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Biological activity and amount of FDA mycelium in mor humus of Scots pine stands (*Pinus sylvestris* L.) in relation to soil properties and degree of pollution

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Abstract. The biological activity and the amount of living fungal mycelium in the mor humus of pine forests around an industrialized city were studied. The activities were lower in the more polluted zone than in a cleaner one but varied between sites within the zones. The relationship of these activities to the microbial environment was determined in both the total data and in the various zones separately. Soil respiration rate was positively related to ammonium nitrogen concentration of the humus in the less polluted zone but negatively in the more polluted zone, while it related negatively to total nitrogen concentration of the humus in the entire data set. DHA was partly accounted for by the variation in acidity parameters, and best by pH($CaCl_2$), with a positive relation. The length of FDA active fungal mycelium showed no significant variation between the zones or sites, and was thus poorly explained by the environmental variables. The weather conditions prevailing at two seasons did not explain any variation of the activities or the length of FDA mycelium, though the biological variables were in general positively related to the moisture of the humus.

Introduction

The potential contribution of an oversupply of nitrogen, in addition to sulphur, to forest damage has been widely discussed in recent years. This is obviously connected with the nutrient imbalance in forest ecosystems arising from nitrogen deposition (Prinz 1987). It is impossible to point to any single agent causing forest damage in the Nordic countries, but nitrogen is one possible stress factor, especially in naturally nitrogen-poor northern forest ecosystems (McLaughlin 1985). The deposition of ammonium and nitrate may cause forest damage in several ways (Nihlgård 1985), for example over-saturation of the ecosystem by nitrogen may cause needle yellowing and reduced vitality in Scots pine (van Dijk & Roelofs 1988).

Soil processes are significant components of the ecosystem and necessary for nutrient cycling. Even small changes in these may cause nutrient accumulation or loss, both leading to an imbalance. Changes in soil processes have been found both after fertilization with nitrogen and due to nitrogen depositon. A surplus of nitrogen compounds has been found to reduce the soil respiration rate and fungal and bacterial biomasses (Bååth et al. 1981; Söderström et al. 1983; Nohrstedt et al. 1989), and nitrogen deposition is connected with changes in the mycorrhizal status of Scots pine (Meyer 1988; Ohtonen et al. 1990) and in the mycoflora, especially a decrease in the number of carpophores of mycorrhizal fungi (Termorshuizen & Schaffers 1987; Ohtonen et al. 1990).

The aim here is to study the biological activity and amount of FDA active fungal mycelium in the mor humus of pine forest sites. The biological analyses are related principally to total and mineral nitrogen and the C/N ratio of the samples. Other chemical data include the acidity parameters, pH(H_2O), pH($CaCl_2$) and exchangeable acidity. The variation is examined in two zones around Oulu, an industrialized city in Finland, distinguished by differences in pollution level and distance from the main sources of emissions. Since moisture and temperature are known to be the most important factors controlling soil respiration (Carlyle & Than 1988), and since there is also strong correlation between soil moisture and FDA active fungal length (Bååth & Söderström 1982), the variation of the biology of the humus is related to the weather conditions during the two growing seasons.

In a preceding paper (Ohtonen et al. 1990) the analyses of N, S and heavy metals, determined from part of this same material, are related to the respiration, DHA and length of the FDA mycelium at the mean level in the autumn in order to use these biological variables as indicators of environmental changes. The accumulation of S in the humus layer in the Oulu area has been discussed earlier (Ohtonen et al. 1989).

Materials and methods

Site description

The area is located around the industrialized city of Oulu (65°N, 25°30′E). Annual SO_4-S deposition in the city centre was 0.9 – 5.6 g m^{-2} in 1986 – 88 (Anon 1989). Forest damage in the area has been studied intensively since the 1970's, and needle damage caused by fertilizer dust, sulphur, chlorine and fluorine deposition has been described (Huttunen 1975, Huttunen & Laine 1983, Huttunen et al. 1985).

The area studied belongs to the middle boreal vegetation zone (Ahti et

al. 1968) and is located in Northern Ostrobothnia, near the coast of the Bothnian Bay (Fig. 1). The mean temperature of the coldest month (February) averaged from 1931 to 1960 is −9.9 °C and that of the warmest (July) 16.6 °C. Mean annual precipitation is 468 mm (Anon. 1988).

Twenty forest sites were selected for investigation, following an inventory of about 60 mature Scots pine (*Pinus sylvestris* L.) forest sites in terms of their vegetation, stand properties and pH of the mor humus layer performed during the period 23.4.–30.5.1987. Attention was particularly paid to the S content of the pine needles (Karhu 1986), which reflect the humus sulphur content (Ohtonen et al. 1989), as an index of the pollution level of the sites. For the preliminary pH measurements, 5–10 humus

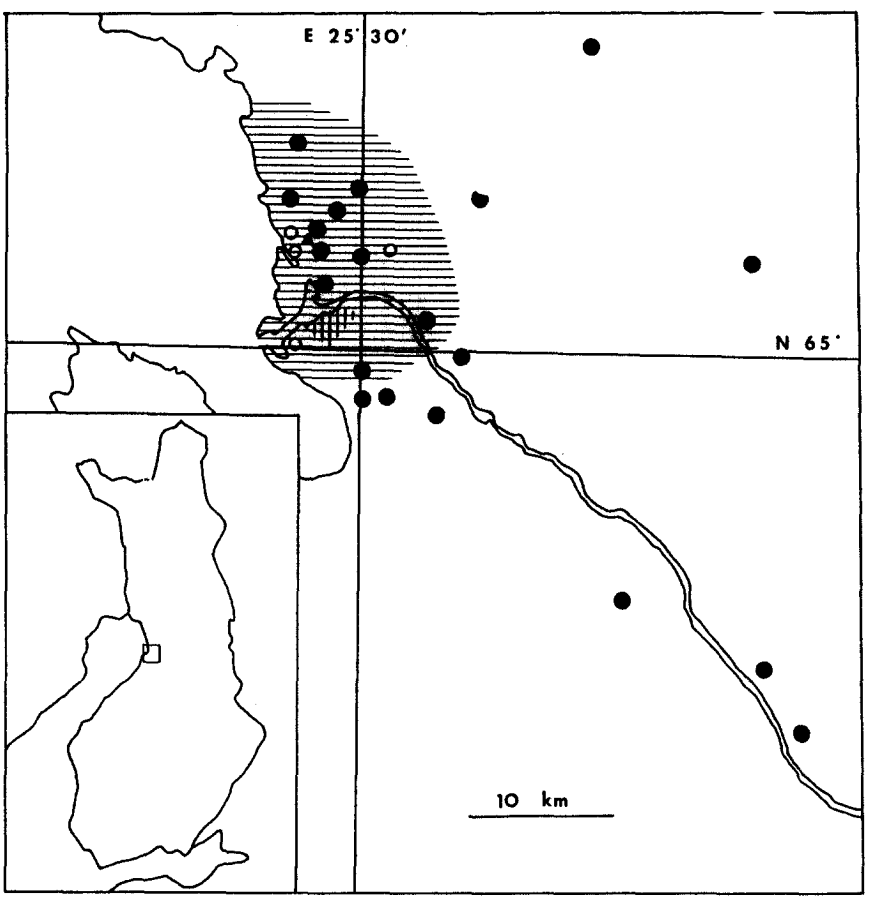

Fig. 1. Location of the sites in Oulu. The sites are indicated by black circles, the main emission sources by open circles, and the meteorological station of the University of Oulu by a black triangle. The hatched area is the more polluted zone and the rest of the area the cleaner zone. The crossed area is the city centre.

cores 30 mm in diameter from each forest site were mixed and homogenized. Roots over 1 mm in diameter, sand and litter were removed. The pH value, measured in a soil-water suspension (1:2 vv), ranged from 3.64 to 5.25. The pH of the sites included ranged from 3.79 to 4.28. These sites had *Vaccinium vitis-idaea, Empetrum nigrum coll.* and *V. myrtillus* in the field layer, and *Pleurozium schreberi, Dicranum* species and *Hylocomium splendens* in the bottom layer. The vegetation was not ideally uniform at all sites, however, especially because those near the city centre were variably influenced by mechanical disturbance, etc., by man, and were quite lush possibly at least partly due to the fertilizer effect of nitrogen deposition. On the other hand, the choice was limited because wide areas around the city are former fields or mires, and the age distribution of forest sites around the city is quite variable. The sites included ranged from about 40 to 100 years in age. Ten of the sites situated at the outer, cleaner zone and ten at the central, more polluted zone (Fig. 1).

Sample preparation

The samples were taken on 1.—3. and 15.—16. June, 3.—4. August, 7.—15. September, 12.—19. October 1987, and 18.—25. April, 15.—23. May, 6.—16. June, 31. July—2. August and 12.—15. September 1988. Not all the sites were sampled in April 1988 as the humus layer was still frozen at most of them. The humus samples of the total humus layer, varying from 2.3 to 8.9 cm in thickness (Table 4), were taken randomly with a stainless steel coring tool 30 mm in diameter, yielding a total of 200—300 g of fresh material at each site. The cores, 15 to 35 per site, were pooled to a bulk sample and homogenized over an ice bath by hand, removing the litter and roots over 1 mm in diameter. One part of each homogenized sample was oven dried, one part deep-frozen and the rest kept in 4 °C for biological analyses, which were performed within 4 days.

Analyses of biological activity

DHA (activity of dehydrogenase enzymes) was measured using a modification of the method of Thalmann (1968). 2.5 g fresh weight (fw) of humus was incubated for 24 hours at 30 °C in 5 ml of 0.5 M Tris-buffer, pH 8.0, containing 1% of TTC (2,3,5-triphenyltetrazoliumchloride, Merck). The resulting TPF (triphenyltetrazoliumformazan) was extracted with 25 ml of absolute ethanol for 2 hours, filtered through MN 649 md and extinction measured at 480 nm using samples of the same kind but without TTC as blanks. A standard curve was prepared for iodonitrotetrazoliumformazan (Sigma). DHA was calculated as μmol TPF g^{-1}dw 24 h^{-1} and μmol TPF g^{-1}OM (organic matter at 550 °C) 24 h^{-1}. Soil respira-

tion rate was measured as duplicates by the alkali absorption method at 12 °C (Coleman 1973) and calculated as μg CO_2 g^{-1}dw h^{-1} and μg CO_2 g^{-1}OM h^{-1}. The activities measured at different points in the season do not represent the current activities, because the temperature used in the analyses was always constant. Thus the analyses describe potential, rather than *in situ* activity, and may reflect more closely the microbial biomasses than the *in situ* activities.

Length of FDA active fungal mycelium

The length of living fungal mycelium present was measured by the FDA method (Söderström 1977) from 3.0 g of fresh humus in triplicate or quadruplicate at five cleaner and five more polluted sites. The samples were homogenized in 60 mM potassium phosphate buffer, pH 7.5, for 1 minute with an Omni Mixer at a speed rating of 10. The fluorescein hyphae were studied microscopically using a Leitz Laborlux D microscope with an AG filter system and NPL fluotar 50 lens and the length of them calculated by the intersection method (Olson 1950) as m g^{-1}dw and m g^{-1}OM.

Other analyses

The pH value was determined in both a soil-water extract and a soil-0.01 M $CaCl_2$ extract (1:2 by volume) with a glass electrode after shaking for 1 hour and allowing to stand for 30 min. Soil moisture was determined gravimetrically after drying at 105 °C for at least 24 hours, and organic matter (OM) by loss on ignition (LOI) at 550 °C (Christensen & Malmros 1982) for 2 hours. Total nitrogen was determined by the micro-Kjeldahl method using tube digestion (Kubin 1978). Carbon content was measured on two or three samples per site by the dry combustion method with an automated C analyzer (Carlo Erba 1106 CHN-analyzer in the University of Joensuu) running five or six replicates per sample, and the conversion factors for each site were calculated from the average C content and average LOI (Christensen & Malmros 1982) of the samples investigated. The C/N ratio was calculated from the conversion factor and the actual LOI and N of the samples. Ammonium and nitrate nitrogen were measured colorimetrically in a 1 M KCl extract (about 1:10 by volume) and exchangeable acidity by a titrimetric method (Black et al. 1965).

Weather data

Soil temperature was measured four times a day beginning from the lst June in 1987 to 31st October in 1988 in a depth of 10 cm in soil and in

the shrub layer of a pine forest at the meteorological station of the University of Oulu (Fig. 1) and averaged for each day (Fig. 2). Rainfall per day was measured in the open field at the same meteorological station (Fig. 2). Mean temperatures and rainfall in the 5- and 10-day periods preceding each sampling were calculated for statistical purposes.

Statistical analyses

Descriptive statistics (mean ± standard error) and Pearson coefficients of

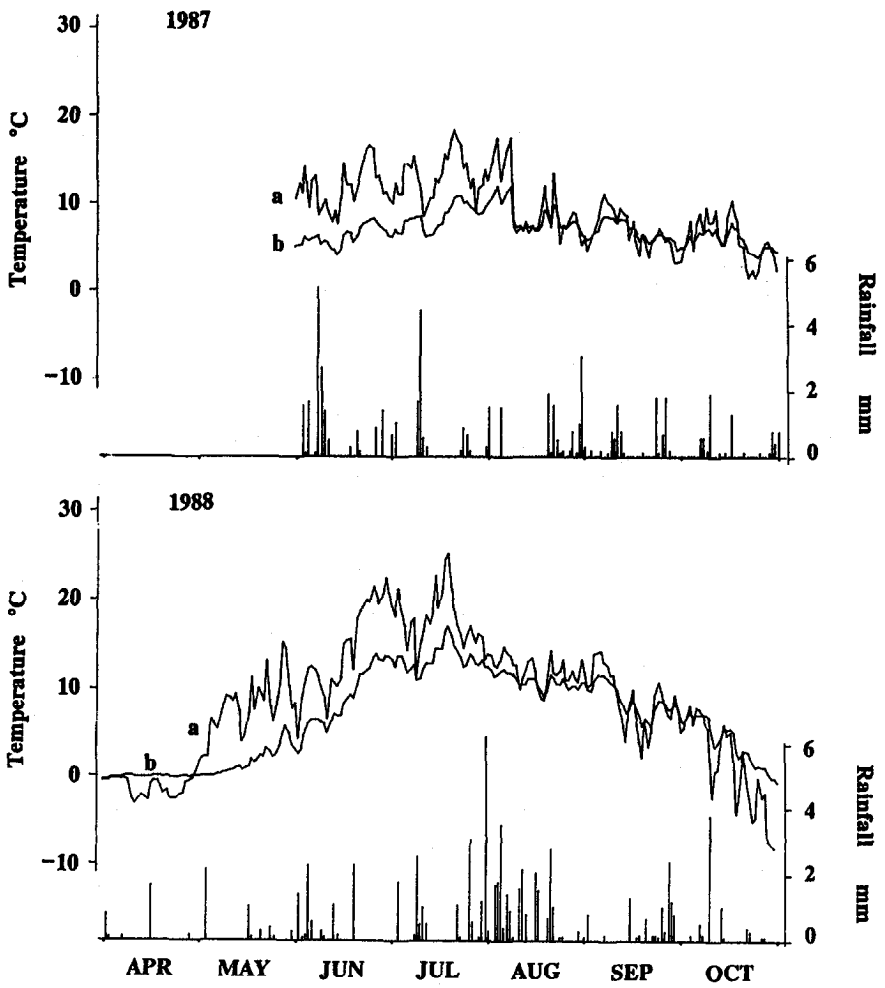

Fig. 2. Daily mean temperature of the shrub layer (*a*) and of the soil at a depth of 10 cm (*b*) in a Scots pine forest and rainfall in an open field (bars), measured at the meteorological station of the University of Oulu (see Fig. 1) from the 1st June to the 30th October in 1987 and from the 1st April to the 30th October in 1988.

linear correlation were used to describe the data. Main effects of zone, site and year and their interactions on soil biology were tested using ANOVA. To test the effect of the pollution zone on soil biology, site nested within zone was used as the total error term. Principal component analysis (PCA) and multiple regression models were employed to search the structure of the data and effects of chemical variables on biological parameters. All statistics was made by the SAS statistical package. Logarithmic values to the base 10 were used to normalize distributions, when necessary.

Results

Variation in the biological activity and the length of FDA mycelium of the mor humus

The DHA of the samples varied from 0.68 to 3.54 averaging 1.88 ± 0.04 μmol TPF g^{-1}OM 24 h^{-1}. The average DHA levels for the sites varied from 1.28 ± 0.11 to 2.56 ± 0.13 μmol TPF g^{-1}OM 24 h^{-1} (Table 1). The sources of this variation were compared by means of an analysis of variance using zone, site and year as the classification variables. The high values measured in April were excluded from this because analyses had not been performed at all the sites. Both ways of expressing the results, per dry weight (dw) or per OM, were tested (Table 2). 52 or 56% of the variation in DHA were explained by the model respectively. The zone vaiable had the most pronounced effect, but significant differences also existed between sites within zones (Table 2). The significance and coefficient of determination (R^2) of the model were independent of the basis used for the calculation, but when calculated on an OM basis the effect of the year on the variation in DHA became insignificant, and thus the differences between the years on a dw basis may be attributed solely to the varying amount of mineral soil in the humus samples.

The respiration rate of the samples varied from 26.2 to 194, averaging 61.1 ± 2.1 μg CO_2 g^{-1}OM h^{-1}. The average respiration rate for the sites varied from 36.2 ± 1.8 to 90.7 ± 4.0 μg CO_2 g^{-1}OM h^{-1} (Table 1). Similar to DHA, an analysis of variance using zone, site and year as the classification variables in respiration, showed 66% and 67% of this variation respectively to be explained by the model. The zone parameter had the most significant effect, although again significant differences existed between sites within zones (Table 2). As with DHA, the significance and R^2 of the model were independent of the basis used for the calculation. On OM basis the effect of the year became insignificant probably for the same reason as in the case of DHA.

Table 1. Biological activity measured in terms of DHA (dehydrogenase activity) and soil respiration rate, and the length of the FDA active mycelium in the mor humus of ten Scots pine forest sites in the cleaner zone and that of ten sites in the more polluted zone in Oulu, averages for 1987 and 1988.

	DHA μmol TPF g^{-1}OM $24\ h^{-1}$	Respiration $\mu g\ CO_2\ g^{-1}$OM h^{-1}	Length of FDA mycelium m g^{-1}OM
Cleaner zone	2.56 ± 0.13	90.7 ± 4.0	1130 ± 240
	2.22 ± 0.14	78.9 ± 13.8	1220 ± 380
	1.96 ± 0.18	64.0 ± 11.5	1180 ± 440
	2.23 ± 0.17	64.7 ± 4.9	790 ± 190
	2.17 ± 0.19	90.5 ± 10.3	1380 ± 380
	1.89 ± 0.18	53.7 ± 3.0	not determined
	2.53 ± 0.20	79.6 ± 4.8	"
	2.00 ± 0.15	74.1 ± 5.9	"
	1.64 ± 0.15	61.2 ± 4.3	"
	1.55 ± 0.21	63.0 ± 3.6	"
$\bar{x}$	2.08 ± 0.06	72.4 ± 2.8	1140 ± 150
More polluted zone	1.58 ± 0.12	48.2 ± 5.4	770 ± 320
	1.90 ± 0.13	78.7 ± 10.1	2020 ± 560
	2.30 ± 0.17	63.1 ± 6.0	1530 ± 420
	1.90 ± 0.20	54.8 ± 12.5	1070 ± 260
	1.56 ± 0.13	36.2 ± 1.8	880 ± 110
	1.28 ± 0.11	41.4 ± 4.7	not determined
	1.62 ± 0.12	45.5 ± 2.8	"
	1.87 ± 0.17	38.2 ± 4.6	"
	1.40 ± 0.10	42.7 ± 3.4	"
	1.36 ± 0.12	38.6 ± 3.3	"
$\bar{x}$	1.68 ± 0.05	48.8 ± 2.5	1210 ± 180

Mean ± standard error (SE). The number of analyses is from 8 to 10 at each site.

The length of FDA mycelium in the samples varied from 140 to 4320, averaging 1180 ± 110 m g^{-1}OM, and the average length of FDA mycelium at the sites varied from 770 ± 320 to 2020 ± 560 m g^{-1}OM (Table 1). About 70% of the variation in FDA mycelium was explained by the model based on zone, site and year, but only year had a significant effect on calculations based on both dw and OM (Table 2).

The activity parameters behaved in a similar manner with respect to the sources of their variation. Both parameters were reduced towards the city

Table 2. Main effects of pollution zone, site and year on DHA, soil respiration rate and the length of FDA mycelium on both a dry weight (dw) and organic matter (OM) basis by ANOVA.

	DHA					
Base of calculation	dw			OM		
Source of variation	df	MS	F	df	MS	F
Year	1	1.681	18.97 ***	1	0.000	0.00 ns
Zone	1	3.188	8.52 **	1	6.859	7.50 *
Site within Zone	18	0.374	4.23 ***	18	0.915	4.39 ***
Year × Zone	1	0.272	4.23 ns	1	0.011	0.08 ns
Year × Site within Zone	18	0.064	0.73 ns	18	0.144	0.69 ns
Error	118	0.089		118	0.209	
	Respiration rate					
Base of calculation	dw			OM		
Source of variation	df	MS	F	df	MS	F
Year	1	0.095	8.48 **	1	0.001	0.12 ns
Zone	1	1.085	24.77 ***	1	0.998	20.21 ***
Site within Zone	18	0.044	3.92 ***	18	0.049	4.37 ***
Year × Zone	1	0.002	0.40 ns	1	0.000	0.05 ns
Year × Site within Zone	18	0.005	0.44 ns	18	0.005	0.41 ns
Error	97	0.011		97	0.011	
	FDA mycelium					
Base of calculation	dw			OM		
Source of variation	df	MS	F	df	MS	F
Year	1	2.412	45.72 ***	1	1.870	36.78 ***
Zone	1	0.113	1.27 ns	1	0.031	0.46 ns
Site within Zone	8	0.087	1.68 ns	8	0.067	1.31 ns
Year × Zone	1	0.019	0.92 ns	1	0.007	0.35 ns
Year × Site within Zone	8	0.021	0.40 ns	8	0.020	0.39 ns
Error	28	0.053		28	0.051	

To test the effect of zone, site nested within zone is used as the total error term. Logarithmic values are used to normalize the distribution in the case of respiration and FDA mycelium. The data for April 1988 are excluded. Level of significance: ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

centre, and they were correlated one with the other (Table 3). The length of FDA mycelium was not different between zones, but was loosely correlated with DHA (Table 3).

Factors affecting the variation in biological activity

Since the zone and site variabilities best explained the variation in the activity parameters, the chemical and physical quality of the mor humus (Table 4), i.e. the environment of the microorganisms, may be responsible for the variability in the soil biological variables. As most humus quality parameters are interrelated, however (Table 3), it is difficult to find causal relationships. In order to investigate the characteristics of the humus samples, principal component (PC) analysis, an ordination method, was used. This technique enables the underlying relationships among variables to be investigated and the classification interpreted in terms of these variables (Peppard 1985).

All the parameters measured in the humus samples, both biological and chemical except for the FDA mycelium, were subjected to PC analysis. The first principal component (PCI) accounted for 31% of the variance in the data (Table 5) and already allowed the differences between the samples for the two zones to be outlined (Fig. 3a). Total nitrogen concentration, C/N ratio and ammonium nitrogen concentration had the highest factor loadings on PCI (Table 5) as seen in Figs. 3b, 3c and 3d. This will be interpreted as reflecting nitrogen deposition in the area. All the samples from the cleaner zone were found on the left-hand side of the scattergrams and the samples from the more polluted zone on the right-hand side, although the dividing line between the zones is not a sharp one and is not marked in the figures. These positions indicate that nitrogen deposition is an important factor among the soil properties and that the division of the sites into two zones had been correct and conformed not only to the sulphur content of the humus layer (Ohtonen et al. 1989) but also to the total and ammonium nitrogen concentrations and the C/N ratio. No other measured environmental parameter affected the structure of the data to any substantial extent. The second component, PC2 includes almost all of the parameters quite equally, while PC3 links exchangeable acidity, moisture and respiration, and PC4 pH($CaCl_2$) and DHA, although the contributions of these components to the model are only 20, 13 and 11%, respectively (Table 5).

The total nitrogen concentration of the humus layer was a significant predictor of respiration rate, calculated from the total data, with a negative relationship (Table 6). In addition, respiration rate was best accounted for by pH($CaCl_2$), exchangeable acidity and moisture, all features which

Table 3. Pearson coefficients of correlation between the parameters measured in the mor humus of the Scots pine forest sites in Oulu.

	DHA									
DHA		Respir.								
Respir.	0.48***		FDA myc.							
FDA myc.	0.37**	0.14		Moisture						
Moisture	0.16*	0.50***	ns		$pH(H_2O)$					
$pH(H_2O)$	ns	ns	0.50***	0.15*		$pH(CaCl_2)$				
$pH(CaCl_2)$	0.31***	0.31***	0.30*	0.30***	0.36***		exc. acid.			
exc. acid.	ns	ns	−0.45***	−0.23**	−0.40***	−0.37***		NH_4-N		
NH_4-N	ns	ns	ns	0.21**	0.45***	0.31***	−0.19*		NO_3-N	
NO_3-N	0.35***	0.28**	ns	0.32***	0.20*	0.36***	−0.23**	0.40***		Total N
Total N	ns	−0.39***	ns	−0.18*	0.27***	0.22**	0.18*	0.55***	ns	
C/N	ns	0.40***	ns	0.19**	−0.29***	−0.21**	−0.20*	−0.53***	ns	−0.93***

DHA, respiration rate, FDA mycelium, exchangeable acidity, and ammonium, nitrate and total nitrogen are calculated on an OM basis. Logarithmic values were used to normalize the distribution, when necessary. ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 4. Characteristics of the mor humus of ten Scots pine forest sites in the cleaner and that of ten sites in the more polluted zone in Oulu, averages for 1987 and 1988.

Zone	Thickness cm	Moisture % fw	LOI $g\,g^{-1}$	pH($CaCl_2$)	pH(H_2O)	Exchangeable acidity $\mu eq\,g^{-1}OM$	NH_4-N $\mu g\,g^{-1}OM$	NO_3-N $\mu g\,g^{-1}OM$	Total N $mg\,g^{-1}OM$	C/N
Cleaner	3.7	69.5 ± 2.1	0.78 ± 0.04	3.18 ± 0.01	4.10 ± 0.04	108 ± 8	21.2 ± 2.2	26.7 ± 2.4	15.9 ± 0.5	38.1 ± 1.2
	4.3	69.2 ± 2.8	0.77 ± 0.03	3.05 ± 0.11	3.87 ± 0.03	121 ± 10	14.8 ± 2.8	20.4 ± 2.4	143. ± 0.4	39.5 ± 1.2
	5.0	70.4 ± 2.8	0.79 ± 0.03	2.95 ± 0.05	3.95 ± 0.03	137 ± 9	13.5 ± 1.5	23.3 ± 2.8	14.6 ± 0.5	36.5 ± 1.2
	3.6	65.2 ± 1.5	0.67 ± 0.04	2.98 ± 0.01	4.01 ± 0.05	168 ± 23	10.4 ± 1.1	21.2 ± 2.2	15.6 ± 1.3	33.3 ± 1.9
	2.3	67.6 ± 3.0	0.67 ± 0.05	3.11 ± 0.05	3.99 ± 0.04	125 ± 12	16.6 ± 3.2	22.7 ± 2.8	13.7 ± 0.5	43.1 ± 1.3
	3.8	67.0 ± 1.3	0.69 ± 0.04	3.05 ± 0.02	4.05 ± 0.04	144 ± 9	13.2 ± 1.3	17.6 ± 1.8	19.5 ± 1.5	31.2 ± 1.7
	4.6	61.9 ± 2.1	0.64 ± 0.03	3.05 ± 0.02	3.98 ± 0.05	131 ± 10	16.2 ± 2.2	23.5 ± 3.2	17.2 ± 0.4	34.0 ± 0.9
	4.2	66.3 ± 1.9	0.72 ± 0.03	3.07 ± 0.03	4.03 ± 0.03	117 ± 8	16.7 ± 1.2	20.5 ± 1.5	15.8 ± 0.4	33.7 ± 0.8
	5.1	67.6 ± 1.5	0.79 ± 0.02	2.97 ± 0.03	4.01 ± 0.04	135 ± 8	16.1 ± 2.6	20.0 ± 2.9	16.3 ± 0.3	34.0 ± 0.7
	4.1	63.9 ± 1.8	0.73 ± 0.03	2.88 ± 0.02	3.87 ± 0.05	152 ± 11	9.5 ± 0.6	15.5 ± 1.8	15.2 ± 0.5	36.2 ± 1.2
$\bar{x}$	4.1	66.9 ± 0.7	0.73 ± 0.01	3.03 ± 0.02	3.99 ± 0.01	134 ± 4	14.8 ± 0.7	21.2 ± 0.8	15.8 ± 0.3	36.1 ± 0.5
More polluted	7.8	62.3 ± 3.0	0.61 ± 0.03	3.17 ± 0.04	4.03 ± 0.03	147 ± 13	49.2 ± 14.1	18.8 ± 2.3	20.7 ± 0.5	26.0 ± 0.6
	6.3	68.8 ± 2.3	0.73 ± 0.02	3.19 ± 0.05	4.06 ± 0.06	153 ± 16	20.9 ± 2.5	22.6 ± 1.0	18.5 ± 0.3	27.9 ± 0.4
	6.9	62.6 ± 1.9	0.60 ± 0.03	3.17 ± 0.02	4.06 ± 0.04	138 ± 6	47.2 ± 9.5	25.9 ± 2.3	22.6 ± 0.6	23.9 ± 0.6
	7.6	70.4 ± 2.4	0.77 ± 0.04	3.10 ± 0.05	4.18 ± 0.06	131 ± 9	108 ± 28	20.1 ± 4.0	22.1 ± 1.2	24.9 ± 1.0
	6.9	56.0 ± 2.2	0.64 ± 0.01	3.20 ± 0.01	4.14 ± 0.05	139 ± 11	41.2 ± 9.4	16.6 ± 1.4	22.6 ± 1.0	23.5 ± 1.1
	8.9	71.0 ± 1.5	0.83 ± 0.02	3.03 ± 0.03	4.12 ± 0.07	109 ± 6	133 ± 26	26.0 ± 4.2	19.6 ± 0.7	28.3 ± 1.0
	4.5	69.7 ± 1.1	0.78 ± 0.03	2.96 ± 0.02	3.98 ± 0.05	130 ± 12	15.5 ± 2.1	18.6 ± 2.1	17.7 ± 0.7	34.8 ± 1.3
	8.7	68.3 ± 1.6	0.77 ± 0.02	3.05 ± 0.04	4.19 ± 0.07	100 ± 8	177 ± 24	35.9 ± 7.8	19.1 ± 0.6	29.4 ± 0.9
	6.5	64.0 ± 1.7	0.74 ± 0.02	3.05 ± 0.03	4.10 ± 0.04	118 ± 6	21.8 ± 4.3	18.1 ± 1.6	21.0 ± 0.5	28.5 ± 0.7
	4.3	65.5 ± 1.6	0.75 ± 0.02	2.96 ± 0.02	4.05 ± 0.03	134 ± 10	14.9 ± 2.2	13.8 ± 1.5	17.9 ± 0.8	30.9 ± 1.3
$\bar{x}$	6.8	65.9 ± 0.8	0.72 ± 0.01	3.09 ± 0.01	4.09 ± 0.02	131 ± 4	62.7 ± 7.9	21.6 ± 1.2	20.1 ± 0.3	27.8 ± 0.4

Mean ± standard error (SE). The number of analyses is from 9 to 10, except in the case of ammonium and nitrate nitrogen, from 7 to 8, and exchangeable acidity, from 5 to 8.

Table 5. Structure of the soil data for Scots pine sites in Oulu, indicated by the first 5 principal components (PC1—PC5). Eigenvalues and values of the eigenvectors are indicated.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue					
Proportion	0.31	0.20	0.13	0.11	0.09
Cumulative	0.31	0.51	0.63	0.75	0.83
Eigenvectors					
Total nitrogen	0.52	−0.09	0.11	0.18	0.03
C/N	−0.52	0.11	−0.15	−0.18	−0.03
NH_4-N	0.41	0.22	0.17	−0.27	−0.20
pH(H_2O)	0.31	0.34	−0.22	−0.22	0.44
Respiration rate	−0.28	0.38	0.33	0.19	0.20
pH($CaCl_2$)	0.25	0.36	−0.09	0.48	0.18
DHA	−0.17	0.38	−0.10	0.57	−0.19
Moisture	−0.13	0.36	0.43	−0.36	0.37
NO_3-N	0.09	0.43	0.15	−0.21	−0.72
Exchangeable acidity	0.01	−0.29	0.74	0.24	0.00

The data for DHA, respiration rate, total, ammonium and nitrate nitrogen and exchangeable acidity are calculated on an OM basis, with logarithmic transformations to normalize the distribution when necessary. The FDA mycelium analyses are excluded because they were not performed at all the sites.

tended to promote respiration (Table 6). Different responses of the activities were expected inside the zones, and the regression models were calculated separately for both zones. The most significant difference in the case of respiration rate was the positive effect of ammonium nitrogen and C/N in the cleaner zone and their negative effect in the more polluted zone (Table 6).

Total nitrogen concentration did not contribute to the regression models for DHA at all (Table 6), and pH($CaCl_2$) accounted for this variation best, having a positive effect both in the total data and when the data for the zones were calculated separately (Table 6). Moisture showed a significant positive effect on DHA only in the more polluted zone (Table 6).

The growing season in 1988 was much warmer than in 1987, and included quite a long rainy period in July—August with mild temperatures (over 10 °C) (Fig. 2). Monthly mean temperatures in the shrub layer were 2.3—4.9 ° warmer in 1988 than in 1987, except in October, and 1.5— 5.3° warmer in the depth of 10 cm. Total rainfall in the period from 1st June to 31st October 1987 and from 1st April to 31st October 1988 was 224 mm and 298 mm, respectively. These clear differences between the years

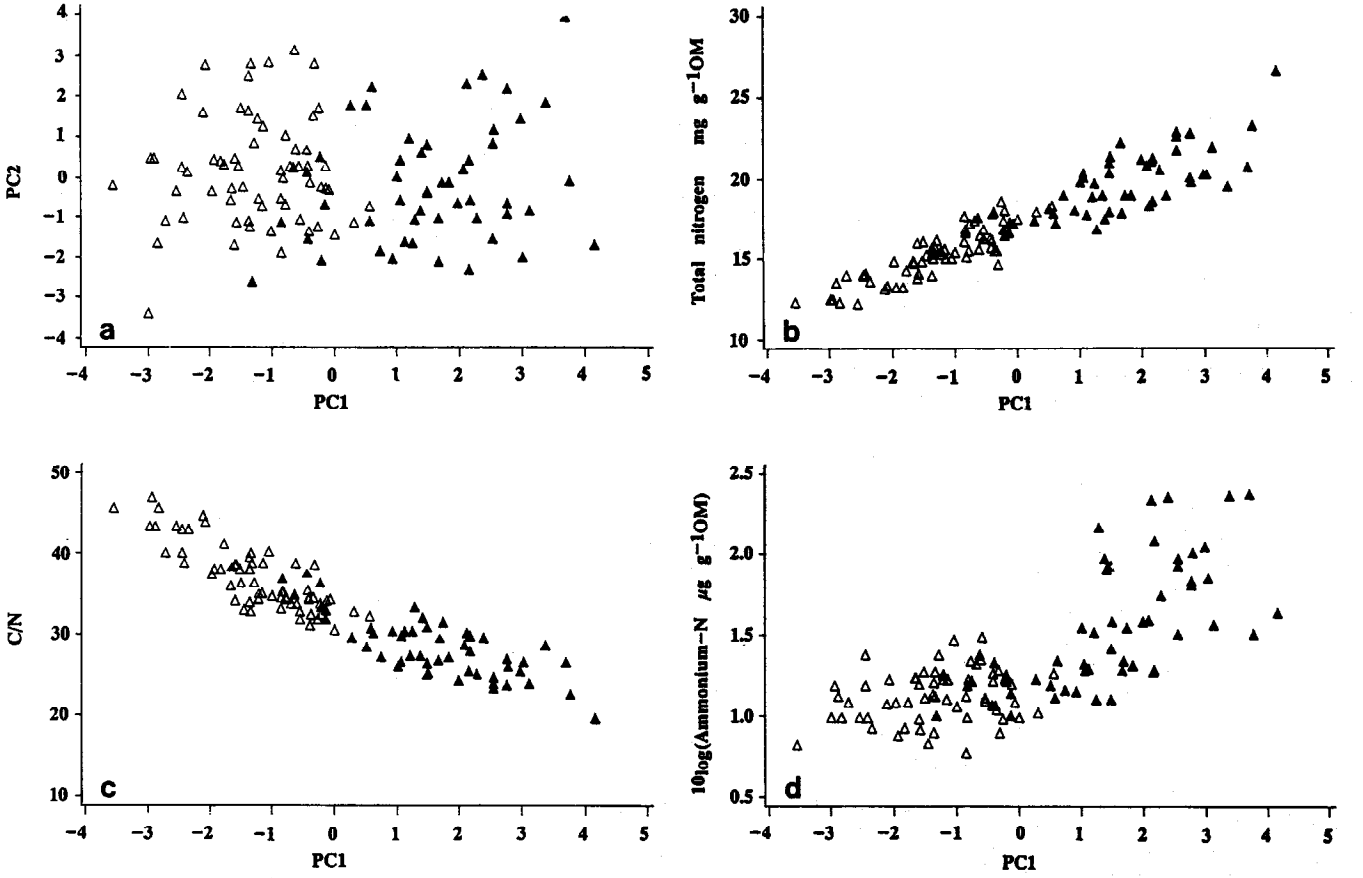

Fig. 3. Structure of the soil data from the Scots pine sites as relations between the first and second principal components (PC1 and PC2, respectively; *a*) and between the first component and total nitrogen concentration (*b*), C/N ratio (*c*) and ammonium nitrogen concentration (*d*) of the humus. Open triangles: cleaner sites; black triangles: more polluted sites. For further information, see Table 5.

Table 6. **Multiple regression models for the biological variables of the mors humus at the Scots pine forest sites in Oulu, calculated on an OM basis.**

	DHA μmol TPF g^{-1}OM 24 h^{-1}		Respiration $\mu g\ CO_2\ g^{-1}OM\ h^{-1}$		FDA mycelium m g^{-1}OM	
All sites						
F of the model	6.7 ***		21.5 ***		5.6 ***	
R^2	0.13		0.42		0.24	
parameter	estimate	signif.	estimate	signif.	estimate	signif.
Moisture % fw	0.01	ns	0.008	***	0.01	**
pH($CaCl_2$)	1.15	***	0.38	***	0.35	ns
exc. acid. μeq g^{-1}OM	−0.004	ns	0.29	*	—	
total N mg g^{-1}	—		−0.02	***	—	
intercept	−2.40	ns	−0.13	ns	1.24	ns
Cleaner sites						
F of the model	9.1 ***		15.7 ***		5.3 **	
R^2	0.17		0.43		0.40	
parameter	estimate	signif.	estimate	signif.	estimate	signif.
Moisture % fw	−0.02	ns	—		−0.009	ns
pH(H_2O)	—		0.15	ns	0.53	ns
pH($CaCl_2$)	1.60	***	—		—	
NH_4-N $\mu g\ g^{-1}$OM	—		0.38	***	—	
C/N	—		0.009	**	—	
exc. acid	—		—		−0.006 *	
Intercept	−1.56	ns	0.44	ns	2.32	ns
More polluted sites						
F of the model	5.8 ***		21.2 ***		1.8 ns	
R^2	0.29		0.60			
parameter	estimate	signif.	estimate	signif.	estimate	signif.
Moisture % fw	0.20	*	0.01	***		
pH(H_2O)	−0.65	ns	—			
pH($CaCl_2$)	2.27	***	0.41	**		
exc. acid μeq g^{-1}OM	−0.51	ns	—			
NH_4-N $\mu g\ g^{-1}$OM	—		−0.12	**		
C/N	—		−0.01			
intercept	−2.89	ns	0.17	ns		

— = parameter not included to the model; ns = not significant parameter.
Logarithmic transformations were used to normalize the distribution when necessary. Only the 1988 FDA mycelium analyses are included. Both total data and data for the two zones separately are presented.

measured in temperatures and rainfall did not have any clear effect on the biological activities or their seasonal variation. The variation in activities

during the growing season was slight in both years, and the clearest seasonal peak concerned the high values measured in early spring in 1988 in both zones (Fig. 4), though only some sites were sampled because of the frozen soil elsewhere. Nonetheless, the seasonal variation of the activities (Fig. 4) showed to follow the moisture patterns of the humus layer (Fig. 5).

Factors affecting the variation in FDA mycelium

The zone and site effects did not account for significant variation in the length of FDA mycelium, and thus no relationships with the chemical and physical quality of the mor humus could be expected. The length of FDA mycelium was best accounted for by moisture in the total data for 1988 (Table 6). Only exchangeable acidity was of any significance for the variation in FDA mycelium in the cleaner zone, and its effect was negative, while no significant regression model could be constructed from the parameters measured in the more polluted zone (Table 6). The seasonal variation in the FDA mycelium was the same as that in biological activities, i.e. high values in spring and smaller variation during the growing season (Fig. 4). The meteorological data showed no relation to this variation, however.

Discussion

The recent discussions concerning the reasons for forest damages in industrial environments have led to many hypotheses including soil changes affecting trees and the availability of nutrients for them. The complexity of soil containing numerous different organisms and with various interrelationships between chemical and biological properties makes the study difficult and many seemingly contradictory results have been presented. The variability in the biochemical quality of the organic soil horizon due to differences in plant cover and litter may directly affect the microflora, and thus, in addition to the direct effect of pollution, qualitative and quantitative changes in the vegetation play a role in soil microbiology.

A similar situation as in the Oulu area with respect to nitrogen compounds in the humus layer and the decreased respiration rate is described by Popovic (1984), who found the depressive effect of artificial acidification on soil respiration to be more pronounced when acid was applied with NPK fertilizers, indicating a harmful effect of a surplus of nitrogen on microorganisms. Bååth et al. (1981) discuss the reduced microbial activity observed after nitrogen application and present various hypotheses based

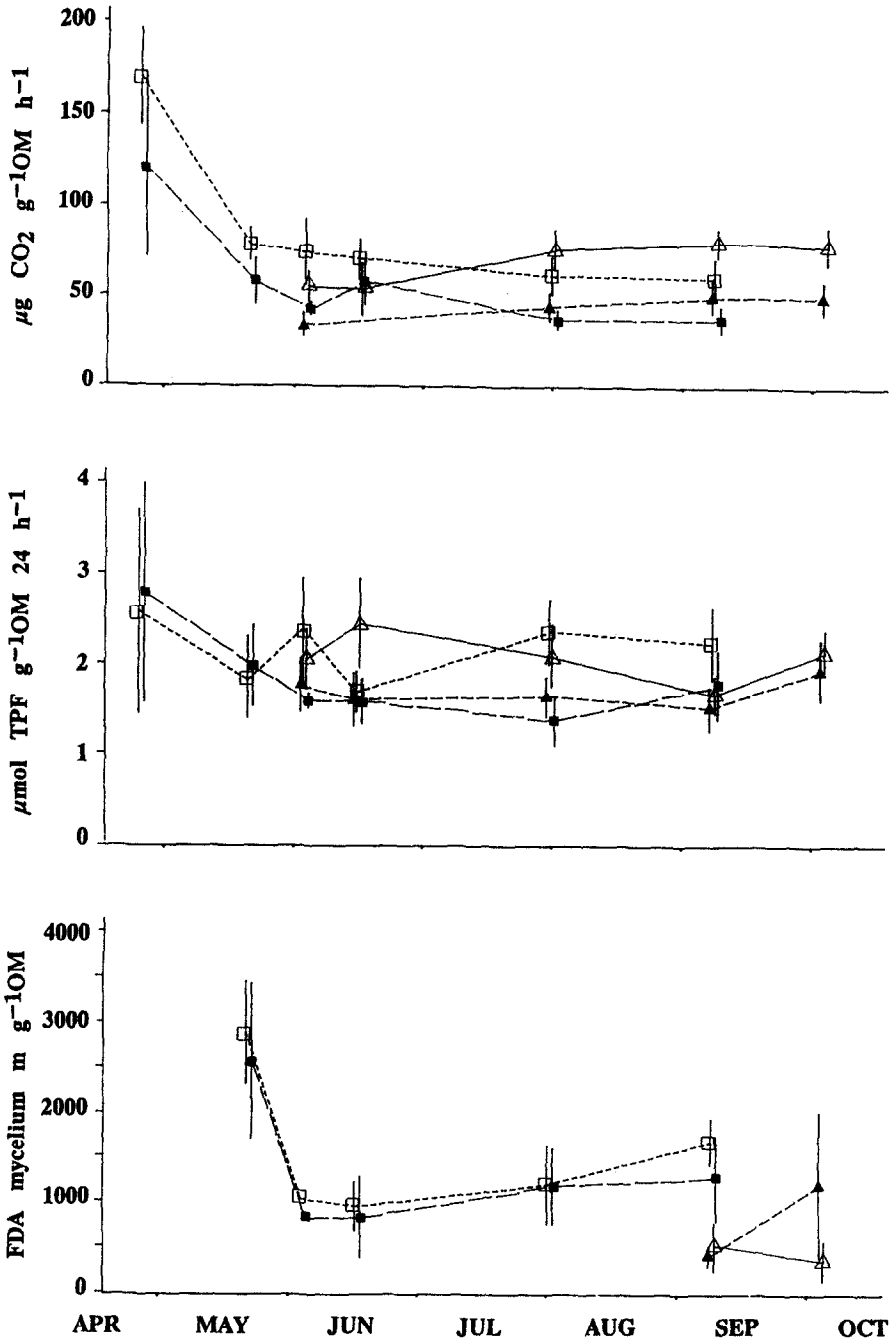

Fig. 4. Variation in respiration rate, DHA and the length of FDA active mycelium in the humus layer of Scots pine forests in the Oulu area with time. Open triangles: cleaner sites in 1987; open squares: cleaner sites in 1988; black triangles: more polluted sites in 1987; black squares: more polluted sites in 1988. The bars indicate the standard error.

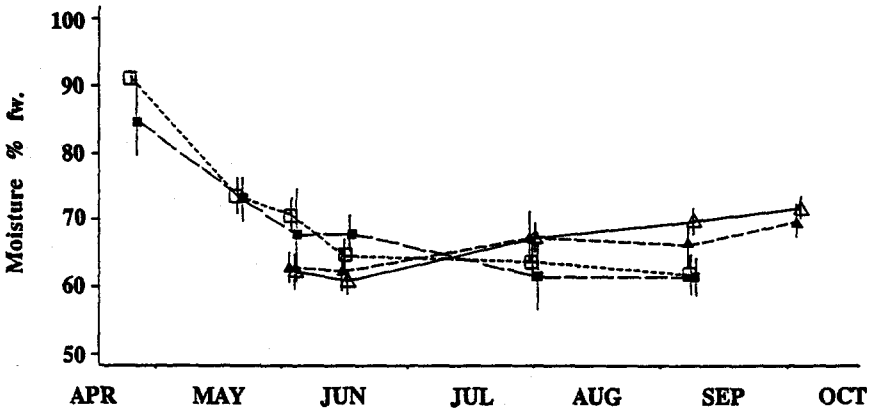

Fig. 5. Variation in moisture in the humus layer of pine forests in the Oulu area with time. Open triangles: cleaner sites in 1987; open squares: cleaner sites in 1988; black triangles: more polluted sites in 1987; black squares: more polluted sites in 1988. The bars indicate the standard error.

on long-term fertilization experiments in particular, a situation which bears some similarity to the long-term nitrogen pollution in the Oulu area. One of the characteristics mentioned by them, reduced mycorrhizal frequency, was found in the Oulu area (Ohtonen et al. 1990). Other changes in the rhizosphere were altered root dynamics, reduced root production rate and reduced root exudation rate (Bååth et al. 1981). Clear differences in the vegetation cover were also noticed between the outer and central zones in Oulu (Ohtonen & Markkola, unpublished), which certainly reflect the root status of the sites. The direct toxic effects of nitrogen compounds themselves on microorganisms are difficult to estimate. The toxic form resulting from the dissolving of gaseous NO_2 in water is NO^{2-}, which is highly bactericidal (Babich & Stotzky 1980) and has been shown to suppress heterotrophic activity (Grant et al. 1979a). In addition, it has been shown to be mutagenic for viruses of bacteria, plants and animals (Babich & Stotzky 1980). This may be of great importance in nitrogen deposition situations and needs further study.

Other hypotheses of Bååth et al. (1981) concerning the decrease in biological activity refer to a direct effect of nitrogen on the quality of the organic matter, an increasing amount of resistent humic materials after nitrogen fertilization, limiting the supply of available carbon for the microorganisms, and nutrient limitation, affecting P, K, Ca or Mg. The alteration in the quality of the organic matter could be relevant in the Oulu area, because the organic layer was thicker at the more polluted sites than at the less polluted ones, indicating reduced decomposition, probably together with increased litter production due to the fertilization effect of nitrogen

deposition. The quality and concentration of the organic carbon will be of great interest for future research in the Oulu area, because these are in general the strongest predictors of soil respiration (Nohrstedt 1985). An altered nutrient status, e.g. enhanced release of Ca, Mg, K and Mn from decomposing litter, as proposed by Berg (1986), Fritze (1988) and Ohtonen et al. (1990) is another future question for the Oulu area.

The destructive effect of surplus N on the microflora may be in interaction with other pollutants in the area. The low R^2 values for the regression models describing the effects of environmental parameters on microorganisms may indicate that there are factors that are not measured here which have an effect on microorganisms and biological activities. Total S and heavy metals in the mor humus showed a negative relationship with the average biological parameters of the soil when calculated as means of the values measured in the autumn of both year (Ohtonen et al. 1990). This was especially evident in the case of the respiration rate, which proved to be a more sensitive parameter in relation to S, N and heavy metals than DHA. Other authors have demonstrated a decrease in soil respiration or microbial biomasses in response to S deposition (Popovic 1984; Bewley & Parkinson 1984; Fritze 1987), bisulphite being the most detrimental for microorganisms (Babich & Stotzky 1980), especially synergistically with nitrite (Grant et al. 1979a). The effects of S deposition are difficult to estimate separately from those of lowered pH, however (Grant et al. 1979b). The concentrations of heavy metals measured in the Oulu area (Ohtonen et al. 1990) were below those found to reduce decomposition rates to a measurable extent (Friedland et al. 1986) and thus the harmful effects of heavy metals detected in the Oulu area (Ohtonen et al. 1990) may be attributable to some interaction with S and N.

The low R^2 values can otherwise be explained by the diverse community structure of the microbial populations in soil, which means that the elimination of one population is not detrimental as a second population may totally or partially fill its niche (Alexander 1980). Such changes in community structure never become apparent in synecological analyses.

Of the various acidity parameters measured, $pH(CaCl_2)$ uniformly showed a positive response to the biological variables. The humus layer in the Oulu area has a very low $pH(CaCl_2)$, from 2.88 to 3.20 on average, which may be below the optimum for soil respiration (Nohrstedt 1985). Thus a positive correlation with pH and biological activity could be expected, and this was indeed found, especially in the case of DHA, both in the total data and in both zones separately, in spite of the quite narrow range of variation in pH at the sites studied in Oulu. The positive relation of DHA to $pH(CaCl_2)$ agrees well with the observations of Nohrstedt

(1985) who found DHA to correlate with soil pH better than with any other soil parameter and suggested that it may serve as a valuable index of soil acidification. The effect of pH(H_2O) and exchangeable acidity on biological activities was not a simple one, however, as the relations varied. This may indicate a situation in which a pH determination from quite a large soil sample is not the correct way to obtain information on the real pH in the environment of the microorganisms at the micro habitat level, and Hovland (1981) indeed claims that the reduced pH of coniferous needle litter due to acid rain may be of little importance for biological activity measured in terms of respiration. pH determinations of this kind may even be entirely devoid of meaning (Boddy 1984). There is certainly a relationship between micro and macro soil pH, however. It can be suggested with some caution that pH measured in a 0.01 M $CaCl_2$ solution will be a better acidity indicator of the microbial environment than pH measured in water or exchangeable acidity.

No effect of site or zone on the amount of living fungal mycelium could be found, as noticed earlier in an urban environment (Fritze 1987). Increased acidity has sometimes been observed to have a depressive effect on the FDA active fungal mycelium, and the percentages hyphae which are FDA-active have been lower at artificially acidified sites than in control plots (Bååth et al. 1980). This may be some kind of shoch effect, because quite high levels of acids commonly are used in short-term acidification experiments. 'Naturally' progressing acidification or contamination leaves more time for the microbial communities to adjust to the changes, and changes in species composition have been found in both bacteria and microfungi (Bååth et al. 1980, 1984).

No significant correlation between the amount of FDA active mycelium and respiration in soil communities in the field has been found. A good correlation has been found between these two parameters, when the community structure is simplified reinoculating some or many soil fungi into sterilized soil (Ingham & Klein 1984; Bååth & Söderström 1988). Bååth & Söderström (1988) used this approach to calculate that 1 m of FDA active mycelium respires to the extent of 11 ng CO_2-C h^{-1} at 20 °C. If this calculation is assumed to be valid in northern forest ecosystems, it will improve the understanding the contribution of fungi to CO_2 evolution from soil, and would suggest that the FDA mycelium accounts for 50—75% of total soil respiration at the cleaner sites in Oulu and 65—100% at the more polluted sites (Table 7). The average contribution of fungi to the total respiration in the cleaner zone is near the bacterial/fungal respiration ratio of 40/60 found in humus of a beech forest by Anderson & Domsch (1975), but too great compared to the ratio 44/52 estimated by Persson et al. (1980) for a Scots pine site. The overestimation might apply to Table 7,

Table 7. Theoretical calculation of respiration of the measured amounts of FDA active fungal mycelium (according to Bååth & Söderström 1988) at the Scots pine sites in Oulu and fungal respiration as a percentage of total measured respiration measured.

	Measured mean respiration rate	Calculated respiration of FDA mycelium	% of the measured
	$\mu g\ CO_2\ g^{-1}OM\ h^{-1}$		
Cleaner zone	90.7	45.65	50.3
	78.9	49.49	62.3
	64.0	47.67	74.5
	64.7	31.92	49.3
	90.5	55.75	61.6
Mean	77.8	46.06	59.6
More polluted zone	48.2	31.11	64.5
	78.7	81.61	103.7
	63.1	61.81	98.0
	54.8	43.23	78.9
	36.2	35.55	98.2
Mean	56.2	50.66	88.7

because Bååth & Söderström (1988) measured respiration at 20 °C whereas in Oulu it was measured at 12 °C, in addition to which the Q_{10} for the respiration of microorganisms at forest sites in Oulu is unknown varying from 1.6 to 3.2 in other soils (Schlesinger 1977). The possible conclusion is, that the activity of bacteria will decrease at polluted sites and the proportion of fungi increase. This hypothesis is in agreement with the observations of Bååth et al. (1980), who found the decrease in the biomass of bacteria under artificially acidified conditions to be more drastic than that in FDA fungi (75% and 40%, respectively). The respiration rate of fungi at more polluted sites may be quite different from that at cleaner sites, however, possibly much lower, which makes the above calculation no more valid at all. It has been noted that the quantity of organic matter present determines the fungal biomass in a quantitative sense but that the nature of the substrate determines rate of fungal activity in decomposition (Flanagan & Van Cleve 1983). This also points to the need for real analyses of fungal and bacterial contributions to respiration.

Also the real activity analyses may be more correct to measure in the field without any disturbance of the soil samples and employing meteorological data from the close vicinity of the microorganisms. CO_2 evolution is usually found to parallel forest floor temperature patterns, the highest levels in the coniferous ecosystems occurring during the summer and autumn (Vogt et al. 1980). About 95 to 99% of the weight loss in the litter

is also attributable to climatic factors, as seen when the same litter was used in different regions (Berg et al. 1984). Also N and S mineralization are increased with temperature (Foster 1989). These results cannot be generalized to the Oulu area according to the present study.

The high activity values measured in the early spring deserve special attention. These may be real activities, as the length of the FDA mycelium showed high amount still in May and Bååth & Söderström (1982) also found high winter values of FDA active mycelium in the southern part of Sweden. Another possibility is that the high activity values may not be true but reflect the cold tolerance organisms which dominate the microbial communities early in the spring and which may have a higher Q_{10} value for their activities than the species which are dominant at other times, a property which would emerge when the analyses are performed at a relatively high temperature compared to the spring temperature in soil. The same phenomena was not found in autumn, but the reason for this may be that no analyses were performed so late that the soil ambient temperature was near 0 °C. The real difference between the spring and autumn conditions in fact is the great amount of meltwater present in spring, which contains all the deposition falling onto the snow during the winter season of some 5 months. The chemical form of the pollutants in the water may be of great importance in this situation, but the moisture effect of the melting snow may be of greater significance than the effects of pollutants.

A more surprising feature is that so little variation existed in the length of the FDA active mycelium except for the high spring values, whereas Bååth & Söderström (1982) noted clear differences in seasonal variation between years characterized by different meteorological conditions. The distances between the sampling times in Oulu may have been too long to allow evaluation of the finer trends in the effect of meteorological conditions on the length of the FDA active mycelium.

Further studies are needed to elucidate many of the results reported here. These include:

- Supporting laboratory experiments performed under controlled conditions and with a limited number of variables, because it may be too complicated to measure the effect of environmental parameters on the activities or biomasses of microorganisms in the field. The effects of the various nitrogen compounds are of particular interest, especially those of ammonium and nitrite nitrogen.
- The various sulphur compounds included in the environmental analysis. This apply particularly bisulphite, which may be the most poisonous sulphur compound for microorganisms.

- The relations between reduced microbial activity, a thicker humus layer, and the altered biochemical nature of the humus.
- Other enzymatic activities than DHA of the microorganisms in relation to an altered environment.
- The contribution of bacterial and fungal respiration to total respiration in relation to the pollution gradient.
- The effects of temperature and other meteorological conditions on microbial activities measured in the field.

The use of parameters of microbial ecology as environmental indicators needs great care, because the reactions of the diverse microbial communities to environmental changes may vary from one biome or ecosystem to another and between different microbe communities. Nitrogen deposition seemed to have a considerable effect on biological activities, and have to take into account when forest damage are studied. Soil respiration rate and DHA proved here to be parallel and relatively good indicators of decreased biological activity in urban polluted forest soil, whereas the length of the FDA mycelia seemed to be of no use in this respect. In spite of small seasonal variation concerning both the activities and FDA mycelium in Oulu, it cannot disregard when comparing soils having possible different microbial communities.

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References

Ahti T, Hämet-Ahti L & Jalas J (1968) Vegetation zones and their sections in northwestern Europe. Ann. Bot. Fennici 5: 169–211

Alexander M (1980) Effects of acidity on microorganisms and microbial processes in soil. In: Hutchinson TC & Havas M (Eds) Proc. NATO Adv. Res. Inst. Conf. on Effects of acid precipitation on terrestrial ecosystems (pp 363–374). Plenum Press, New York

Anderson JPE & Domsch KH (1975) Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Can. J. Microbiol. 21: 314—322

Anon (1988) Kuukausikatsaus Suomen ilmastoon, yhteenveto 1988. Ilmatieteen laitos, Helsinki (in Finnish)

Anon (1989) Oulun kaupungin ympäristöhygienian osasto (The municipal authority in Oulu, unpublished)

Bååth E, Berg B, Lohm U, Lundgren B, Lundkvist H, Rosswall T, Söderström B, Wiren A (1980) Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. Pedobiol. 20: 85—100

Bååth E, Lundgren B & Söderström B (1981) Effects of nitrogen fertilization on the activity and biomass of fungi and bacteria in a podzoilic soil. Zbl. Bakt. Hyg., I. Abt. Orig. C 2: 90—98

—— (1984) Fungal populations in podzolic soil experimentally acidified to simulate acid rain. Microb. Ecol. 10: 197—203

Bååth E & Söderström B (1982) Seasonal and spatial variation in fungal biomass in a forest soil. Soil Biol. Biochem. 14: 353—358

—— (1988) FDA-stained fungal mycelium and respiration rate in reinoculated sterilized soil. Soil Biol. Biochem. 20: 403—404

Babich H & Stotzky G (1980) Environmental factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. Crt. Rev. Microbiol. 8: 99—145

Berg B (1986) The influence of experimental acidification on nutrient release and decomposition rates of needle and root litter in the forest floor. Forest Ecol. Man. 15: 195—213

Berg B, Jansson PE & Meentemayer V (1984) Litter decomposition and climate — regional and local models. In: Gren G (Ed) State and Change of Forest Ecosystem — Indicators in Current Research (pp 389—404). Swedish Univ. Agr. Sci., Dept Ecol. Environ. Res., Report No 13

Bewley RJF & Parkinson D (1984) Effects of sulphur dioxide pollution on forest soil microorganisms. Can. J. Microbiol. 30: 179—185

Black CA, Evans DD, White JL, Ensminger LE, Clark FE & Dinauer RC (Eds) (1965) Methods in Soil Analysis. Part 2, Chemical and Microbiological Properties. Am. Soc. Agr. Inc. Publ. Madison

Boddy L (1984) The micro-environment of basidiomycete mycelia in temperate deciduous woodlands. In: Jennings DH & Rayner ADM (Eds) The Ecology and Physiology of the Fungal Mycelium (pp 261—289). Cambridge University Press, Cambridge

Carlyle JC & U Ba Than (1988) Abiotic controls of soil respiration beneath an eighteen-year-old *Pinus radiata* stand in south-eastern Australia. J. Ecol. 76: 654—662

Christensen BT & Malmros PA (1982) Loss-on-ignition and carbon content in a beech forest soil profile. Holarctic Ecol. 5: 376—380

Coleman DC (1973) Compartmental analysis of "total soil respiration": an exploratory study. Oikos 24: 361—366

van Dijk FG & Roelofs JGM (1988) Effects of excessive ammonium deposition on the nutritional status and condition of pine needles. Physiol. Plant. 73: 494—501

Flanagan PW & Van Cleve K (1983) Nutrient cycling in relation to decomposition and organic-matter quality in taiga ecosystem. Can. J. Forest Res. 13: 795—817

Foster NW (1989) Influences of seasonal temperature on nitrogen and sulfur mineralization/immobilization in a maple-birch forest floor in central Ontario. Can. J. Soil Sci. 69: 501—514

Friedland AJ, Johnson AH & Siccama TG (1986) Coniferous litter decomposition on Camels Hump, Vermont: a review. Can. J. Bot. 64: 1349—1354

Fritze H (1987) The influence of urban air pollution on soil respiration and fungal hyphal length. Ann. Bot. Fennici 24: 251—256

Fritze H (1988) Influence of urban air pollution on needle litter decomposition and nutrient release. Scand. J. Forest Res. 3: 291—297

Grant IF, Bancroft K & Alexander M (1979a) SO_2 and NO_2 effects on microbial activity in an acid forest soil. Microb. Ecol. 5: 85—89

Grant IF, Bancroft K & Alexander M (1979b) Effect of SO_2 and bisulfite on heterotrophic activity in an acid soil. Applied Environ. Microbiol 38: 78—83

Hovland J (1981) The effect of artificial acid rain on respiration and cellulase activity in Norway spruce needle litter. Soil Biol. Biochem. 13: 23—26

Huttunen S (1975) The influence of air pollution on the forest vegetation around Oulu. Acta Univ. Ouluensis, Ser. A, Scientiae Rerum Naturalium 33, Biol. 2

Huttunen S & Laine K (1983) Effects of airborne pollutants on the surface wax structure on *Pinus sylvestris* needles. Ann. Bot. Fennici 20: 79—86

Huttunen S, Laine K & Torvela H (1985) Seasonal sulphur contents of pine needles as indices of air pollution. Ann. Bot. Fennici 22: 343—359

Ingham ER & Klein DA (1984) Soil fungi: relationship between hyphal activity and staining with fluorescein diacetate. Soil Biol. Biochem. 16: 273—278

Karhu M (1986) Männyn neulaset ja koivun lehdet ilman rikkikuormituksen ilmentäjinä. Dept. of Botany, Univ. of Oulu (in Finnish)

Kubin E (1978) Kasvimateriaalin typpipitoisuuden määrittämisestä. (Abstract: The determination of the organic nitrogen in plant material). Oulun yliopiston kasvitieteen laitoksen monisteita 7 (in Finnish)

Meyer FH (1988) Ectomycorrhiza and decline of trees. In: Jansen AE, Dighton J & Bresser AHM (Eds) Ectomycorrhize and acid rain. Workshop on Ectomycorrhize / Expert Meeting, Berg en Dal, The Netherlands (pp 9—31)

McLaughlin SB (1985) Effects of air pollution on forests. J. Air poll. Contr. Ass. 35: 512—534

Nihlgård B (1985) The ammonium hypothesis — An additional explanation to the forest dieback in Europe. Ambio 14: 2—8

Nohrstedt HÖ (1985) Biological activity in soil from forest stands in Central Sweden, as related to site properties. Microb. Ecol. 11: 259—266

Nohrstedt H Ö, Arnebrandt K, Bååth E & Söderström B (1989) Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen-fertilized pine forest soils in Sweden. Can. J. Forest Res. 19: 323—328

Ohtonen R, Markkola AM, Heinonen-Tanski H & Fritze H (1990) Soil biological parameters as indicators of changes in Scots pine forests (*Pinus sylvestris* L.) caused by air pollution. In: Kauppi P, Kenttämies K & Anttila P (Eds) Acidification in Finland (pp 373—393). Springer-Verlag, Berlin Heidelberg

Ohtonen R, Markkola AM & Torvela H (1989) Total sulfur content in the humus layer of urban polluted forest soils. Water Air Soil Poll. 44: 135—141

Olson FCW (1950) Quantitative estimates of filamentous algae. Trans. Am. Microsc. Soc. 59: 272—279

Peppard TL (1985) Principal component analysis — its application in monitoring the consistency of beer quality. J. Inst. Brew. 91: 325—328

Persson T, Bååth E, Clarholm M, Lundkvist H, Söderström BE & Sohlenius B (1980) Trophic structure, biomass dynamics and carbon metabolism of soil organisms in a Scots pine forest. In: Persson T (Ed) Structure and function of northern coniferous forests. Ecol. Bull. (Stockholm) 32: 419—459

Popovic B (1984) Mineralization of carbon and nitrogen in humus from field acidification studies. Forest Ecol. Man. 8: 81—93

Prinz B (1987) Causes of forest damage in Europe. Environment 29: 10—37

Schlesinger WH (1977) Carbon balance in terrestrial detritus. Ann. Rev. Ecol. Sys. 8: 51—81

Söderström BE (1977) Vital staining of fungi in pure cultures and in soil with fluorescein diacetate. Soil Biol. Biochem. 9: 59–63

Söderström B, Bååth E & Lundgren B (1983) Decrease in soil microbial activity and biomasses owing to nitrogen amendments. Can. J. Microbiol. 29: 1500–1506

Termorshuizen AJ & Schaffers AP (1987) Occurrence of carpophores of ectomycorrhizal fungi in selected stands of *Pinus sylvestris* in the Netherlands in relation to stand vitality and air pollution. Plant Soil 104: 209–217

von Thalman A (1968) Zur Methodik der Bestimmung der Dehydrogenaseaktivität in Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtschaftliche Forshung 21: 249–258

Vogt KA, Edmonds RL, Antons GC, Vogt DJ (1980) Relationships between CO_2 evolution, ATP concentrations and decomposition in four forest ecosystems in western Washington. Oikos 35: 72–79