measured in our study. However, other studies have shown that long-term annual urea fertilization may induce strong acidification of mineral soils (10–20 cm) (Tamm and Popovic 1995; Tamm et al. 1999).

The potential impacts of these belowground changes on long-term ecosystem health and function are difficult to predict. Brockley and Simpson (2004) reported a 49% increase in tree basal area growth in ON1 relative to that in the control over 9 years at our study site, with no further tree growth response at the more intense ON2 treatment. Because of larger tree mortality in the ON2 treatment, area-based response (i.e., cubic metres per hectare) was larger in the ON1 treatment regime. Negative dose–response relationships have been reported from other long-term "optimum nutrition" experiments with *Pinus* (Tamm et al. 1999; Kishchuk et al. 2002). Foliar nutrient imbalance was implicated as a probable contributing factor in the negative impact of heavy N additions on the growth of *Pinus* in these studies. Despite the frequent use of multinutrient fertilizer prescriptions, critical thresholds for foliar N/P, N/K, and N/Mg ratios have been exceeded at our study site and at several other lodgepole pole pine "maximum productivity" field installations in central British Columbia (Brockley and Simpson 2004). Our results suggest that decreased mycorrhizal colonization and reduced fine-root biomass and vigour might be contributing factors in impaired foliar nutrient balance in high-N treatments. Also, reduced fine-root vitality and a shift in growth allocation from below- to above-ground components might potentially be problematic under droughty soil conditions (Linder et al. 1987; Persson et al. 1995) or if nutrient additions were abruptly stopped. Finally, the reduction in ectomycorrhizas and the protection provided by them against root pathogens (reviewed by Whipps 2004) could potentially lead to root disease problems.

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