DIVISION S-7—FOREST & RANGE SOILS

Changes in Microbial Nitrogen Across a 100-Year Chronosequence of Upland Hardwood Forests

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ABSTRACT

Soil microorganisms mediate many of the major processes involved in soil N cycling. Also, they are strong competitors with plants for available soil N. Thus, changes in microbial N because of forest harvesting may have significant impacts on N availability and overall forest N cycling. A chronosequence of upland hardwood forest stands in southern Indiana, USA, ranging in age from 1 to 100 yr since last harvest, was chosen to evaluate changes in microbial N with stand development. Microbial N was measured in the A and B soil horizons during different seasons from 1997 to 1999. Peak levels of microbial N were highest in the youngest forest stand (1 to 3 yr old), but seasonal variability was greater than differences by stand age. Microbial N concentration (mg kg^{-1}) varied significantly by season and soil horizon within stands ranging in age from 1 to 30 yr since harvest, but not in the mature, 80- to 100-yr-old stand. There were few significant differences in microbial N content (kg ha^{-1}). Harvesting did not appear to have long-term effects on microbial N, but spatial variability in microbial N appeared to be greater in the younger forest stands.

Timber harvesting is a major disturbance in forest ecosystems that may impact microbial biomass and nutrient content. Immobilization of N in microbial biomass can reduce N losses from the forest ecosystem (Vitousek and Matson, 1984); whereas, increases in microbially mediated nitrification and denitrification can exacerbate ecosystem N losses (Holmes and Zak, 1999). Short-term changes in microbial N content are to be expected, as there can be significant changes in forest floor mass (Mroz et al., 1985), litter inputs and litter quality (Gholz et al., 1985a; Hinesley et al., 1991; Kim et al., 1996), soil water availability (Jordan et al., 1999), soil temperature (Mattson and Swank, 1989), soil pH (Morris and Boerner, 1998), plant N uptake (Gholz et al., 1985b), fine-root activity (Idol et al., 2000), and fluctuations in these conditions after a harvest. Because microbes mediate many soil N cycling processes, such as N mineralization, nitrification, and denitrification, and are strong competitors with plants for inorganic soil N, it is important to understand the effects of forest harvesting on microbial N.

Several studies have assessed the effects of forest harvesting on soil microbial biomass. Hendrickson et al. (1985) found increases in bacterial populations after conventional harvest in a mixed conifer and hardwood forest, but Houston et al. (1998) found no change in microbial biomass after harvesting in a similar forest type. In a chronosequence study of a northern hardwood forest, Taylor et al. (1999) found higher microbial biomass in early (<20 yr) and late (>120 yr) successional stands than in midsuccessional stands (20 to 80 yr). The effects of harvesting on other measures of microbial biomass and activity, such as soil enzyme activity (Bolton et al., 1993; Eivazi and Bayan, 1996), soil and microbial respiration (Hendrickson et al., 1985; Chang and Trofymow, 1996; Houston et al., 1998), and the shifts in microbial populations among fungi and bacteria (Pietikäinen and Fritze, 1995; Houston et al., 1998), have also been of interest. Changes because of harvesting in the relative size of the microbial N pool, however, have not been as thoroughly studied.

Individually, measurements of microbial biomass or activity can yield inferences concerning the role of the microbial community in forest nutrient cycling and, thus, the effects of harvesting on nutrient dynamics. Microbial biomass can serve as an estimate of microbial N immobilization; however, the microbial C/N ratio can vary from 15:1 in fungi to 4.5:1 in bacteria (Paul and Clark, 1996). Therefore, microbial biomass alone is not an accurate indicator of the microbial-N pool size. Soil enzyme activity and microbial respiration can give an indication of the rate and nature of organic matter decay and thus of potential rates of N mineralization and immobilization. The interpretation of this type of data, however, is still uncertain with respect to actual rates of nutrient cycling. Thus, nutrient cycling studies that seek to evaluate the contribution of the soil microbial community need to include estimations of microbial nutrient pool size as well as microbial nutrient immobilization and mineralization.

Although microbial biomass and activity generally decline with soil depth (Bolton et al., 1993; Winkler et al., 1996), few studies have investigated the effects of harvesting or disturbance on patterns of microbial biomass, nutrient content, or activity by soil depth or horizon. Because harvesting can change forest floor cover, incident solar radiation (Yin et al., 1991), and rates of fine-root growth and turnover by soil horizon (Yin et al., 1989; Idol et al., 2000), patterns of microbial-N content and turnover by soil horizon may differ with forest stand age. Because soil horizons can differ with respect to ion-exchange capacity and redox status, shifts in microbial-N content and turnover may impact N leaching and denitrification. Also, because plant rooting density and N uptake by soil horizon may change during forest

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Table 1. Selected soil properties across a 100-yr chronosequence of upland and hardwood forests.

Sand age (Year harvested)	Soil series	Horizon	Depth sampled	Bulk density	pH	C	N	S	P
			mm	Mg m^{-3}		g kg^{-1}			mg kg^{-1}
1–3 (1996)	Gilpin/Wellston	A	0–80	1.35	5.39	33.8	4.74	0.448	71.6
		B	80–300	1.74	4.53	7.56	1.38	0.381	31.3
6–8 (1991)	Gilpin	A	0–80	1.20	5.41	35.1	4.63	0.308	91.6
		B	80–300	1.32	4.88	6.10	1.26	0.000	55.5
12–14 (1985)	Gilpin	A	0–80	1.05	5.50	31.2	4.43	0.240	37.4
		B	80–300	1.35	4.81	5.84	1.01	0.052	8.93
31–33 (1966)	Gilpin/Wellston	A	0–80	0.95	4.59	31.3	4.68	0.581	50.0
		B	80–300	1.10	4.71	5.42	1.14	0.093	16.8
80–(around 1910)	Wellston	A	0–80	1.02	4.59	27.4	4.12	0.387	122
		B	8-–300	1.20	4.49	6.75	1.41	0.216	68.9

regeneration, changes in microbial N by horizon could impact N bioavailability and plant-N uptake.

The objective of the present study was to investigate the influence of forest stand age on annual and seasonal microbial N by soil horizon. First, since forest harvesting leads to increased soil temperature and water availability, it was hypothesized that microbial N would be highest in recently harvested stands. Second, because of the soil disturbance generally associated with standard harvesting techniques, it was hypothesized that seasonal variability in microbial N would be greater in younger forest stands. Finally, it was hypothesized that microbial-N concentration in the A horizon would consistently be greater than in the B horizon because of the higher organic matter content, N availability, and biological activity in the A horizon.

MATERIALS AND METHODS

Study Site Descriptions

A chronosequence of upland, temperate, deciduous forest stands within the Central Hardwoods Region, ranging in age from 1 to 100 yr, was chosen for this study. All stands were within the Southern Indiana Purdue Agricultural Center in Dubois County, IN (38.4° N lat., 86.7° W long., elevation approximately 200 m). The soils are of the Gilpin-Wellston complex, a mixture of fine-silty, mixed, mesic Ultic Hapludalfs and Typic Hapludults (Soil Survey Staff, 1980). Small differences in soil base saturation and depth to the C horizon (broken sandstone) are the major features distinguishing the two soil series, with the Wellston silt loam being generally a deeper soil with greater base saturation. General soil properties for the study sites are given in Table 1. Overstory plant species composition is a mixture of temperate deciduous species, typically dominated by oak (*Quercus* L.) and hickory (*Carya* Nutt.) at maturity. Site 1 was clearcut-harvested in 1996. Site 2 was clearcut-harvested in 1991. Site 3 was clearcut-harvested in 1985. Site 4 was clearcut-harvested in 1966. Site 5 was a mature, 80- to 100-yr-old forest stand that represented preharvest site conditions. A more detailed vegetation inventory is given Table 2. Litter pools and litter inputs are given in Table 3. Forest biomass removal during harvesting of Site 1 is listed in Table 4. Further descriptions of much of this data can be found in Idol et al. (1998, 2000, 2001).

Because of the desire to maintain consistency among sites with respect to climate, soils, landform and aspect, vegetation,

Table 2. Plant species composition across a 100-yr chronosequence of upland hardwood forests.†

Stand age	Seedlings			Saplings			Overstory		
(year harvested)	ha^{-1}	Major species	Percent stems	ha^{-1}	Major species	Percent stems	ha^{-1}	Major species	Percent stems (m^2 ha^{-1})
1–3‡ (1996)	–			3 780	*Acer saccharum*	63	384	*Quercus alba*	18 (15.9)
					Nyssa sylvatica	15		*Quercus rubra*	10 (12.6)
								Acer saccharum	32 (5.6)
								Carya glabra	10 (4.7)
6–8 (1991)	79 000	*Smilax* spp.	14	95 000	*Liriodendron tulipifera*	30	–		
		Rubus spp.	13						
		Parthenocissus quinquefolia	13		*Quercus alba*	20			
					Acer saccharum	12			
12–14 (1985)	107 000	*Parthenocissus quinquefolia*	22	16 600	*Asimina triloba*	23	1 340	*Prunus serotina*	16
					Sassafras albidum	19		*Quercus rubra*	15
		Rubus spp.	16		*Cercis canadensis*	12		*Cercis canadensis*	13
		Smilax spp.	14					*Rhus copallina*	12
		Rhus radicans	12					*Acer saccharum*	12
31–33 (1966)	146 000	*Rhus radicans*	40	1 950	*Acer saccharum*	38	1 950	*Acer saccharum*	36
		Prunus serotina	25		*Asimina triloba*	28		*Prunus serotina*	21
								Sassafras albidum	10
80–100 (around 1910)	18 300	*Parthenocissus quinquefolia*	26	770	*Acer saccharum*	96	445	*Quercus alba*	23 (19.0)
		Acer rubrum	24					*Acer saccharum*	57 (3.9)
		Rhus radicans	10						
		Prunus serotina	10						

† For all sites, only those species which represent 10% or greater of the stem counts were used.
‡ Represents preharvest vegetation inventory.

Table 3. Litter pools and litter inputs across a 100-yr chronosequence of upland hardwood forests.

Stand age	Down dead wood	Forest floor (Oi+Oe)	Fine roots (0–30 cm)	Litterfall	Fine root mortality
	kg ha^{-1}				
1–8	156	54	44	50–70	55
10–14	nd†	69	26	27	69
30–35	119	98	18	38	62
80–100	156	139	15.8	52	60

† No data.

Table 4. Stem volume removed during harvesting from Site 1, an upland hardwood forest.

Species	Volume removed
	m^3 ha^{-1}
Quercus alba	26.8
Q. rubra	22.6
Q. velutina	2.7
Carya spp.	6.1
Fraxinus americana	1.8
Other	5.8
Total	65.8

and management history, only one forest stand per age class was chosen for the study. Plots were chosen based on surveys of geographic location, vegetation, soil, slope, aspect, and management history. Three 0.5-ha plots were established in Sites 1, 2, 3, and 5. Only two plots with similar physiography and management history could be located within Site 4. All plots were located on 10 to 20% sideslopes with south- and southwest-facing aspects. Plots within sites were generally within 5 to 20 m of each other. Sites 1, 2, and 3 were within 30 to 50 m of each other. Sites 4 and 5 were adjacent to one another and were within 1 km of Sites 1, 2, and 3.

Soil Microbial Biomass Nitrogen

In September and December 1997, April, August, and December 1998, and April and September 1999, three 30-cm deep bulk soil samples were collected from each plot and separated into A and B horizon samples (0–8 cm for the A horizon and 8–30 cm for the B horizon). Samples were sealed in polyethylene bags and transported back to the lab within 12 h where they were kept at 4°C until analysis (average time <1 mo; maximum time approximately 3 mo).

Microbial N was determined using the chloroform fumigation-extraction technique of Brookes et al. (1985), as modified by Mueller et al. (1992) to remove live roots. Approximately 60 g of field-moist soil from each sample was placed into a 250-mL bottle and shaken for 1h in a 100-mL solution of 0.05 mol L^{-1} K_2SO_4 to remove soluble organic C. The samples were filtered through a 2-mm sieve to remove roots and particulate organic matter. The filtered samples were centrifuged to separate the soil and extract, and the supernatant was discarded. Each soil sample was then divided, half to be used as a control and half to be fumigated. Control samples were weighed into fresh 250-mL bottles, and approximately a 10-g (dry soil weight) subsample was taken to determine water content. The subsample was oven-dried at 105°C until a constant weight was obtained (24–48 h). Control samples were extracted in a 100-mL solution of 0.5 mol L^{-1} K_2SO_4 for 1 h, centrifuged to separate the soil and extract, and the supernatant was collected and kept frozen for analysis. The fumigation samples were placed into 50-mL beakers inside a vacuum dessicator with a vial of ethanol-free chloroform. A vacuum was applied until the chloroform boiled for 2 min., then the dessicator was sealed and left in the dark for 5 d. After the fumigation period, the dessicator was evacuated and the fumigation samples were extracted in a 0.5 mol L^{-1} K_2SO_4 solution, as previously described.

The supernatant extracts were analyzed for total N content using a modification of the micro-Kjeldahl method (Nelson and Sommers, 1973). Fifteen milliliters of extract was digested in 10 mL of 95% H_2SO_4 (w/v) and a catalyst composed of 100:10:1 mixture of K_2SO_4/$CuSO_4$/Se in 50-mL Folin-Wu test tubes. This mixture was digested at 280°C (±20°C) until all of the organic compounds were decomposed (6–8 h). The solution was brought to a 50-mL volume with deionized water. A 15-mL subsample was steam-distilled in a strong base (10 mol L^{-1} NaOH), the distillate collected in a boric acid mixed indicator solution, and the solution back-titrated (Keeney and Nelson, 1982).

The fumigation-extractable N minus the control-extractable N was taken as the measure of microbial N. A correction factor has been employed in various studies to account for the incomplete extraction of microbial N, but the value of this factor often varies by method of analysis (Joergensen, 1996), biomass calculation (Voroney and Paul, 1984; Smith et al., 1994), or comparison standard (Ross and Sparling, 1993). A correction factor was not used in this study because the objective was to compare changes in microbial N by site, soil horizon, and season, not to exactly quantify the microbial-N pool size. Microbial N was calculated based on concentration (mg kg^{-1}) and content (kg ha^{-1}). Individual soil horizon bulk densities and depths were used to convert concentration to content. Both types of calculations were considered important because comparison of microbial-C or N pool size among forest stands with different horizon depths or bulk densities requires that data be reported on a per area or volume basis, for example, kilograms per hectare or grams per cubic meters.

Statistics

Because of the unique nature of the chronosequence in this study, there is no true replication of stand age. Although multiple plots were sampled in each age class, they were not randomly distributed across the sites. Thus, the use of inferential statistics to compare stand ages is subject to the error of pseudoreplication (Hurlbert, 1984). Only trends in the data by stand age can be discussed.

Repeated measures analyses were used to compare microbial N over time and between the two soil horizons within each stand age (SAS Institute Inc., 1989). After analysis of variance (ANOVA) to test the significance of the main effects, regression analysis was used to partition the variability by treatments into single degree of freedom orthogonal polynomial contrasts (Montgomery, 1991). Significance of the main effects (time and horizon) was tested by the plot × effect error terms. Differences were considered significant at $p < 0.05$. Adjusted limits of error were calculated (Andrews et al., 1980) and used as confidence intervals around the mean values.

RESULTS

Soil microbial N concentration (mg kg^{-1}) varied significantly by time of sampling and horizon depth for Sites 1 through 4, the stands ranging from 1 to 30 yr since last harvest (Fig. 1A). Although variability by soil horizon was significant for Site 5, the 80- to 100-yr-old stand (Table 5), the overall ANOVA model, including time, horizon, and their interaction, was not significant ($P = 0.19$). The adjusted limits of error (confidence limits) about the mean values of microbial N were

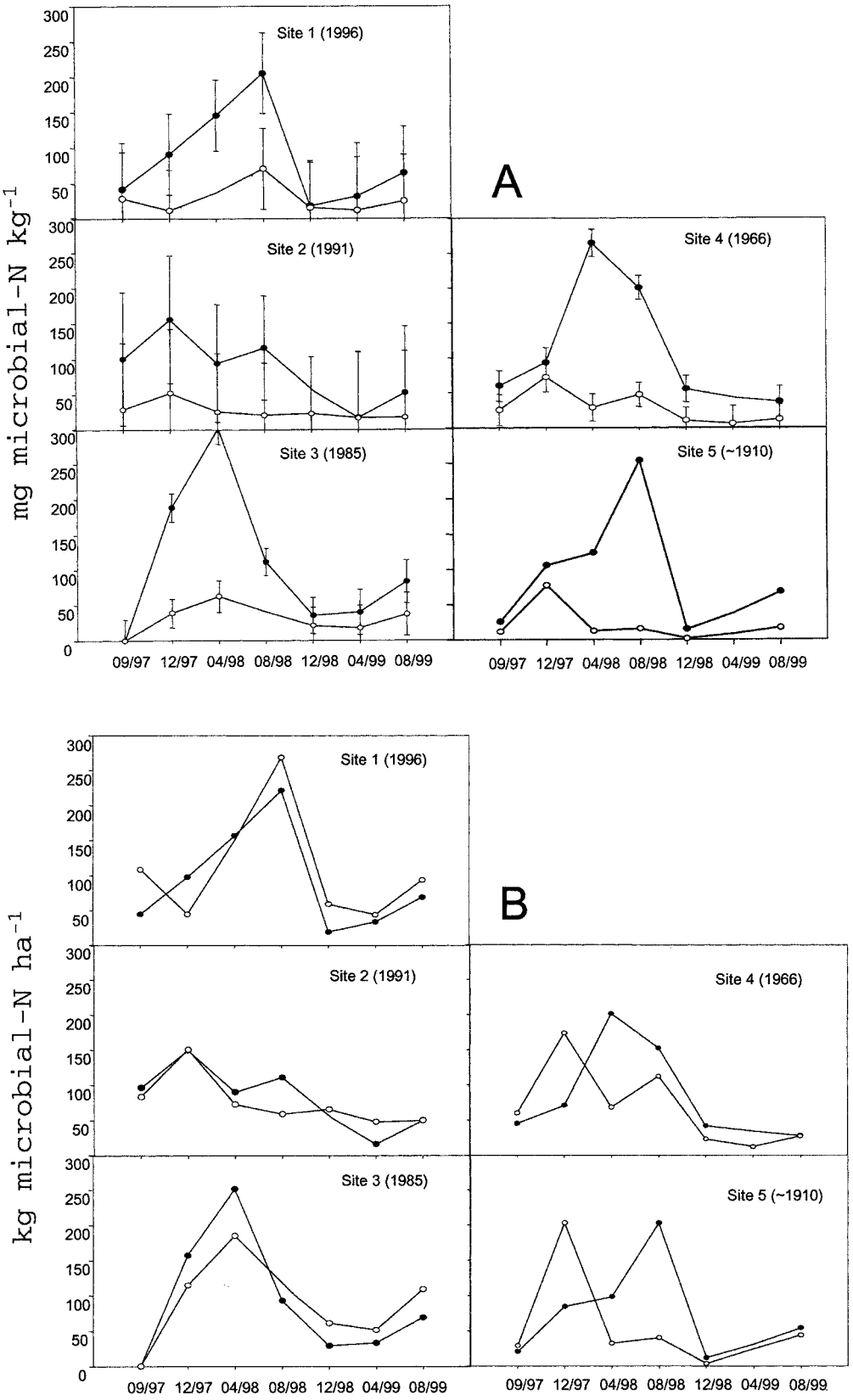

Fig. 1. Soil Microbial N Across a 100-yr chronosequence of Upland Hardwood Forests. A, microbial-N concentration (mg kg^{-1}); B, microbial-N content (kg ha^{-1}). Closed circles, A horizon (0–8 cm); open circles, B horizon (8–30 cm). Error bars represent the adjusted limit of error (confidence limit). Where error bars are not included, no significant differences were found.

Table 5. Repeated measures analysis of variance within site (year harvested) for the effect of time and soil horizon on soil microbial N by concentration (mg kg^{-1}) and content (kg ha^{-1}).

		mg kg^{-1}		kg ha^{-1}	
Source	**df**	***F*-value**	***P* > *F***	***F*-value**	***P* > *F***
		Site 1 (1996)			
Plot	**2**	**1.63**	**ns†**	**1.57**	**ns**
Time	**5**	**6.86**	*	**4.90**	*
linear	**1**	**0.02**	**ns**	**0.04**	**ns**
quadratic	**1**	**7.48**	*	**4.36**	**ns**
cubic	**1**	**3.79**	**ns**	**0.21**	**ns**
quartic	**1**	**16.90**	**	**13.40**	**
quintic	**1**	**5.07**	**ns**	**5.68**	*
Horizon	**1**	**30.20**	**	**<0.01**	**ns**
Time × Horizon	**5**	**3.75**	*	**1.40**	**ns**
linear	**1**	**0.43**	**ns**		
quadratic	**1**	**4.46**			
cubic	**1**	**10.46**	*		
quartic	**1**	**5.30**	*		
quintic	**1**	**0.39**	**ns**		
		Site 2 (1991)			
Plot	**2**	**0.93**	**ns**	**1.11**	**ns**
Time	**6**	**10.27**	**	**3.75**	*
linear	**1**	**60.39**	**	**8.25**	*
quadratic	**1**	**0.60**	**ns**	**0.15**	**ns**
cubic	**1**	**25.70**	**	**3.45**	**ns**
quartic	**1**	**2.72**	**ns**	**0.06**	**ns**
quintic	**1**	**3.41**	**ns**	**0.13**	**ns**
sextic	**1**	**8.84**	*	**2.36**	**ns**
Horizon	**1**	**75.96**	*	**0.24**	**ns**
Time × Horizon	**6**	**9.66**	**	**1.07**	**ns**
linear	**1**	**2.70**	**ns**		
quadratic	**1**	**1.31**	**ns**		
cubic	**1**	**2.99**	**ns**		
quartic	**1**	**5.20**	*		
quintic	**1**	**3.85**	**ns**		
sextic	**1**	**21.46**	**		
		Site 3 (1985)			
Plot	**2**	**0.99**	**ns**	**1.08**	**ns**
Time	**5**	**8.63**	*	**2.24**	**ns**
linear	**1**	**1.10**	**ns**		
quadratic	**1**	**3.73**	**ns**		
cubic	**1**	**35.12**	**		
quartic	**1**	**0.10**	**ns**		
quintic	**1**	**3.12**	**ns**		
Horizon	**1**	**59.82**	*	**0.47**	**ns**
Time × Horizon	**5**	**6.41**	**	**0.33**	**ns**
linear	**1**	**2.13**	**ns**		
quadratic	**1**	**3.78**	**ns**		
cubic	**1**	**25.26**	**		
quartic	**1**	**0.10**	**ns**		
quintic	**1**	**0.79**	**ns**		
		Site 4 (1966)			
Plot	**1**	**1.58**	**ns**	**0.72**	**ns**
Time	**6**	**6.60**	*	**3.36**	**ns**
linear	**1**	**9.62**	*		
quadratic	**1**	**8.15**	*		
cubic	**1**	**16.95**	**		
quartic	**1**	**1.66**	**ns**		
quintic	**1**	**2.89**	**ns**		
sextic	**1**	**0.30**	**ns**		
Horizon	**1**	**22.87**	*	**0.99**	**ns**
Time × Horizon	**6**	**21.74**	**	**4.87**	*
linear	**1**	**9.64**	*	**0.39**	**ns**
quadratic	**1**	**29.94**	**	**4.17**	**ns**
cubic	**1**	**40.35**	**	**2.22**	**ns**
quartic	**1**	**11.86**	*	**3.40**	**ns**
quintic	**1**	**35.48**	**	**14.01**	**
sextic	**1**	**3.18**	**ns**	**5.23**	**ns**
		Site 5 (~1910)			
Plot	**2**	**0.70**	**ns**	**2.29**	**ns**
Time	**5**	**2.03**	**ns**	**0.91**	**ns**
Horizon	**1**	**8.52**	*	**0.40**	**ns**
Time × Horizon	**5**	**1.87**	**ns**	**3.14**	**ns**

*** Significant at the 0.05 probability level.**
**** Significant at the 0.01 probability level.**
† Not significant.

greater for Sites 1 and 2, the stands harvested in 1996 and 1991, than in Sites 3 and 4, harvested in 1985 and 1966. This may indicate a larger degree of spatial variation in microbial N in recently harvested stands. Thus, despite overall higher mean values of microbial N in the A compared with the B horizon, there were few time periods during which these differences were significant for Sites 1 and 2.

There were few significant differences in microbial-N content (kg ha^{-1}) in any of the sites (Table 5). Seasonal patterns of microbial N in the A and B horizons within Sites 1 through 3 were similar, although patterns across sites differed (Fig. 1B). Simultaneous peaks in microbial N in both horizons in Sites 1 and 3 led to very high microbial N during periods of 1998. In Site 1, peak microbial N across both horizons was 490 kg ha^{-1}; in Site 2, peak microbial N was 437 kg ha^{-1}. In Sites 2, 4, and 5, microbial N never peaked above 300 kg ha^{-1}.

Although seasonal variability in microbial-N content was not significant in most stands, it did range widely, even from one measurement period to the next. Across both horizons, the difference between maximum and minimum microbial N ranged from 387 kg ha^{-1} in Site 1 to 119 kg ha^{-1} in Site 2. Within horizons, differences in maximum and minimum microbial N were at least 100 kg ha^{-1} for all sites.

DISCUSSION

Estimates of microbial N found in the present study are within a range of biomass N contents seen in studies of other temperate forest ecosystems, including European common beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L. Karst.) (Martikainen and Palojärvi, 1990), Scots pine (*Pinus sylvestris* L.) and European common alder (*Alnus glutinosa* L. Gaertn.) (Joergensen and Scheu, 1999), and New Zealand southern beech (*Nothofagus* spp.) (Ross and Tate, 1993; Sparling et al., 1994). Because no correction factor was used in the present study, however, these values are not directly comparable.

A basic hypothesis of this study was that microbial N would be highest in recently harvested stands and decline back to preharvest levels with increasing stand age. Results gave only cursory support to this hypothesis. Although the highest level of microbial-N content occurred in Site 1 (Fig. 1B), fluctuations by time of sampling and soil horizon were larger than any apparent differences by stand age.

This lack of a clear difference by site is perhaps due to the fact that, in general, soil conditions after harvesting were favorable for microbial activity in all the forest stands (Table 2). Soil pH, C, N, S, and P content in Sites 1 through 4 were generally similar. Litterfall and fine-root mortality were also high in the younger stands (Table 3). Only the higher bulk density in Site 1 could be considered unfavorable for microbial activity (Table 2). Thus, the degree of soil disturbance associated with harvesting was probably not sufficient to negatively affect microbial N in these forest stands.

A second hypothesis was that seasonal variability in

microbial N would be higher in younger forest stands because of soil disturbance associated with forest harvesting. Because microbial-N content generally did not differ significantly within any of the sites, assessments of spatial and seasonal variability can only be made on microbial-N concentration (mg kg^{-1}). Results by concentration actually contradicted this hypothesis, as seasonal variation was higher in Sites 3 and 4, stands harvested in 1985 and 1996 than in Sites 1 and 2, harvested in 1996 and 1991 (Fig. 1A). Although seasonal variation was not significant in Site 5, the mature forest stand, variation in mean values was greater than in Sites 1 and 2.

Finally, it was hypothesized that microbial-N concentration in the A horizon would consistently be greater than in the B horizon. Higher organic matter concentration, greater fine-root activity, and the closer proximity to the forest floor all suggest that the activity and concentration of microbes would be greater in the A horizon. Results generally supported this hypothesis, as A horizon microbial-N concentration was significantly greater than in the B horizon for all sites (Table 5). Because of large seasonal fluctuations, however, differences were significant for at most three of the seven time periods studied within any one stand (Fig. 1A). This was mostly due to a significant decrease in A horizon microbial-N concentration rather than a significant increase in B horizon microbial N. The magnitude and significance of this decline, as mentioned previously, was generally greater in the older forest stands. Thus, although the A horizon is generally a more favorable environment for microbial activity, microbes in this horizon may be more exposed to changing environmental conditions (e.g., soil temperature and water), subject to greater competition for N with plant roots, and subject to greater predation by soil protists, nematodes, and microarthropods.

Although not statistically significant, the large declines in A horizon microbial-N content between time periods are not easily accounted for in this system. Measurements of net N mineralization, changes in soil available N, and inorganic N leaching can account for perhaps half of these declines (Idol, unpublished data, 2000). It is unlikely that denitrification or leaching of organic N could account for a large proportion of the remainder. Much of the missing microbial N may simply be residing in the labile soil organic-N pool, available for mineralization or reimmobilization when conditions become more favorable for microbial activity.

Even though this study was not designed to specifically assess spatial variability in microbial N, the disturbance associated with forest harvesting may be expected to lead to greater spatial variation in many soil and site characteristics. For Sites 1 and 2, both <10-yr postharvest during this study, the variation in microbial N not accounted for by time of sampling or soil horizon was much greater than in Sites 3 and 4, 12- to 33-yr postharvest. This is evident in the larger adjusted limits of error surrounding the mean values in Fig. 1A. Previous research in these stands has shown similar trends for soil temperature, fine-root growth and turnover, and forest floor mass, including down dead wood (Idol et al., 2000, 2001).

As stands recover from disturbance, the spatial heterogeneity of soil properties and processes likely declines because of the insulating cover of the forest floor, extensive colonization by plant root systems, and the general dominance of the growing overstory plant community. Although the specific cause for this higher degree of variability in microbial N is unknown, it may be related to variation in forest floor cover, soil temperature, root activity, litter substrate availability and quality, and general soil disturbance, for example, compaction, mineral soil exposure, mechanical turbation, incorporation of litter, etc. Thus, assessments of the effects of forest harvesting on microbial N and nutrient cycling at the individual stand or site level might yield better information if studies focused on the extent and causes of spatial variation rather than on potential changes in mean values.

CONCLUSIONS

Our first hypothesis predicted that microbial N would be greatest in recently harvested stands but similar at most stand ages. Although peak values of microbial N were highest in the youngest stand, seasonal variations were much greater than differences by stand age. Our second hypothesis predicted that seasonal fluctuations in microbial N would be greater in the younger stands of the chronosequence, especially in the A horizon. This hypothesis was rejected as evidence suggested that seasonal differences in microbial-N concentration were actually greater in older forest stands. It was further hypothesized that microbial N in the A horizon would be consistently greater than in the B horizon. Although results supported this hypothesis for microbial-N concentration averaged over the 3-yr study period, differences between horizons within specific time periods were often not significant. Finally, these results suggest that the spatial variability of microbial N may be influenced by forest harvesting more than mean values averaged across stands or sites. Thus, future studies should focus on the causes and extent of this variability and how it is associated with forest harvesting or stand age.

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