Douglas-fir soil C and N properties a decade after termination of urea fertilization

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Abstract: Chemical and microbial soil properties were assessed in paired unfertilized and urea fertilized (>89 g $N \cdot m^{-2}$) plots in 13 second-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands distributed throughout western Washington and Oregon. A decade following the termination of fertilization, fertilized plots averaged 28% higher total N in the O layer than unfertilized plots, 24% higher total N in surface (0–5 cm) mineral soil, and up to four times the amount of extractable ammonium and nitrate. Decreased pH (0.2 pH units) caused by fertilization may have been due to nitrification or enhanced cation uptake. In some soil layers, fertilization decreased cellulase activity and soil respiration but increased wood decomposition. There was no effect of fertilization on concentrations of light and heavy fractions, labile carbohydrates, and phosphatase and xylanase activities. No increase in soil organic C was detected, although variability precluded observing an increase of less than ~15%. Lack of a regionwide fertilization influence on soil organic C contrasts with several site-specific forest and agricultural studies that have shown C increases resulting from fertilization. Overall, the results indicate a substantial residual influence on soil N a decade after urea fertilization but much more limited influence on soil C processes and pools.

Résumé : Les propriétés chimiques et microbiennes du sol ont été évaluées dans des paires de parcelles non fertilisées ou fertilisées à l'urée (>89 g $N \cdot m^{-2}$) établies dans 13 peuplements de seconde venue de douglas de Menzies (*Pseudotsuga menziesii* (Mirb.) Franco) répartis dans l'ouest des états de Washington et de l'Oregon. Une dizaine d'années après l'arrêt de la fertilisation, les parcelles fertilisées avaient en moyenne 28% plus de N total dans l'horizon O que les parcelles non fertilisées, 24% plus de N total en surface (0–5 cm) du sol minéral et jusqu'à quatre fois plus de nitrate et d'ammonium extractibles. La diminution du pH (0,2 unités de pH) causée par la fertilisation pourrait être due à la nitrification ou à une plus grande absorption de cations. Dans certains horizons du sol, la fertilisation a causé une diminution de l'activité de la cellulase et de la respiration du sol mais a accéléré la décomposition du bois. La fertilisation n'a eu aucun effet sur la concentration des fractions légère et lourde, sur les hydrates de carbone labiles, ainsi que sur l'activité de la phosphatase et de la xylanase. Aucune augmentation de C dans le sol organique n'a été observée. Par contre, la variabilité ne permettait pas d'observer une augmentation inférieure à ~15%. L'absence d'effet de la fertilisation dans l'ensemble de la région sur le C dans le sol organique contraste avec plusieurs études réalisées sur des sites forestiers et agricoles spécifiques qui ont rapporté une augmentation de C due à la fertilisation. Dans l'ensemble, les résultats indiquent une importante influence résiduelle sur N du sol une dizaine d'années après la fertilisation à l'urée mais une influence beaucoup plus limitée sur les processus et les réserves de C dans le sol.

[Traduit par la Rédaction]

Introduction

Fertilization with N is a common practice in intensively managed forests (Allen 1987), including those in the Pacific Northwest Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) region (Chappell et al. 1991). Regional evaluations have indicated N fertilization increased tree growth in many second-rotation Douglas-fir stands (Edmonds and Hsiang 1987; Stegemoeller and Chappell 1990), but a sustained increase in N cycling and N availability was not observed in the forest floor 8 years after fertilization (Chappell et al. 1999). In contrast, enhanced N availability in the mineral soil was found at a site more than a decade after termination of fertilization (Binkley and Reid 1985; Strader and Binkley 1989). The long-term influences on mineral soil properties at other sites across the region are not known.

The magnitude and duration of fertilization effects may be related to the magnitude of soil properties themselves. For example, Prescott et al. (1993) and Miller (1988) hypothesized that long-term enhancement of soil N cycling would be more likely on sites with high soil N content. However, in second-growth Douglas-fir stands in western Washington, N fertilization did not result in a sustained increase in N cycling and N availability in the forest floor (Chappell et al. 1999). Further, the magnitude of the N fertilization effect in

Received March 30, 2001. Accepted September 19, 2001. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on December 7, 2001.

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 DOI: 10.1139/cjfr-31-12-2225

the forest floor was not related to the initial soil N capital (Chappell et al. 1999). The effects on the mineral soil were not evaluated.

Long-term soil responses to N fertilization may extend beyond N availability, as many chemical, microbiological, and physical soil properties are altered by land-use practices (Gessel et al. 1990). In particular, N fertilization may be one of several forest-management practices that change soil C stores (Johnson 1992*a*), which may be important in terrestrial C sequestration as part of the global C cycle (Lal et al. 1998). Responses of soil C amounts and processes vary among forest ecosystems. Fertilization with N, sometimes combined with other nutrients, increased soil organic C in trembling aspen (*Populus tremuloides* Michx.) (Van Cleve and Moore 1978), Monterey pine (*Pinus radiata* D. Don) (Baker et al. 1986), and Scots pine (*Pinus sylvestris* L.) (Nohrstedt et al. 1989) but not in a slash pine (*Pinus elliottii* Engelm. var. *elliotti*) forest (Harding and Jokela 1994). Slower decomposition may contribute to long-term increases in soil C (Kaye et al. 2000) through several mechanisms. Litter from N fertilized stands of Scots pine decomposed more slowly than litter from unfertilized stands, but Norway spruce (*Picea abies* (L.) Karst.) decomposition was not affected by fertilization (Berg 2000). Nitrogen additions decreased microbial activity per gram of C in Norway spruce and Scots pine forests (Söderström et al. 1983; Nohrstedt et al. 1989; Smolander et al. 1994) but not in aspen (Van Cleve and Moore 1978). Nitrogen additions decreased ligninolytic enzyme activities, which may decrease detrital decay rates (Carreiro et al. 2000).

Forest management might also influence other soil properties relevant to long-term site productivity or C sequestration. These include pH, microbial biomass, soil carbohydrates, enzymes, and light fraction amount and its characteristics (Gregorich et al. 1994). The light fraction of the soil, defined as material that floats in a salt solution of a chosen density, is more responsive to some soil management practices than are whole-soil characteristics (Gregorich et al. 1994). Fertilization of agricultural systems with macronutrients increased light fraction amounts (Gregorich et al. 1996, 1997; Fließbach and Mäder 2000), while application of lime decreased light fraction, apparently because of effects on aggregate stability (Chan and Heenan 1999). Reduction in forest aboveground litter can decrease light fraction (Boone 1994). The decrease in light fraction from cultivation (Gregorich et al. 1994) can continue for decades after reversion to forest (Compton and Boone 2000).

Evaluation of the effect of urea fertilization on second-growth Douglas-fir forests has been undertaken through a regional experiment encompassing more than 80 sites distributed throughout western Washington and Oregon (Edmonds and Hsiang 1987). Urea fertilization increased average tree growth at these sites (Stegemoeller and Chappell 1990, 1991). The magnitude of the tree response was related to soil properties; in particular, tree response was positively correlated with forest floor C/N ratio (Edmonds and Hsiang 1987). An investigation of forest floor response at eight of the sites indicated a tendency for forest floor N content to be higher on fertilized plots, but there was no effect of fertilization on net N mineralization (Chappell et al. 1999). A study at three of the sites did not show a fertilization influence on forest floor and mineral soil C content (Canary et al. 2000), but the limited sample size may have prevented observing an effect.

In this study, we investigated forest floor and mineral soil responses over a broader array of sites to increase statistical power and allow a regional assessment. We evaluated a larger variety of soil properties in both the forest floor and mineral soil. Our objectives were (*i*) to determine the effects of urea fertilization on soil chemical and microbiological properties in second-growth Douglas-fir forests of the western Washington and Oregon region, a decade after termination of fertilizer application, and (*ii*) to determine if the magnitudes of the fertilizer effects are related to amounts of soil C and N. This study is important in developing a better understanding of regional effects of N fertilization on long-term productivity and soil C storage. Subsequent papers will report the influence of N application on the stability of organic matter and the contributions of density fractions to N availability and microbial respiration responses (Swanston 2000).

Materials and methods

Study sites and sample collection

The study was conducted at 13 second-growth Douglas-fir fertilization sites of the Stand Management Cooperative Nutrition Project (previously called the Regional Forest Nutrition Research Project; Hazard and Peterson 1984) distributed throughout western Oregon and Washington, U.S.A. (Table 1). The sites cover a broad range of physical attributes (Table 1) and soil characteristics (Table 2). At each site, six treatment plots were established in 1969–1970. Each plot had an area of $\geq$400 m^2 plus a treated buffer. We examined two plots at each site: a no-treatment control, and a plot that received multiple urea applications between 1969 and 1986 for a total N addition of 89.6 or 112 $g \cdot m^{-2}$. The initial fertilizer application was 44.8 g $N \cdot m^{-2}$ in 1969–1970; an additional 22.4 g $N \cdot m^{-2}$ was applied in 1977–1978, in 1981–1982, and on all but two sites (No. 1, Cedar Falls, and No. 43, Skykomish) in 1985–1986. The fertilizer was applied between October and March; the time of application varied among sites and among years. The overstory was primarily composed of 45- to 72-year-old Douglas-fir at time of soil sampling in 1995. A fourteenth site (No. 87, Crochline Saddle) initially included in the study was dropped, because the difference in soil N between the fertilized and control plots was substantially greater than the amount in added fertilizer, suggesting a large pretreatment difference between the control and fertilized plots.

Both plots at a given site were sampled on the same day. Soils were sampled in August and September 1995 on 4.5 × 4.5 m grids to yield a minimum of 16 sampling points per plot. At each sampling point, separate Oi and Oe+a layers were collected from a 15 × 15 cm area. These two layers constitute the entire O layer or forest floor. The Oi layer, also known as L layer, is the surface layer that consists of needles, twigs, cones, and bark fragments that have undergone little decomposition. The Oe+a layer aggregates the Oe and the Oa layers. The Oe layer, also known as F layer, consists of partially decomposed plant material, some of which is recognizable. The Oa layer, also known as H layer, is well-decomposed organic matter of unrecognizable origin.

After collection of the Oi and Oe+a layers, a 10 × 10 cm column of the 0- to 5-cm mineral soil and a 5 × 5 cm column of the 5- to 15-cm mineral soil were collected from the side of a small soil pit at each sampling point. Samples from alternating grid points were immediately composited, resulting in two composited samples per plot for each of the four layers.

Table 1. Site characteristics of second-growth Douglas-fir stands in western Washington and Oregon, U.S.A.

Site No.*	Site name*	Latitude (°N)	Longitude (°W)	Elevation (m)	Slope (%)	Aspect	Precipitation ($cm \cdot year^{-1}$)†	Site index (m at 50 years)‡
1	Cedar Falls	47.41	121.82	344	0	—	200	35
5	Cedar Falls Power Line	47.38	121.90	274	10	E	180	31
17	Little Ohop Creek	46.92	122.16	670	20	SE	170	40
20	Deep Creek	45.96	123.31	373	10	SE	140	42
25	Wolf Creek/Swing Log Road	43.92	123.36	350	0	—	140	34
26	Walton/Bishop Road	44.08	123.54	305	40	W	150	38
43	Skykomish	47.71	121.23	457	10	NW	230	26
53	Camp Grisdale	47.25	123.59	420	15	W	300	37
57	Headquarter Camp	46.22	122.73	536	0	—	220	40
64	Gibson Creek	44.15	123.82	189	25	SE	230	38
65	Fourth Creek	43.80	122.38	756	20	SE	140	37
89	Elk Creek	43.37	123.87	957	30	E	180	44
103	Cristy Flats	43.90	122.30	914	20	SE	180	32

*Official numbers and names used by the Stand Management Cooperative, University of Washington, Seattle.
†Mean annual precipitation for 1961–1990 is from Daly and Taylor (2000).
‡Based on height measurements of 20 or more trees at each site (Edmonds and Chappell 1994).

Table 2. Soil properties of second-growth Douglas-fir stands in western Washington and Oregon, U.S.A.

					Organic layer‡		0–15 cm mineral soil‡	
Site No.*	Parent material	Soil series	Soil classification†	Surface soil texture	C ($g \cdot m^{-2}$)	N ($g \cdot m^{-2}$)	C ($g \cdot kg^{-1}$)	N ($g \cdot kg^{-1}$)
1	Glacial till	Alderwood	Dystroxerept	Gravelly loam	740	19	72	2.7
5	Glacial till	Everett	Dystroxerept	Loam	nd§	nd	nd	nd
17	Sandstone	Wilkeson	Haploxeralf	Loam	530	13	127	3.5
20	Glacial sediments	Astoria	Dystrudept	Loam	1250	32	35	1.9
25	Sandstone	nd	nd	Silty loam	1010	22	39	1.9
26	Sandstone	Honeygrove	Palehumult	Silty loam	870	17	45	1.7
43	Granite	Tenneriffe	Haplorthod	Fine sand	1770	36	25	0.7
53	Glacial till	Hoquiam	Fulvudand	Loam	820	25	62	3.2
57	Igneous	Olympic	Palehumult	Loam	630	17	41	2.1
64	Sandstone	nd	nd	Loam	650	15	48	2.1
65	Pumice	nd	nd	Loam	1230	33	45	1.9
89	Sandstone	Preacher	Dystrudept	Loam	850	19	65	2.7
103	Pumice and ash	nd	nd	Loam	730	20	98	3.4

*Official numbers used by the Stand Management Cooperative, University of Washington, Seattle.
†USDA great-group classification (Soil Survey Staff 1998).
‡Carbon and N values at time of plot establishment in 1969–1970. Organic layer values are based on two forest floor samples in the control plot, and mineral soil values are based on one nearby soil pit (Edmonds and Chappell 1993).
§nd, not determined.

Sample analysis

Each Oi sample was oven-dried (55°C) and weighed. Each Oe+a sample was weighed, and a subsample analyzed for moisture content. A separate subsample was sieved to yield a field-moist fine (<2 mm) fraction, of which a portion was air-dried. The remainder of the sample was oven-dried (55°C). Each mineral soil sample was sieved to yield a field-moist fine (<2 mm) fraction, of which portions were air-dried or frozen at –20°C. Field-moist samples were stored in coolers and refrigerated at 4°C prior to analysis.

The field-moist fine Oe+a and mineral soil subsamples were extracted with 0.5 M K_2SO_4 within 2 days of collection. Extracts were analyzed for nitrite plus nitrate (hereafter called nitrate) and ammonium on an Alpkem autoanalyzer (OI Corp., College Station, Tex.), and for total extractable N on a Lachat Rapid flow injection analyzer (Lachat Instruments, Milwaukee, Wis.) following persulfate digestion (Cabrera and Beare 1993). To measure short-term respiration, field-moist fine subsamples equivalent to 4 g dry mass were adjusted to 200% moisture content for Oe+a or 75% moisture content for mineral soil, incubated at 15°C for 14 days, and head space CO_2 concentrations were analyzed by gas chromatography. The activities of key enzymes responsible for hydrolysis of two major detrital polymers, cellulose and xylan, and for the mineralization of organic phosphate monoesters were measured in refrigerated, field-moist fine Oe+a and mineral soil subsamples within 2 weeks of collection. Subsamples were suspended in distilled water (10:1, water:soil). For cellulase and xylanase, 1.0 mL of suspension was incubated with 1.0 mL of 1% substrate (carboxymethyl-cellulose and xylan, respectively) in 0.1 M acetate buffer (pH 5) at 30°C. Toluene (200 mL) was added as a bacteriostatic agent. After 24 h, the reaction mixtures were centrifuged, and 1.0 mL of supernatant was assayed for reducing sugar content with dinitrosalicylic acid reagent (Spalding 1977) using glucose as a standard. Acid phosphatase activity was measured

colorimetrically by the release of *p*-nitrophenol from the corresponding phosphate ester (adapted from Tabatabai 1994; Caldwell et al. 1999). One millilitre of sample suspension was incubated with 1 mL of 50 mM *p*-nitrophenylphosphate in acetate buffer at 30°C. After 1 h, the reaction was terminated by adding 0.5 mL 0.5 M $CaCl_2$ and 2.0 mL 0.5 M NaOH. After centrifugation, released *p*-nitrophenol was measured at 410 nm and compared with a standard curve. For all assays, controls of sample without substrate and substrate without sample were run to account for contamination. Results were converted to a dry-mass basis, based on moisture contents of soil samples.

Air-dried fine Oe+a and mineral soil subsamples were analyzed for pH (McLean 1982) using 10:1 water:substrate ratio for Oe+a and 2:1 ratio for mineral soil. Air-dried subsamples were analyzed for carbohydrates by the method of Martens and Frankenberger (1990).

Frozen and subsequently thawed fine mineral soil subsamples were separated into light (<1.65 $g \cdot cm^{-3}$) and heavy (>1.65 $g \cdot cm^{-3}$) density fractions in solutions of sodium polytungstate (Sometu-US, Van Nuys, Calif.) by sonication, mixing, floatation, and aspiration (Sollins et al. 1999). Density fractions were oven-dried (70°C) and weighed. Density fractions and ovendried Oi, Oe+a, and fine Oe+a were analyzed for total C and N with a Leco 2000 CNS analyzer (Leco, St. Joseph, Mich.).

Soil respiration and concentrations of extractable N and enzymes were converted to an ovendried (100°C) basis with results from soil-moisture measurements. Areal masses of C and N in Oi and Oe+a layers were calculated by multiplying C and N concentrations by sample mass and dividing by sample area. Extractable organic N was calculated by subtracting extractable nitrate-N and ammonium-N from total extractable N. The C and N concentrations for the total fine (<2 mm) soil were calculated as mass-weighted values of the light and heavy fractions.

On each plot, 20 individually weighed birch sticks (1.5 × 19.5 × 152 mm, Puritan regular tongue depressors, Hardwood Products Co., Guilford, Maine) were placed at each of three soil depths: on the O layer surface, at interface between O and mineral soil, and within the upper 10 cm of mineral soil. Ten sticks were collected from each depth after 1 year of field incubation, and the other 10, after 2 years. They were brushed free of soil, dried to constant mass at 55°C, and weighed. Percent mass loss was calculated from the difference between initial moisture-corrected mass and final mass.

Statistical analysis

The plot was the experimental unit. The replicate soil or stick measurements for each plot were averaged to yield plot-level values, which were then evaluated statistically. The influence of urea fertilization was tested with a paired *t* test (Zar 1999), where the control and fertilized plots at each site formed the pair. Relations among variables were examined with Pearson correlation (Zar 1999).

Results

The light fraction, as a proportion of the mineral soil, was not affected by urea fertilization (Table 3). There was no observed effect of fertilization on C mass or concentration in the O or mineral soil layers (Table 3, Fig. 1). Because of natural variability and sample size, an actual fertilizer-caused soil C increase of up to ~15% may have gone undetected. This assessment is based on the 95% confidence intervals of the actual fertilizer responses, which as a percentage of the control, were –6 to 19% for Oi C mass, –6 to 34% for Oe+a C mass, –8 to 13% for 0- to 5-cm mineral soil C concentration, and –7 to 16% for 5 to 15-cm mineral soil C concentration. Fertilization resulted in higher N mass in the Oi and Oe+a layers and higher N concentration in fine fractions of both the Oe+a and mineral soil (Table 3, Fig. 2), resulting in decreased C/N ratios (Table 3).

The N concentration in both light and heavy fractions was enhanced by urea fertilization in the 0- to 5-cm mineral soil, but this was less evident in the 5- to 15-cm soil (Table 3). As a result of fertilization, average N concentration was 23% higher in the light fraction of the 0- to 5-cm mineral soil and 21% higher in the heavy fraction. Of the enhanced N in the 0- to 5-cm mineral soil, 38% was in the light fraction. This is somewhat greater than the proportion of N that occurs in the light fraction in the control soil (32%) and indicates a slight relative preference for incorporation of added N into the light fraction compared with the heavy fraction.

Fertilization enhanced all forms of extractable N in the Oe+a, and the fertilizer effect decreased with depth (Table 4). In response to fertilization, extractable organic N averaged 27% higher in the Oe+a, 20% higher in the 0- to 5-cm mineral soil, and 15% higher in the 5- to 15-cm mineral soil. Nitrate was enhanced by a factor of four in Oe+a and a factor of two in the 0- to 5-cm mineral soil but was not detectably affected in the 5- to 15-cm mineral soil. Ammonium doubled in the Oe+a but was not detectably influenced in the mineral soil. The pH declined by an average of 0.2 pH units in all layers in response to fertilization (Table 4).

Soil respiration was decreased by fertilization in the 0- to 5-cm mineral soil, but no effect was observed in the other layers (Table 4). Paralleling the decrease in respiration in the 0- to 5-cm mineral soil was a decrease in cellulase activity (Table 4). In contrast, activities of other enzymes were not influenced nor were labile carbohydrates (Table 4). Fertilization had a small positive effect on the decomposition of the birch sticks in the surface O layer after 1 year, but after 2 years this effect was absent (Table 5). At the interface of the organic and mineral layers, there was no effect of fertilization on wood decomposition after year 1, and by year 2 there was a small positive effect.

Correlation between response to fertilization, as indicated by the difference between fertilized plot and paired control plot, and magnitude of control soil properties was not consistent among the soil layers. A statistically significant correlation of soil C and N responses with soil C mass occurred in the Oe+a layer (Table 6); but removal from the analysis of a single site (No. 43, Skykomish) that had high C mass resulted in a nonsignificant correlation. The responses of extractable N were not significantly correlated with control total soil N. Some responses of extractable N were significantly correlated with control extractable N (Table 7), but removal from the analysis of a single site (No. 17, Little Ohop Creek) from the Oe+a resulted in nonsignificant correlations. In the 0- to 5-cm mineral soil, response of ammonium was negatively correlated with control ammonium, while response of nitrate was positively correlated with control nitrate.

Discussion

The amount of fertilizer N added to the forest stands was more than five times the amount of N added in wet-plus-dry

Table 3. Light-density fraction (<1.65 g·cm^{-3}) of fine (<2 mm) surface (0–5 cm, 5–15 cm) mineral soil, and C and N of O layer and fine, heavy, and light density fractions from paired control and urea-fertilized plots, a decade after last fertilization at second-growth Douglas-fir stands in western Washington and Oregon, U.S.A.

Variable	Layer	Control	Fertilized	Difference*	$P^{\dagger}$
Light fraction	0–5 cm	11.03 (1.37)	11.15 (1.02)	0.13 (0.78)	ns
(% of fine)	5–15 cm	3.93 (0.30)	4.01 (0.37)	0.07 (0.28)	ns
Total C mass (g C·m^{-2})	Oi	705 (29)	752 (38)	47 (41)	ns
	Oe+a	934 (117)	1064 (184)	130 (87)	ns
Fine C concentration	Oe+a	252 (10)	270 (11)	18 (10)	0.10
(g C·kg^{-1} fine)	0–5 cm	74.3 (6.0)	76.4 (5.7)	2.1 (3.6)	ns
	5–15 cm	42.8 (3.1)	44.7 (3.5)	1.9 (2.3)	ns
Light C concentration	0–5 cm	282.2 (6.4)	275.9 (7.9)	–6.3 (9.8)	ns
(g C·kg^{-1} light)	5–15 cm	289.9 (7.5)	295.6 (14.6)	5.8 (11.4)	ns
Heavy C concentration	0–5 cm	50.3 (4.5)	52.5 (4.7)	2.2 (3.5)	ns
(g C·kg^{-1} heavy)	5–15 cm	32.7 (2.6)	34.5 (2.9)	1.9 (1.8)	ns
Total N mass (g N·m^{-2})	Oi	14.11 (0.81)	16.10 (1.11)	1.99 (0.83)	0.03
	Oe+a	25.13 (2.35)	34.03 (4.27)	8.90 (2.70)	0.006
Fine N concentration	Oe+a	8.50 (0.40)	10.94 (0.52)	2.45 (0.35)	<0.0001
(g N·kg^{-1} fine)	0–5 cm	2.48 (0.24)	3.06 (0.29)	0.57 (0.20)	0.01
	5–15 cm	1.57 (0.15)	1.80 (0.17)	0.23 (0.10)	0.04
Light N concentration	0–5 cm	7.30 (0.37)	8.99 (0.57)	1.69 (0.46)	0.003
(g N·kg^{-1} light)	5–15 cm	6.75 (0.45)	7.40 (0.30)	0.65 (0.33)	0.07
Heavy N concentration	0–5 cm	1.93 (0.21)	2.34 (0.26)	0.41 (0.18)	0.04
(g N·kg^{-1} heavy)	5–15 cm	1.36 (0.14)	1.55 (0.17)	0.20 (0.10)	0.06
Total C/N	Oi	52.11 (3.12)	48.58 (2.84)	–3.53 (1.99)	0.10
	Oe+a	36.88 (1.64)	30.98 (1.60)	–5.90 (1.21)	0.0004
Fine C/N	Oe+a	30.14 (1.09)	25.10 (1.01)	–5.04 (0.66)	<0.0001
	0–5 cm	30.87 (1.24)	26.14 (1.33)	–4.73 (0.84)	0.0001
	5–15 cm	28.40 (1.36)	26.10 (1.38)	–2.30 (0.61)	0.003
Light C/N	0–5 cm	39.72 (1.55)	32.28 (1.92)	–7.45 (1.26)	0.0003
	5–15 cm	44.77 (2.10)	40.28 (1.56)	–4.50 (2.10)	0.05
Heavy C/N	0–5 cm	27.42 (1.46)	23.73 (1.30)	–3.69 (0.51)	<0.0001
	5–15 cm	25.43 (1.40)	23.52 (1.36)	–1.91 (0.46)	0.001

Note: Values are means with SE given in parentheses.
*Difference = fertilized plot – paired control plot.
†Fertilized treatment versus control was evaluated with paired *t* test of 13 pairs of plots. ns, not significant ($P > 0.10$).

atmospheric deposition over the 26-year period from initiation of fertilization to soil sampling. The atmospheric deposition in a western Washington forest close to fertilizer sites was ~0.5 g N·m^{-2}·year^{-1} (Johnson and Lindberg 1992), yielding ~13 g N·m^{-2} over the 26-year period. This contrasts with the 89–112 g N·m^{-2} added in fertilizer. Effects of this fertilization on a number of soil properties were evident a decade after application, while other soil properties were unaffected.

The lack of fertilizer influence on light fraction in our study indicates that both its production and depletion either increased or decreased or that neither was influenced substantially. The origin of light fraction in forest mineral soils is indicated by its composition of dead root fragments, hyphae, charcoal, pumice, and adsorbed or entrapped colloidal particles (Spycher et al. 1983). Fertilization can decrease fine root growth in forests (Haynes and Gower 1995) and, thus, reduce the production of light fraction. This might be offset by a decrease in decomposition rates resulting from fertilization (Fog 1988), thereby reducing the depletion of light fraction. Fragments of aboveground forest litter mixed into mineral soil by animals might also contribute to the light fraction. Reduction in aboveground forest litter decreased light fraction in oak forest (Boone 1994).

Long-term agricultural studies have found fertilization to either increase light fraction or have no influence (Bremer et al. 1994; Gregorich et al. 1996, 1997; Fließbach and Mäder 2000). Other management practices have much larger effects on the light fraction. Cultivation decreased light fraction by 80% (Gregorich et al. 1996, 1997). This reduced light fraction level may continue for decades following reversion of cultivated fields to forest (Compton and Boone 2000).

The lack of an influence of fertilization on soil C mass or concentration (Table 3, Fig. 1) is consistent with several agricultural studies. Nitrogen fertilization for 12–36 years increased 0- to 15-cm mineral soil C at only two of three Iowa Mollisol sites (Robinson et al. 1996). In a 35-year study in New Brunswick, N fertilization of timothy did not consistently enhance surface soil C concentration (Bélanger et al. 1999). The lack of response of soil C to N fertilization has also been observed in Saskatchewan (Campbell et al. 1991). These observations contrast with C increases resulting from N addition in a variety of forest and agricultural ecosystems (Van Cleve and Moore 1978; Baker et al. 1986; Nohrstedt et

Fig. 1. Carbon mass in O layers and C concentration of fine (<2 mm) 0- to 15-cm mineral soil from paired control and urea-fertilized plots in 13 second-growth Douglas-fir stands in western Washington and Oregon, 9 years or more after last fertilizer application. The *P* values are for fertilized treatment versus control based on paired *t* tests of 13 pairs of plots.

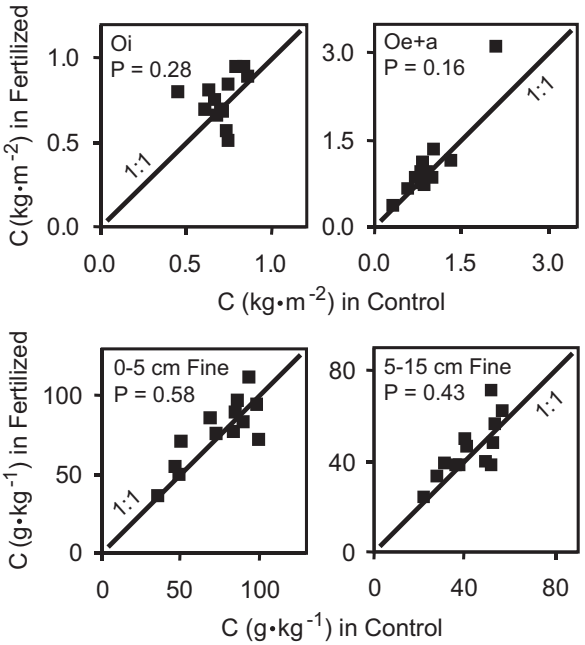
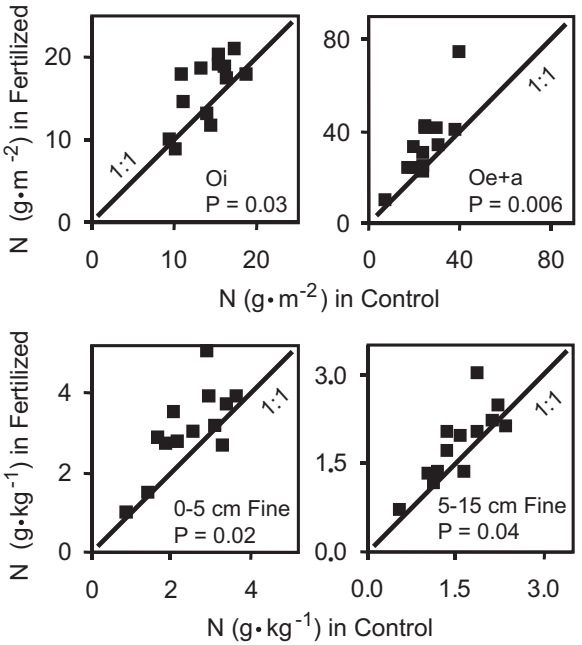


Fig. 2. Nitrogen mass in O layers and N concentration of fine (<2 mm) 0- to 15-cm mineral soil from paired control and urea-fertilized plots in 13 second-growth Douglas-fir stands in western Washington and Oregon, 9 years or more after last fertilizer application. The *P* values are for fertilized treatment versus control based on paired *t* tests of 13 pairs of plots.



al. 1989). In a dryland no-till cropping system, ammonium nitrate fertilization for 11 years increased detrital inputs and soil C concentrations in the 0- to 7.5-cm layer (Halvorson et al. 1999). Urea plus phosphate application for 11 years yielded higher C concentration in 0- to 15-cm soil (Goyal et al. 1999).

An actual soil C increase resulting from fertilization of up to ~15% may have gone undetected in our study. Such an increase would be equivalent to ~1000 g C·m^{-2} for the O layers plus 0- to 15-cm mineral soil, based on C values from this study (Table 3) and bulk densities and coarse fragments from previous assessments (Edmonds and Chappell 1994; Stand Management Cooperative, University of Washington, unpublished data). Trees in western Oregon and Washington show ~20% increased volume growth to the fertilizer regime examined in this study (Stegemoeller and Chappell 1990). At three of the sites examined in our study, this increased growth was equivalent to ~2700 g C·m^{-2} (Canary et al. 2000). Comparison of this actual tree response with the possible, but undetected, soil response indicates vegetation will be much more important than soil in enhancing ecosystem C sequestration in these forests.

In spite of the limited or no effects on C pools, decomposition processes were affected by the fertilization (Tables 4 and 5). Enhanced wood decomposition resulting from fertilization (Table 5) is consistent with substantial N additions nearly doubling the mass loss from decomposing Douglas-fir litter in a laboratory study (Homann and Cole 1990) and with fertilization enhancing forest litter decomposition in the field (Prescott et al. 1992). Other field studies have shown litter decomposition to decrease (Titus and Malcolm 1987) or be unaffected by fertilization (Prescott et al. 1993), root decomposition to be unaffected by fertilization in forest soils (King et al. 1997), and wood decomposition to be unaffected by nutrient enhancement in streams (Wold and Hershey 1999). The contrasting results may be due to the extent to which decomposition had proceeded in the various studies. Although higher N availability may stimulate mass loss during the early stages of decomposition, it may have an opposite effect during the later stages of decomposition when cellulose has been degraded and lignin remains (Berg and Matzner 1997). Repression of lignin-degrading enzyme synthesis and formation of structurally more complex, and hence recalcitrant, compounds are possible mechanisms by which N suppresses lignin degradation (Fog 1988).

Enhanced wood decomposition resulting from fertilization (Table 5) contrasts with the suppression of soil respiration (Table 4). The wood substrate was the same for both control and fertilized plots. The more rapid decomposition and higher extractable N (Table 4) as a result of fertilization are consistent with the wood decomposition being limited by low availability of N. The residual N from fertilization partially offset the N limitation and resulted in enhanced wood decomposition. The substrate for soil respiration, however, likely differed between the control and fertilized plots. Incorporation of added N into the soil organic matter during years of detrital production and decomposition may have made the organic matter more recalcitrant with respect to microbial breakdown (Fog 1988). In addition, the parallel decreases of respiration and cellulase activity in the 0- to 5-cm mineral soil (Table 4) are consistent with a mechanism of enzyme control on availability of respirable substances.

Table 4. Extractable N, pH, enzyme activities, labile carbohydrates, and soil respiration of fine (<2 mm) Oe+a and mineral soil from paired control and urea-fertilized plots, a decade after last fertilization at second-growth Douglas-fir stands in western Washington and Oregon, U.S.A.

Variable	Layer	Control	Fertilized	Difference*	$P^{\dagger}$
Extractable organic N (mg N·kg^{-1} fine)	Oe+a	48.14 (5.69)	61.46 (7.13)	13.32 (5.91)	0.04
	0–5 cm	16.05 (1.63)	19.26 (2.16)	3.21 (1.09)	0.01
	5–15 cm	11.98 (1.25)	13.82 (1.30)	1.83 (0.45)	0.001
Ammonium (mg N·kg^{-1} fine)	Oe+a	6.18 (2.39)	13.83 (5.04)	7.65 (2.82)	0.02
	0–5 cm	1.02 (0.35)	1.20 (0.23)	0.18 (0.34)	ns
	5–15 cm	0.53 (0.12)	0.74 (0.13)	0.22 (0.14)	ns
Nitrate (mg N·kg^{-1} fine)	Oe+a	3.15 (1.20)	12.63 (4.79)	9.48 (4.21)	0.04
	0–5 cm	1.73 (0.80)	5.10 (1.96)	3.37 (1.36)	0.03
	5–15 cm	1.10 (0.44)	2.09 (0.70)	0.99 (0.57)	ns
Nitrate-N/(Nitrate-N + Ammonium-N) (%)	Oe+a	35.8 (5.5)	43.1 (8.1)	7.2 (6.9)	ns
	0–5 cm	41.6 (7.6)	53.2 (8.8)	11.6 (9.0)	ns
	5–15 cm	54.8 (5.2)	58.7 (7.9)	3.9 (5.4)	ns
pH	Oe+a	4.97 (0.14)	4.83 (0.14)	–0.14 (0.06)	0.03
	0–5 cm	5.28 (0.09)	5.07 (0.09)	–0.21 (0.05)	0.002
	5–15 cm	5.36 (0.06)	5.15 (0.06)	–0.21 (0.04)	0.0001
Respiration (mg CO_2-C·kg^{-1}·14 days^{-1})	Oe+a	494 (23)	429 (36)	–64.3 (40.3)	ns
	0–5 cm	200 (15)	173 (10)	–27.2 (12.2)	0.05
	5–15 cm	71.2 (6.1)	64.8 (3.3)	–6.4 (4.7)	ns
Carbohydrate (g glucose·kg^{-1})	Oe+a	92.2 (8.3)	89.5 (5.9)	–2.7 (5.5)	ns
	0–5 cm	31.0 (3.0)	28.5 (2.6)	–2.5 (1.4)	0.10
	5–15 cm	23.7 (1.9)	23.0 (2.0)	–0.7 (1.4)	ns
Cellulase	Oe+a	73.4 (7.2)	65.4 (6.6)	–8.0 (6.3)	ns
(mmol glucose equivalent·kg^{-1}·day^{-1})	0–5 cm	19.6 (2.8)	14.9 (2.3)	–4.7 (1.9)	0.03
	5–15 cm	4.3 (0.7)	4.1 (0.7)	–0.2 (0.4)	ns
Xylanase	Oe+a	121.3 (12.0)	110.9 (10.6)	–10.4 (6.3)	ns
(mmol glucose equivalent·kg^{-1}·day^{-1})	0–5 cm	32.1 (4.3)	31.0 (4.3)	–1.1 (2.8)	ns
	5–15 cm	9.1 (1.4)	9.8 (1.6)	0.7 (1.4)	ns
Phosphatase (mmol *p*-nitrophenol·kg^{-1}·h^{-1})	Oe+a	45.0 (4.3)	48.1 (3.6)	3.9 (4.0)	ns
	0–5 cm	12.4 (1.7)	12.9 (1.2)	0.5 (0.9)	ns
	5–15 cm	7.2 (1.0)	7.2 (0.8)	0.0 (0.5)	ns

Note: Values are means with SEs in parentheses.
*Difference = fertilized plot – paired control plot.
†Fertilized treatment vs. control was evaluated with paired *t* test of 13 pairs of plots. ns, not significant ($P > 0.10$).

Table 5. Percent mass lost from birch sticks incubated in the field for 1 or 2 years in paired control and urea-fertilized plots, a decade following termination of fertilization at second-growth Douglas-fir stands in western Washington and Oregon, U.S.A.

Duration (years)	Location	Mass loss (%) Control	Fertilized	Difference*	$P^{\dagger}$
1	O surface	10.5 (1.3)	13.4 (1.6)	2.9 (1.2)	0.03
	O – mineral soil interface	31.5 (4.4)	32.7 (4.2)	1.3 (2.7)	ns
	Upper mineral soil	25.2 (4.2)	28.4 (4.0)	3.3 (3.2)	ns
2	O surface	34.9 (4.8)	38.9 (4.5)	3.9 (2.7)	ns
	O – mineral soil interface	56.6 (4.9)	62.8 (5.0)	6.2 (2.4)	0.03
	Upper mineral soil	52.7 (5.8)	56.5 (5.1)	3.8 (3.2)	ns

Note: Values are means with SEs in parentheses.
*Difference = fertilized plot – paired control plot.
†Fertilized treatment versus control was evaluated with paired *t* test of 12 pairs of plots for 1 year duration and 10 pairs of plots for 2 year duration. Other pairs of plots were deleted from the study because of harvesting. ns, not significant ($P > 0.10$).

Different decomposer populations in response to different chemical environments (Fog 1988) also provide a possible explanation. Over shorter periods, the mechanisms of the fertilizer effect may be different. For example, in a short-term, laboratory evaluation of the influence of urea on lodgepole pine forest floor, microbial respiration was ini-

Table 6. Pearson's correlation coefficients for total soil C and N masses and concentrations, a decade after last fertilization at second-growth Douglas-fir stands in western Washington and Oregon, U.S.A.

				Pearson's correlation coefficient			
Soil layer	Variable	Minimum	Maximum	C difference	N difference	Control C mass or concentration	Control N mass or concentration
Oi	C difference (kg C·m^{-2})†	–0.24	0.34				
	N difference (g N·m^{-2})	2.7	6.9	0.73**			
	Control C mass (kg C·m^{-2})	0.45	0.95	–0.46	–0.14		
	Control N mass (g N·m^{-2})	8.8	21.0	0.03	–0.07	0.33	
	Control C/N	32.7	74.6	–0.44	–0.19	0.34	–0.75**
Oe+a	C difference (kg C·m^{-2})	–0.19	1.02				
	N difference (g N·m^{-2})	–1.3	34.6	0.93***			
	Control C mass (kg C·m^{-2})	0.31	3.11	0.62*	0.64*		
	Control N mass (g N·m^{-2})	7.6	74.5	0.35	0.43	0.90***	
	Control C/N	24.9	53.1	0.65*	0.56*	0.58*	0.17
0–5 cm	C difference (g C·kg^{-1})	–29	19				
	N difference (g N·kg^{-1})	–0.68	2.11	0.83***			
	Control C concentration (g C·kg^{-1})	35	111	–0.37	–0.03		
	Control N concentration (g N·kg^{-1})	0.9	5.1	–0.40	–0.12	0.91***	
	Control C/N	20	40	0.14	0.07	–0.33	–0.65*
5–15 cm	C difference (g C·kg^{-1})	–14	18				
	N difference (g N·kg^{-1})	–0.29	1.14	0.92***			
	Control C concentration (g C·kg^{-1})	22	71	–0.18	–0.10		
	Control N concentration (g N·kg^{-1})	0.5	3.0	–0.26	–0.06	0.91***	
	Control C/N	18	40	0.11	–0.14	–0.48	–0.75**

Note: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
†Difference = fertilized plot – paired control plot.

tially enhanced but was subsequently suppressed following urea application, a temporal pattern that could be due to early exhaustion of easily utilizable C compounds (Thirukkumaran and Parkinson 2000).

The enhanced soil N on fertilized plots was equivalent to an average of 40% of the added N, based on N values from this study (Table 3) and bulk density and coarse fragment values from previous assessments (Edmonds and Chappell 1994; Stand Management Cooperative, University of Washington, unpublished data). This is the same mean percent recovery as occurred in a variety of forests subjected to fertilizer applications of ≥40 g N·m^{-2} (summarized from Johnson 1992*b*). Although there was a slight relative preference for incorporation of added N into the light fraction compared with the heavy fraction, the consequence for future N dynamics is unclear. Limited involvement of the light fraction in soil N dynamics in some forest soils is indicated by its contributing only 2% to whole-soil N mineralization potential in a maple soil and 13% in a pine soil (Boone 1994). In contrast, the positive correlation between light fraction N mass and N mineralization in agricultural soils (Barrios et al. 1996; Curtin and Wen 1999) may indicate greater importance of the light fraction in those systems. The decreased C/N ratios resulting from fertilization (Table 3) may alter both soil C and N dynamics. In 300-day incubations of previously N fertilized and unfertilized soils, N fertilized soils had lower C/N ratios and lower C mineralization rates in both the heavy and light fractions as well as the whole soil (Swanston 2000). In anaerobic (Sollins et al. 1984) and aerobic incubations (Swanston 2000), N mineralization was positively correlated with C/N of the heavy fraction but negatively correlated with C/N of the light fraction. Thus, the ultimate effect of different whole-soil C/N ratios on N availability depended on the relative amounts of the light and heavy fractions.

The enhanced extractable organic N, ammonium, and nitrate a decade after fertilization with urea (Table 4) is consistent with enhanced N availability in the mineral soil of a Douglas-fir stand in southern Washington 22 years after fertilization with ammonium nitrate (Binkley and Reid 1985; Strader and Binkley 1989). In contrast to these results, no effect was observed on extractable ammonium and nitrate in a Scots pine forest 7 years after terminating ammonium nitrate applications that totaled 216 g N·m^{-2} (Quist et al. 1999), and no effect was observed on net N mineralization in second-growth Douglas-fir on Vancouver Island, Canada, 15 years after fertilization with either urea or ammonium nitrate (Strader and Binkley 1989).

Urea has substantial effects on soil properties other than N. Immediately after application, the hydrolysis of urea and protonation of ammonia consume protons, which lead to a substantial increase in soil pH and mobilization of organics (Homann and Grigal 1992). Dissolved P concentrations also increase following hydrolysis of urea (Shand et al. 2000). Douglas-fir growth response to urea is positively correlated to total mineral soil P along the Washington coast (Edmonds and Hsiang 1987), suggesting possible growth limitation resulting from low P availability as well as low N availability in that area. Under those conditions, enhanced growth from urea application might be from greater P availability through solubilization of P, as well as enhanced N availability. Both the increased pH and the increased dissolved P, however, are

Table 7. Pearson's correlation coefficients for extractable soil N concentrations, a decade following termination of fertilization at second-growth Douglas-fir stands in western Washington and Oregon, U.S.A.

				Pearson's correlation coefficient				
Soil layer	Variable	Minimum	Maximum	Organic N difference	Ammonium difference	Nitrate difference	Control organic N	Control ammonium
Oe+a	Organic N difference (mg N·kg^{-1})†	–6.8	72.0					
	Ammonium difference (mg N·kg^{-1})	–0.2	35.6	–0.12				
	Nitrate difference (mg N·kg^{-1})	–3.2	42.0	0.34	0.03			
	Control organic N (mg N·kg^{-1})	22.1	98.1	–0.24	0.23	–0.27		
	Control ammonium (mg N·kg^{-1})	0.1	32.5	–0.01	0.87***	0.42	–0.11	
	Control nitrate (mg N·kg^{-1})	0.1	15.1	–0.01	0.62*	0.37	–0.28	0.86***
0–5 cm	Organic N difference (mg N·kg^{-1})	0.1	14.5					
	Ammonium difference (mg N·kg^{-1})	–2.7	2.5	0.31				
	Nitrate difference (mg N·kg^{-1})	–0.1	15.8	–0.15	–0.48			
	Control organic N (mg N·kg^{-1})	4.5	26.6	0.23	0.42	–0.32		
	Control ammonium (mg N·kg^{-1})	<0.1	3.7	–0.41	–0.77**	0.52	–0.06	
	Control nitrate (mg N·kg^{-1})	<0.1	8.7	–0.24	–0.44	0.62*	–0.07	0.72**
5–15 cm	Organic N difference (mg N·kg^{-1})	–1.7	4.3					
	Ammonium difference (mg N·kg^{-1})	–0.7	1.0	0.13				
	Nitrate difference (mg N·kg^{-1})	–1.4	5.7	–0.34	–0.21			
	Control organic N (mg N·kg^{-1})	3.1	19.6	–0.06	0.66*	–0.23		
	Control ammonium (mg N·kg^{-1})	<0.1	1.2	0.08	–0.53	0.19	–0.27	
	Control nitrate (mg N·kg^{-1})	<0.1	4.7	0.01	–0.63*	–0.04	–0.31	0.68*

Note: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
†Difference = fertilized plot – paired control plot.

transient responses that return to near control levels within several months (Homann and Grigal 1992; Shand et al. 2000).

The long-term decline in pH as a result of urea fertilization (Table 4) is opposite to the initial increase (Homann and Grigal 1992). This may be caused by nitrification, which produces protons and can decrease soil pH (Van Miegroet and Cole 1984). The combined processes of urea hydrolysis and nitrification yield a decrease in pH from the net production of protons:

$$(NH_2)_2CO + 2\,H^+ + H_2O == 2\,NH_4^+ + CO_2$$

$$2NH_4^+ + 4\,O_2 == 2\,NO_3^- + 4\,H^+ + 2\,H_2O$$

$$(NH_2)_2CO + 4\,O_2 == 2\,NO_3^- + 2H^+ + H_2O + CO_2$$

This provides a viable explanation of the pH decrease. However, other processes could have contributed to net production of protons, including enhanced uptake of the ammonium in N-deficient stands and enhanced uptake of cationic nutrients (Binkley and Richter 1987) in response to increased tree growth (Chappell et al. 1999; Edmonds and Hsiang 1987).

Several studies have proposed the concept that the magnitude of the response of a soil property to fertilization may be related to the magnitude of the soil property itself. For example, little or no C response might occur on high-C soils (Bélanger et al. 1999), and response of N cycling and availability to urea fertilization might be greater and (or) last longer on sites with higher initial N capital or cycling (Miller 1981; Prescott et al. 1993). In the O layer of this study, the

few significant correlations between responses and magnitudes of soil properties were attributed to single sites, the elimination of which led to nonsignificant correlations and lack of support for this concept. In stands with fertilizer treatments similar to those of this study, O layer turnover rates and O layer N mineralization did not support the concept either (Chappell et al. 1999). In the 0- to 5-cm mineral soil, however, correlations provided some support (Table 7). Higher ammonium response resulting from fertilization was associated with lower control ammonium, while higher nitrate response was associated with higher control nitrate. These relations are consistent with nitrification being restricted on sites that have natural low ammonium availability.

Conclusions

This study demonstrated the complexity of soil responses to previous ecosystem manipulation. A decade after termination of urea fertilization, residual soil responses were observed in some, but not all, chemical properties and microbial indicators. Responses differed both in their magnitude and their location within the soil profile. Fertilization increased total and extractable N in both the O layer and the 0- to 15-cm mineral soil, which may continue to enhance tree productivity. The magnitude of total N response was not related to total N, total C, or C/N, while the magnitude of extractable N response was related to extractable N concentration in the mineral soil. Fertilization decreased mineral soil pH by an average of 0.2 pH units, which may be due to nitrification or enhanced uptake of cations. Decreased soil respiration as a result of fertilization was consistent with decreased cellulase activity but could also be from formation of recalcitrant organic matter. There were no effects detected on light and heavy density fractions as proportions of the mineral soil, labile carbohydrates, C masses in O layers, or C concentrations in mineral soil, although increases in soil C of up to ~15% of control values could have occurred and gone undetected. These results indicate urea fertilization of Pacific Northwest forests would not likely increase soil C storage substantially on a regional basis. In contrast to specific site assessments, this regional approach allows generalization over a broad area, which is important for gauging long-term effects on forest productivity and soil C sequestration in the context of regional and global change.

Acknowledgements

This material is based upon work supported by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture under Agreement No. 95-37107-1642. Additional support was provided by the Stand Management Cooperative, University of Washington; the Long-Term Ecosystem Productivity Program of the USDA Forest Service, Pacific Northwest Research Station, Corvallis, Oregon; and the Bureau for Faculty Research, Western Washington University. We thank Bob Gonyea and Bert Hasselberg for field help and Carol Glassman, Noah Fierer, Adrien Elseroad, and Ed Curtis for laboratory assistance.

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