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Seasonal changes in microbial biomass and nutrient flush in forest soils

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Abstract Microbial biomass and N, P, K, and Mg flushes were estimated in spring, summer, autumn, and winter samples of different forest soils. The microbial biomass showed significant seasonal fluctuations with an average distribution of $880 \pm 270\ \mu g\ C\ g^{-1}$ soil in spring, $787 \pm 356\ \mu g\ C\ g^{-1}$ soil in winter, $589 \pm 295\ \mu g\ C\ g^{-1}$ soil in summer, and $560 \pm 318\ \mu g\ C\ g^{-1}$ soil in autumn. The average annual concentrations of C, N, P, K, and Ca in the microbial biomass were 704, 106, 82, 69 and $10\ \mu g\ g^{-1}$ soil, respectively. Microbial C represented between 0.5 and 2% of the organic soil C whereas the percentage of microbial N with respect to the total soil N was two- to threefold higher than that of C; the annual fluctuations in these percentages followed a similar trend to that of the microbial biomass. Microbial biomass was positively correlated with soil pH, moisture, organic C, and total N. The mean nutrient flush was 31, 15, 7, and $4\ \mu g\ g^{-1}$ soil for N, K, P, and Mg, respectively, and except for K, the seasonal distribution was autumn ≥ spring ≫ winter ≥ summer. The average increase in available nutrients due to the mineralization of dead microbial cells was 240% for N, and 30, 26, and 14% for P, K, and Mg, respectively. There was a positive relationship between microbial biomass and the N, P, K, and Mg flushes. All the variables studied were significantly affected by the season, the type of soil, and the interaction between type of soil and season, but soil type often explained most of the variance.

Key words Microorganisms · Macronutrients · N, P, K, and Mg · Atlantic European ecosystem · Temperate humid zone · Annual changes

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Introduction

Numerous studies on the measurement of microbial C, N, and P in different natural and disturbed ecosystems have shown that the soil microbial biomass contains important labile pools of C and mineral nutrients (Anderson and Domsch 1980; Smith and Paul 1990; von Lützow et al. 1992; Wardle 1992; Díaz-Raviña et al. 1993a, b), which are liberated after the death of microorganisms. Changes in the microbial population in response to variations in soil conditions (moisture, C, nutrients, temperature, pH) have important implications for nutrient cycling, with microorganisms acting as a source and sink for nutrient elements. Seasons influence microbial numbers (Díaz-Raviña et al. 1993c) and mass (Granatstein et al. 1987; Lynch and Panting 1980) either directly, by inducing microbial responses to soil changes, or indirectly, by influencing plant metabolism. However, information on the fluctuations in microbial biomass within the annual cycle is scarce and most estimates have been made for agricultural soils with some authors finding large annual fluctuations in the microbial biomass (Lynch and Panting 1980; Ross et al. 1981) while others observed only small annual changes (Schnürer et al. 1986; Patra et al. 1990).

The aim of the present research was to study seasonal fluctuations in the microbial biomass and its contribution to available nutrient concentrations in five forest soils. The flushes of N, P, K, and Mg after 10 days of incubation following chloroform fumigation was used to determine this contribution. The relative importance of the type of soil and season on the microbial biomass and nutrient flushes was estimated.

Materials and methods

Area and soils studied

Four pinewoods of 30- to 40-years-old *Pinus pinaster* Sol. (RGR1, RGR2, HCBS, and FC) and one oakwood of 100- to 130-years-old *Quercus robur* L. (HCAS) located in Galicia (NW Spain) were used for this study. The average air temperature and annual precipitation were, respectively, from 6.7 to 13.8 °C and 1799 mm for RGR1, from 4.9 to 17.4 °C and 1424 mm for RGR2, from 5.4 to 16.0 °C and 1227 mm for HCAS, from 6.2 to 18.5 °C and 1169 mm for HCBS, and from 7.8 to 16.9 °C and 1264 mm for FC. The climate is temperate humid, the highest temperature being reached in summer (July–August) and the lowest in winter (December–February); around 38, 28, 24, and 10% of the rainfall occurred in winter, spring, autumn, and summer, respectively. The soils, classified as Rankers (RGR1 and RGR2), Humic Cambisols (HCAS and HCBS), and Ferralic Cambisol (FC), were developed over different kinds of parent material (granite, granite, acid schist, basic schist, and gabbro, respectively). The pH was acid, the organic C and total N content medium to high, and the C : N ratio relatively low. Ca was the most abundant available nutrient, followed by K, Na, Mg, and P, inorganic N being the least abundant (Table 1).

The soils were studied in spring, summer, autumn, and winter. For each sampling, after removing the litter layer, 30–50 soil subsamples of about 100 g each were collected randomly from the top 15 cm of the A horizon and were mixed to obtain a composite sample. Sampling and sample manipulation were performed aseptically and without the samples drying or heating. The soils were sieved and the fraction <2 mm was used for all analyses.

Microbial biomass

The procedure used to determine microbial biomass C was that of Jenkinson and Powlson (1976) with some minor modifications (Díaz-Raviña et al. 1993b). Three out of six 50-g replicates of each soil were fumigated with purified $CHCl_3$ for 24 h and then re-inoculated with 1 g of the corresponding fresh soil. The six fumigated and unfumigated samples were then brought to 60% water-holding capacity and incubated for 10 days at 25 °C in a water bath. The CO_2-C released was determined by evacuating it with a stream of moist CO_2-free air that was then bubbled through a NaOH solution of known strength which was subsequently titrated against HCl. Microbial biomass C was calculated as $F/0.45$, where F is the flush of CO_2-C defined as the CO_2 evolved by the fumigated soil during the 10 days of incubation minus the CO_2 evolved by the corresponding non-fumigated control soil in the same period. The N, P, K, and Ca contents of the microbial biomass were estimated using the factors provided by Anderson and Domsch (1980) for conversion of biomass C into biomass mineral content: N : C = 0.15, P : C = 0.116, K : C = 0.098, and Ca : C = 0.014.

Nutrient flush

Inorganic N and the available P, K, and Mg content of the soils were estimated after incubating the fumigated and unfumigated samples. The nutrient flush, which was calculated as the difference between the available nutrient contents in the fumigated and the unfumigated samples, was used to measure the contribution by the microbial biomass to the concentration of the plant-available nutrients. Organic C, total N, and available nutrient measurements were carried out as described by Díaz-Raviña et al. (1993b, c). Organic C was determined by combustion and measurement of CO_2 in a Carmhograph 12 (Wosthoff OHG, Bochum, Germany) and total N by Kjeldahl digestion and steam distillation. Inorganic N (NH_4^+-N+NO_3^--N+NO_2^--N) was estimated in 2 N KCl extracts, labile P in 0.5 M $NaHCO_3$ extracts, and available K and Mg in 0.5 N

Table 1 Main characteristics of the soils. Means of samples taken in spring, summer, autumn and winter. Ranges of values for the four seasons are given in parentheses

Soils	Vegetation	pH (H_2O)	Moisture (%)	Organic C (%)	Total N (%)	C : N	Available nutrients (μg g^{-1} soil)					
							N	Ca	Mg	Na	K	P
RGR1	Pinewood	4.6	45	12.2	0.73	17	38	203	25	82	60	23
		(4.5–4.7)	(38–48)	(11.5–13.2)	(0.69–0.79)	(17–17)	(22–78)	(145–277)	(11–40)	(46–125)	(38–103)	(18–32)
RGR2	Pinewood	4.7	28	5.6	0.27	21	9	167	16	39	56	33
		(4.6–4.9)	(10–39)	(4.9–6.3)	(0.24–0.30)	(20–21)	(1–23)	(19–268)	(7–22)	(18–67)	(24–81)	(17–44)
HCAS	Oakwood	5.0	21	2.6	0.19	14	14	165	40	41	114	30
		(4.9–5.1)	(11–29)	(2.3–2.9)	(0.18–0.20)	(13–15)	(5–29)	(97–330)	(30–52)	(30–47)	(66–156)	(17–40)
HCBS	Pinewood	5.6	29	4.2	0.29	14	11	420	88	94	77	23
		(5.2–5.8)	(13–37)	(3.5–4.5)	(0.26–0.31)	(13–15)	(5–23)	(291–557)	(71–112)	(84–104)	(43–97)	(14–28)
FC	Pinewood	5.8	44	9.7	0.72	14	20	207	23	88	51	25
		(5.6–5.9)	(34–49)	(8.7–10.5)	(0.69–0.73)	(13–14)	(5–39)	(120–330)	(13–29)	(50–134)	(22–77)	(20–27)

acetic acid extracts. Inorganic N was determined by steam distillation in a Büchi 432 in the presence of H_3BO_3 and $Na_2B_4O_7 \cdot 7H_2O$ (1:5 w/w) buffer reagent adding Devarda's alloy. P was estimated colorimetrically measuring the absorption at 882 nm, K by emission spectroscopy, and Mg by atomic absorption spectroscopy.

All results were obtained by triplicate determinations, and are expressed on the basis of oven-dry (105 °C) weight of soil (dry soil). To reveal the variations in soil samples taken in different seasons, the values for nutrient flush ($\bar{X}ij$) were reduced to the standardized variables $Zij = (\bar{X}ij - \bar{X})/SD$, where $\bar{X}ij$ is the value for soil i in season J, and $\bar{X}$ and SD are the mean and the standard deviation, respectively, for all the samples. The data were processed by a standard analysis of variance and, in cases of a significant F statistic, Tukey's minimum significant difference test was used to separate the means. The percentage of data variation attributable to the type of soil and season was calculated using a two-way analysis of variance.

Results and discussion

Microbial biomass

Microbial biomass C in the soils ranged from 282 to 1275 μg C g^{-1} soil (Fig. 1) and microbial N from 42 to 191 μg N g^{-1} soil (Table 2). Microbial biomass C represented between 0.5 and 2% of the organic soil C but microbial N as a percentage of total soil N was two- to three-fold higher, ranging from 1.5 to 4.5% (Fig. 2). Quantities of P, K, and Ca in the microbial biomass ranged from 33 to 148 μg g^{-1} soil, from 28 to 125 μg g^{-1} soil, and from 4 to 18 μg g^{-1} soil, respectively (Table 2). The levels of C and nutrients in the microbial biomass fell within the range given for other forest ecosystems (Smith and Paul 1990; von Lützow et al. 1992). The microbial C seasonal change was similar in all the soils (Fig. 1); there were two maxima, one in spring (mean 880 μg C g^{-1} soil) and another in winter (mean 787 μg C g^{-1} soil), with summer (mean 589 μg C g^{-1} soil) and autumn (mean 560 μg C g^{-1} soil) values being significantly ($P \leq 0.001$) lower. The annual percentage distribution of the microbial biomass was 31, 28, 21, and 20% for spring, winter, summer, and autumn, respectively. In a spruce forest soil,

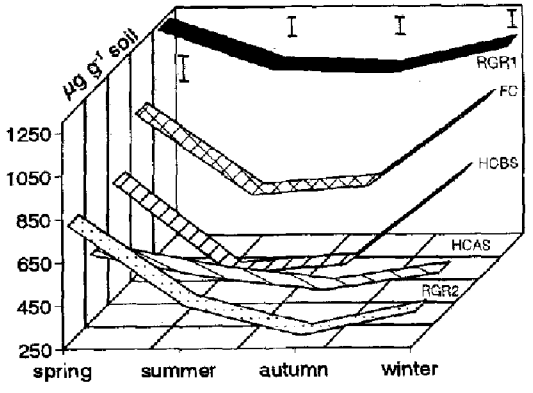

Fig. 1 Seasonal variation in the microbial population biomass. *RGR1 RGR2* Rankers, *HCAS HCBS* Humic Cambisols, *FC* Ferralic Cambisol; minimum significant difference ($P \leq 0.05$) shown as *bars* at all sampling times

Table 2 Carbon and nutrients in the microbial biomass (μg g^{-1} soil). Values are means ± SD

Soil	Season	C	N	P	K	Ca
RGR1	Spring	1275 ± 47	191 ± 7	148 ± 6	125 ± 5	18 ± 1
	Summer	1087 ± 13	163 ± 2	126 ± 2	107 ± 2	15 ± 0
	Autumn	1072 ± 48	161 ± 7	124 ± 5	105 ± 4	15 ± 1
	Winter	1189 ± 40	178 ± 6	138 ± 4	116 ± 4	17 ± 1
RGR2	Spring	793 ± 58	119 ± 9	92 ± 7	78 ± 6	11 ± 0
	Summer	423 ± 27	63 ± 4	49 ± 3	41 ± 3	6 ± 0
	Autumn	282 ± 32	42 ± 5	33 ± 4	28 ± 3	4 ± 1
	Winter	391 ± 46	59 ± 7	45 ± 5	38 ± 4	5 ± 1
HCAS	Spring	555 ± 32	83 ± 5	64 ± 4	54 ± 3	8 ± 1
	Summer	452 ± 33	68 ± 5	52 ± 4	44 ± 3	6 ± 0
	Autumn	387 ± 53	58 ± 8	45 ± 6	38 ± 5	5 ± 1
	Winter	464 ± 27	70 ± 4	54 ± 3	45 ± 3	6 ± 0
HCBS	Spring	782 ± 58	117 ± 9	91 ± 8	77 ± 7	11 ± 1
	Summer	363 ± 38	54 ± 6	42 ± 4	35 ± 4	5 ± 1
	Autumn	399 ± 47	60 ± 7	46 ± 5	39 ± 4	5 ± 1
	Winter	813 ± 59	122 ± 9	94 ± 8	80 ± 7	11 ± 0
FC	Spring	994 ± 33	149 ± 5	115 ± 4	97 ± 3	14 ± 1
	Summer	620 ± 59	93 ± 9	72 ± 9	61 ± 7	9 ± 1
	Autumn	658 ± 46	99 ± 7	76 ± 6	64 ± 5	9 ± 1
	Winter	1078 ± 60	162 ± 9	125 ± 7	106 ± 6	15 ± 1

von Lützow et al. (1992) recorded the highest biomass N values in autumn and spring and the lowest in summer. Ding Ming Mao et al. (1992) found seasonal fluctuations in the collective microbial biomass in tropical forest soils and Söderström (1979) reported fluctuations in the fungal biomass in a Swedish podzolized pine-forest soil. In agricultural soils, several authors (Lynch and Panting 1980; Ross et al. 1981; Granatstein et al. 1987; Van Gestel et al. 1992) observed clear annual variations in the microbial biomass in different soils, while Schnürer et al. (1986), Patra et al. (1990), and Wheatley et al. (1990) found no significant temporal changes. In the present study, the highest microbial biomass level was generally found in the pinewood soil RGR1, followed by FC, HCBS, RGR2, and HCAS.

The variation in microbial biomass values in the present study was largely explained by the type of soil and the time of year. Type of soil which explained 71% of the variance (Table 3), season accounted for 18%, and the interaction between season and soil type explained a further 8%. Among the soil characteristics analyzed (Table 1), pH ($r = 0.82$, $P \leq 0.001$, $n = 16$; soil RGR1 excluded), organic C ($r = 0.82$, $P \leq 0.001$, $n = 20$), total N ($r = 0.79$, $P \leq 0.001$, $n = 20$), and moisture content ($r = 0.78$, $P \leq 0.001$, $n = 20$) were significantly and positively related to microbial biomass. Similar relationships between most of these properties and the microbial biomass have been observed by other authors in several forest (Díaz-Raviña et al. 1988) and cultivated (Ross et al. 1980; Van Gestel et al. 1992) soils. The effect of pH and moisture on the microbial biomass in the present study was similar to that reported for both microbial density and activity in forest soils (Díaz-Raviña et al. 1993c); nevertheless, while microbial numbers were related to organic matter quality (C : N ratio) and available Ca and Mg contents, the micro-

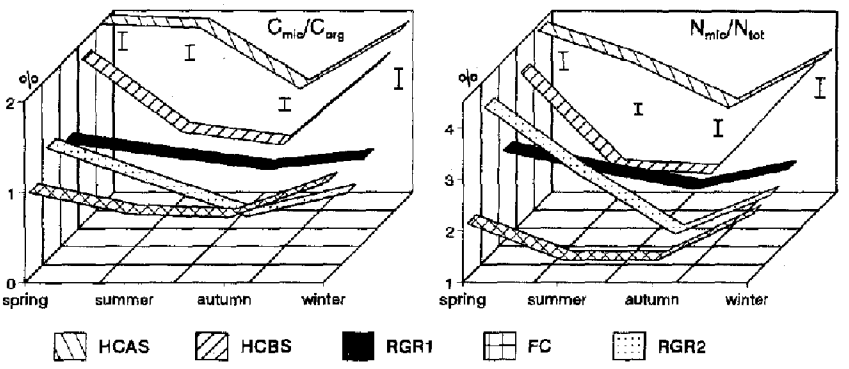

Fig. 2 Seasonal variation in the percentage of microbial C and N with respect to organic C (C_{mic}/C_{org}) and total N (N_{mic}/N_{tot}). For other explanations, see Fig. 1

bial biomass was also affected, as previously reported for microbial activity (Díaz-Raviña et al. 1993c), by organic matter quantity and N content. The microbial biomass annual fluctuation can be partly attributed to the soil characteristics mentioned above. Moisture is particularly important for temperate forest soils because humidity is one of the main factors controlling the microbial population density and distribution (Acea and Carballas 1990). The lack of water seemed to limit the microbial biomass more than temperature, since lower microbial biomass contents were observed in summer than in winter. In addition to these factors, the supply of nutrients or substrate, the relative abundance of the different groups of microorganisms, and other environmental factors may have been involved. Thus, the relatively high level of microbial biomass in spring may have been due to a sudden increase in available substrate derived from roots (Lynch and Panting 1980), which favored the proliferation of microorganisms, whereas the increase in microbial biomass in winter may have been due to a larger proportion of fungi which are

Table 3 Two-way analysis of variance of the effect of soil and season on microbial biomass and flush of nutrients. All analyses significant at $P < 0.0001$

	Source of variation	Degrees of freedom	Sums of squares	Proportion of variation (%)	F ratio
Microbial C	Season	3	1077176	18	155
	Soil	4	4155355	71	448
	Season × soil	12	493095	8	18
Microbial C: organic C	Season	3	3	23	104
	Soil	4	9	62	214
	Season × soil	12	2	12	13
Microbial N: total N	Season	3	18	33	115
	Soil	4	26	47	124
	Season × soil	12	8	16	14
N flush	Season	3	7216	16	510
	Soil	4	32734	73	1735
	Season × soil	12	4436	10	78
P flush	Season	3	421	34	131
	Soil	4	486	40	114
	Season × soil	12	272	22	21
K flush	Season	3	1756	44	187
	Soil	4	784	20	62
	Season × soil	12	1303	33	35
Mg flush	Season	3	106	27	76
	Soil	4	231	59	124
	Season × soil	12	39	10	7
N flush: available N	Season	3	291154	14	104
	Soil	4	422756	16	114
	Season × soil	12	1852447	71	116
P flush: available P	Season	3	6461	30	115
	Soil	4	8966	44	121
	Season × soil	12	4587	22	21
K flush: available K	Season	3	4946	31	147
	Soil	4	7431	46	166
	Season × soil	12	3936	21	25
Mg flush: available Mg	Season	3	1626	11	41
	Soil	4	8227	59	156
	Season × soil	12	3760	26	24

particularly favored by humidity (Acea and Carballas 1990).

Values of microbial C and N, expressed as a percentage of organic C and total N (Fig. 2), respectively, were also higher in spring (mean 1.4% of organic C and 3.4% of total N) and winter (mean 1.3% organic C and 2.9% of total N) and lower in summer (mean 1.1% of organic C and 2.4% of total N) and autumn (mean 0.8% of organic C and 1.9% of total N). The percentage of organic soil C present as microbial C was mainly determined by the type of soil, which accounted for most of the variance (62%), followed by season (23%), and the soil×season interaction (12%), while the variance in the percentage of total N present as microbial N was closely distributed between soil and season (47 and 33%, respectively), with the interaction of both factors explaining a further 16% (Table 3).

Flush of nutrients

A positive flush of decomposition following fumigation and incubation was observed in all soil samples. The flush of N, K, P, and Mg ranged from 7 to 115, from 6 to 35, from 0 to 17, and from 0 to 9 $\mu g\, g^{-1}$ soil, respectively (Table 4). N had the highest flush values (mean 31 $\mu g\, N\, g^{-1}$ soil), followed by K (mean 15 $\mu g\, K\, g^{-1}$ soil), P (mean 7 $\mu g\, P\, g^{-1}$ soil), and Mg (mean 4 $\mu g\, Mg\, g^{-1}$soil), which is in agreement with values reported previously for several forest soils (Díaz-Raviña et al. 1993b), but the mineral N flush was high compared with that for some agricultural soils (Carter and MacLeod 1987).

Significant seasonal changes in the nutrient flush were detected (Fig. 3). The N, P, and Mg flushes (Table 4) reached maxima in spring (mean 34 $\mu g\, N\, g^{-1}$ soil, 11 $\mu g\, P\, g^{-1}$ soil, and 5 μg Mg g^{-1} soil) and autumn (mean 47 $\mu g\, N\, g^{-1}$ soil, 8 $\mu g\, P\, g^{-1}$ soil, and 5 $\mu g\, Mg\, g^{-1}$ soil) and minima in summer (19 $\mu g\, N\, g^{-1}$ soil, 4 $\mu g\, P\, g^{-1}$ soil, and 3 $\mu g\, Mg\, g^{-1}$ soil) and winter (23 $\mu g\, N\, g^{-1}$ soil, 7 $\mu g\, P\, g^{-1}$ soil, and 2 $\mu g\, Mg\, g^{-1}$ soil). The K flush was relatively high in winter and spring and low in autumn and summer (mean 23, 17, 10, and 9 $\mu g\, K\, g^{-1}$ soil, respectively; Table 4). The seasonal distribution of the flush of most nutrients (N, P and Mg) was in keeping with the order followed by the microbial population density (Díaz-Raviña et al. 1993c).

The flush in N, P, and Mg was strongly dependent on soil type, which explained between 40 and 73% of the variation (Table 3), N flush being the most influenced by the soil factor, followed by Mg and P, whereas this factor only explained 20% of the K flush variation. Season also explained a significant percentage of the nutrient flush, explaining from 16 to 44% of the variation; this influence was relatively high for K and P and low for Mg and N. The soil×season interaction explained a further 10–33% of the variation. There was a positive correlation between microbial C and the flush of N ($r = 0.68$, $P \leq 0.001$), P ($r = 0.62$, $P \leq 0.005$), and K ($r = 0.61$, $P \leq 0.005$), and Mg ($r = 0.60$, $P \leq 0.005$). All these relationships were consistent with data previously reported for forest soils (Díaz-Raviña et al. 1993b). Ross et al. (1980) also found a close relationship between biomass C and N flush in grassland soils. The seasonal order for the flushes of N, P, and Mg (autumn ≥ spring > > winter ≥ summer) or the flush of K (winter > spring > autumn ≥ summer) frequently differed

Table 4 Flush of nutrients and flush value relative to the soil content of the corresponding available nutrient. Values are means ± SD

Soil	Season	N flush ($\mu g\, g^{-1}$ soil)	P flush ($\mu g\, g^{-1}$ soil)	K flush ($\mu g\, g^{-1}$ soil)	Mg flush ($\mu g\, g^{-1}$ soil)	Ratio N flush to available N (%)	Ratio P flush to availabe P (%)	Ratio K flush to available K (%)	Ratio Mg flush to available Mg (%)
RGR1	Spring	87±3	13±1	21±2	8±1	394± 14	49±2	53±4	70±5
	Summer	55±3	6±1	12±1	7±1	223± 13	31±3	32±3	21±2
	Autumn	115±4	12±2	6±1	9±1	148± 2	37±6	7±1	23±2
	Winter	50±5	12±1	30±3	5±1	172± 17	73±9	51±4	29±3
RGR2	Spring	24±2	12±2	16±2	5±1	103± 9	38±2	22±3	21±3
	Summer	8±1	3±1	8±2	2±1	383± 29	7±3	35±7	17±8
	Autumn	25±1	4±1	9±2	4±1	180± 4	9±1	12±1	20±3
	Winter	9±1	6±1	14±1	1±1	867±115	37±3	32±2	9±8
HCAS	Spring	15±2	9±2	14±2	4±1	53± 6	30±6	9±1	9±1
	Summer	12±1	0±1	7±2	2±1	253± 23	0±1	11±4	7±3
	Autumn	24±2	11±1	10±1	4±1	160± 13	32±2	8±1	12±1
	Winter	10±1	5±1	7±1	2±1	148± 8	31±3	6±1	4±2
HCBS	Spring	15±1	4±1	15±2	6±2	62± 5	15±4	18±3	7±1
	Summer	7±1	1±1	10±1	1±1	67± 5	2±2	24±1	1±1
	Autumn	36±2	4±1	8±1	4±1	720± 40	15±4	8±1	4±1
	Winter	16±1	4±1	35±4	2±1	283± 16	31±5	39±4	3±1
FC	Spring	29±3	17±2	18±2	2±1	108± 11	63±7	34±4	5±2
	Summer	12±1	9±1	10±1	0±1	240± 20	36±2	47±4	0±1
	Atumn	36±3	10±1	19±2	3±1	92± 7	38±2	25±2	10±3
	Winter	29±2	6±1	29±3	1±1	226± 19	30±5	55±6	5±4

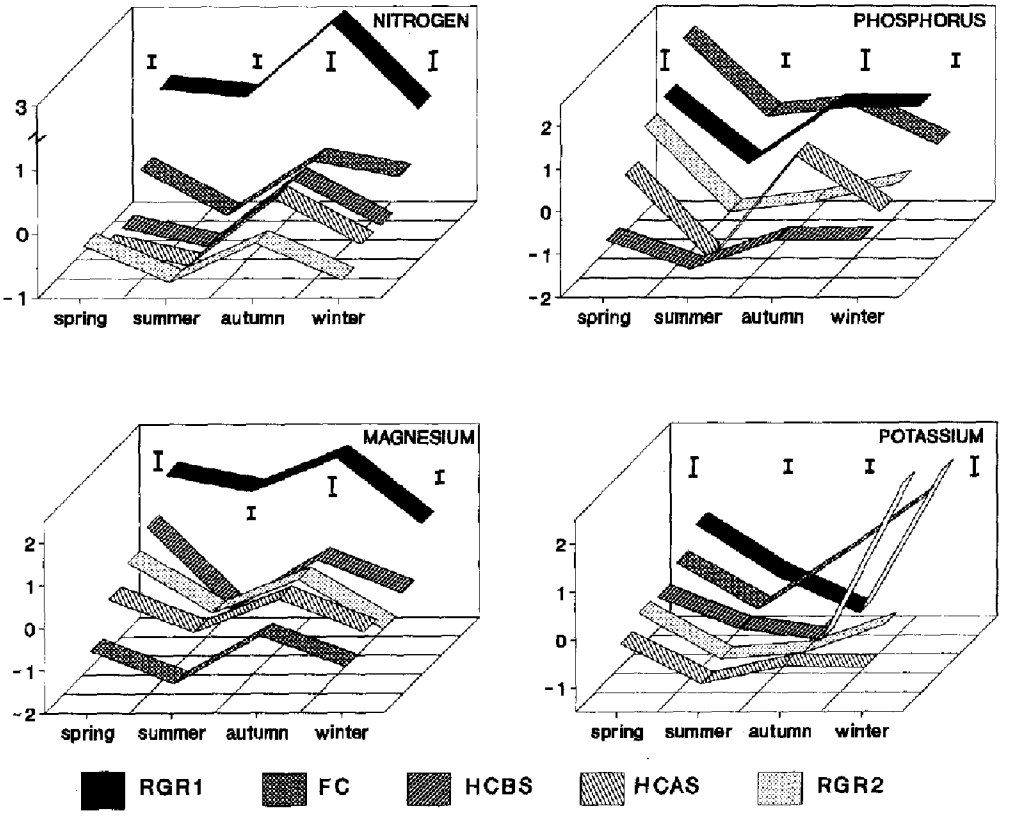

Fig. 3 Seasonal variation (normalized values) in the flush of nutrients. For other explanations, see Fig. 1

from the order of microbial biomass (spring > winter > summer ≥ autumn). This finding suggests that the flush of these nutrients is unsuitable as an index of temporal variations in forest soil microbial biomass; this is in accord with findings by Ross et al. (1981) and Sarathchandra et al. (1984) on N flush as an index of microbial C in cultivated soils.

In the present study, the microbial biomass contributed substantially to plant-available nutrients (Table 4). As a consequence of the mineralization of dead microbial cells, the inorganic N content of the soils increased from 0.5- to 8-fold (mean 2.5) and the increase in the other nutrients ranged from 0 to 73% for P (mean 30%), from 6 to 55% for K (mean 26%), and from 0 to 70% for Mg (mean 14%). Although no uniform trends were apparent, significant seasonal fluctuations in the ratio of each nutrient flush to the available nutrient were detected in the different soils. Generally, the maximum values for N, P, and K were found in winter and the minimum values in spring, summer, and autumn, with no consistent seasonal effect found for Mg (Table 4).

The contribution from the microbial biomass to the plant-available nutrient concentrations varied significantly with the type of soil, the season, and the soil × season interaction. The type of soil, which explained between 16 and 59% of the variation, was the most important factor in the ratios of Mg, P, and K flushes to the corresponding available nutrient concentration, while the interaction between soil and season, which represented from 21 to 71% of the variation, mainly affected the mineral N flush to available N ratio. The season also significantly affected this contribution, particularly for K and P, explaining a further 11–31% of the variability.

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