

Effects of long-term mineral fertilization on microbial biomass, microbial activity, and the presence of *r*- and *K*-strategists in soil

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Abstract Long-term effects of mineral fertilization on microbial biomass C (MBC), basal respiration (R_B), substrate-induced respiration (R_S), β -glucosidase activity, and the *r*-*K*-growth strategy of soil microflora were investigated using a field trial on grassland established in 1969. The experimental plots were fertilized at three rates of mineral N (0, 80, and 160 kg ha⁻¹ year⁻¹) with 32 kg P ha⁻¹ year⁻¹ and 100 kg K ha⁻¹ year⁻¹. No fertilizer was applied on the control plots (C). The application of a mineral fertilizer led to lower values of the MBC and R_B , probably as a result of fast mineralization of available substrate after an input of the mineral fertilizer. The application of mineral N decreased the content of C extracted by 0.5 M K₂SO₄ (C_{ex}). A positive correlation was found between pH and the proportion of active microflora (R_S /MBC). The specific growth rate (μ) of soil heterotrophs was higher in the fertilized than in unfertilized soils, suggesting the stimulation of *r*-strategists, probably as the result of the presence of available P and rhizodepositions. The cessation of fertilization with 320 kg N ha⁻¹ year⁻¹ (NF) in 1989 also stimulated *r*-strategists compared to C soil, probably as the result of the higher content of available P in the NF soil than in the C soil.

Keywords Mineral fertilization · Microbial biomass · Respiration · Growth curves · *r*-Strategy

Introduction

Input of mineral fertilizers to soil can affect the biomass and composition of soil microflora (Chu et al. 2007). Microorganisms are indispensable for key soil functions like decomposition of soil organic matter, degradation of xenobiotics, and formation of soil aggregates (Sparling 1997; Stenberg 1999). They can also act as a source and sink for plant nutrients (Houot and Chaussod 1995; Salinas-Garcia et al. 1997). Contradictory results have been reported on the effect of fertilization on biomass and activity of soil microflora (Moore et al. 2000; Dodor and Tabatabai 2005; Majumder et al. 2007), and are due to various environmental variables like soil texture and carbon content (Böhme and Böhme 2006; Lugato et al. 2006), plant cover (Dijkstra et al. 2005), and the period of fertilization (Liu et al. 2007). Input of fertilizer can stimulate passage from dormant to active microbial cells (Lugato et al. 2006), which can affect nutrient cycling in soil. Fertilizers can have both direct and indirect effects on soil microflora (Šimek et al. 1999). The direct effects can include enhanced nutrient availability, accumulation of toxic metals (Šimek et al. 1999), and shift in soil pH with negative effects on soil microflora (Dodor and Tabatabai 2005; Chu et al. 2007; Enwall et al. 2007; Liu et al. 2007). Indirect effects are connected with the stimulation of plant growth, with increases in rhizodepositions and plant residues input to soil and thus stimulation of growth and activity of microorganisms of fertilized soil (Salinas-Garcia et al. 1997; Wu et al. 2004; Dodor and Tabatabai 2005; Chu et al. 2007). However, too high amounts of fertilizer can have negative effects by stressing soil microorganisms (McCarty and Meisinger 1997).

The content of soil microbial biomass and C mineralizing activity have been widely used to determine the effect

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of fertilization on soil microflora. Both positive (Majumder et al. 2007; Prakash et al. 2007) and negative (Bittman et al. 2005; Dijkstra et al. 2005) effects of fertilization on these microbial properties have been reported. Acosta-Martínez et al. (1999), Moore et al. (2000), and Liu et al. (2007) reported that high rates of mineral fertilizer had no influence on microbial biomass and microbial activity.

Enzyme activities can be measured to assess the effects of soil management on microbial activity (Dodor and Tabatabai 2005). The activity of β -glucosidase is involved in the degradation of cellulose to glucose (Böhme and Böhme 2006), and both positive and negative effects of fertilization have been reported on this enzyme activity (Acosta-Martínez et al. 1999; Marx et al. 2005).

Soil microorganisms can be classified as *r*- and *K*-strategists, with the former growing fast when substrate is abundant, whereas the latter can grow when resources are limited (van Elsas et al. 2007). Because fertilization leads to higher fluctuation of the substrate, it may have an influence on the distribution of *r*- and *K*-strategists in soil, and this can influence the nutrient flow through microflora.

Long-field trials are important for evaluating the long-lasting effects of agricultural practices on soil properties including microbial biomass and microbial activity (Liu et al. 2007). The aim of this investigation was to determine the effects of long-term mineral fertilization at four rates on soil microbial biomass, basal respiration, substrate-induced respiration, β -glucosidase activity, and on the growth strategy of soil microflora. Soils of plots fertilized since 1969 were sampled in 2003, 2004, and 2005. A soil where application of fertilizers was ceased in 1989 after 20 years of fertilization was also monitored. The soil microbial biomass was chosen as a labile pool of soil organic matter and respiration as a parameter reflecting C mineralizing capacity (Dilly et al. 2003). The β -glucosidase activity was selected as it can be an indicator of increasing soil organic matter of plant origin (Acosta-Martínez et al. 1999). The specific growth rate and the time to reach the maximum respiration rate after substrate addition to soil were calculated from respiration growth curves and used to indicate the predominance of *r*–*K* strategists.

Materials and methods

Field experiment

We sampled soils from a long-term experiment involving the effects of fertilization on production and quality of fodder and soil quality. The experimental plots are situated at Závěšín (latitude 49°59', longitude 12°45', altitude 750 m a.s.l.) near Mariánské lázně (Czech Republic) in an area with a moderately cold and wet climate with a mean annual

temperature of 6.4°C and a mean annual rainfall of about 700 mm (measurements in the vicinity of the plots). Soil is a sandy loam classified as Haplic Cambisol (Dystric) (IUSS Working Group WRB 2006) with C_{org} 2.57%, N_{tot} 0.21%, and pH_{KCl} 3.9. A commercial mixture for grassland with predominance of meadow fescue (*Festuca pratensis* Huds.) and timothy (*Phleum pratense* L.) was seeded after reclamation in 1968 when the experiment was set up. The experiment consisted of a completely randomized block design with four replicates. Each plot is a rectangle with an area of 15 m² (2.5×6.0 m).

The treatments can be grouped in three different periods:

1. 1969–1989: the control plots (C) were not fertilized. The other plots were fertilized with 32 kg P ha⁻¹ as superphosphate, 100 kg K ha⁻¹ as KCl, and with N fertilizer at four rates: no N (PK), 80 kg ha⁻¹ (80 N), 160 kg ha⁻¹ (160 N), and 320 kg ha⁻¹ (NF). Ammonium nitrate with limestone was used as a nitrogen fertilizer. Limestone was not applied in the control plots. Fertilization was performed at the beginning of a growing season.
2. 1990–1994: all plots were not fertilized, and the sward was cut once in a year.
3. After 1995: fertilizers were applied at the same rate and modality as they were applied in the period 1969–1989 except the fertilization with 160 kg N ha⁻¹, which was split in two doses of 80 kg N ha⁻¹ (the first in spring and the second at the first harvest), and the treatment NF which was not fertilized.

During the years of sampling, harvests were carried out twice a year on 14/6/2003, 24/08/2003, 19/06/2004/, 11/09/2004, 14/06/2005, and on 30/08/2005. The yield of aboveground plant biomass was determined at each harvest.

Sampling, sieving, and storage of soil

Soil was sampled by a corer (0–15 cm) on 22/07/2003, 19/07/2004, and 20/7/2005. Ten subsamples were taken out from each plot, pooled, and transported to a laboratory. Soil was sieved (<2 mm) and stored at 4°C. Microbial analyses were carried out within 6 weeks on moist soil, whereas a portion of soil was dried at room temperature prior to chemical analyses. Organic carbon was determined after soil grinding and successive sieving at 0.25 mm sieve.

Chemical analyses

Chemical analyses were performed with soil samples taken in 2004, whereas soil moisture and water holding capacity were determined at each sampling date. Moisture was assessed as a weight decrease after 6-h drying at 105°C, and the water content was expressed as a percentage of the

dry weight. Water-holding capacity was determined according to ISO 11274 (1998a). The pH was determined according to ISO 10390 (1994). Soil (5 g) was shaken with 25 ml 1 M KCl for 5 min, and then pH was recorded by a glass electrode after 2-h standing.

Organic C (C_{org}) was determined by wet digestion of 1 g soil sample with 5 ml 0.27 M $\text{K}_2\text{Cr}_2\text{O}_7$ solution and 7.5 ml concentrated H_2SO_4 according to ISO 14235 (1998b). Total N (N_{tot}) was determined by treating soil (5 g) with 13 ml of the mineralizing mixture containing 10 g of selenium mixture (Merck) and 10 g salicylic acid in 1 l of concentrated H_2SO_4 . After 12 h, H_2O_2 (30%) was added, and the samples were mineralized for 2 h at 420°C. Soil N was determined after distillation into boric acid (Kjeltec Auto 1030 Analyzer, Tecator; Novozámský et al. 1983).

Mineral N was extracted by shaking soil (10 g on dry basis) with 1 M KCl (1:5 w/v) for 1 h. The suspension was centrifuged for 3 min at 9,000×g. Ammonium was determined spectrophotometrically at 660 nm by a reaction with salicylate and hypochlorite (Forster 1995). Nitrates were measured directly at 210 nm by subtracting the absorbance of the extract reduced with a Cu/Zn reductor (Kandeler 1993). A spectrophotometer UV2 ATI Unicam was used for both measurements. Macroelements were determined after their extraction from soil with a Mehlich III extraction solution (Mehlich 1984). An AAnalyst 400 (Perkin-Elmer) spectrophotometer was used for determination of Mg, Ca (atomic absorption spectrometry), and K (atomic emission spectrometry). Phosphorus was determined at 750 nm using a UV-Cary 50 Conc (Varian) spectrophotometer.

Microbial analyses

Determination of soil microbial C and N biomass (MBC, MBN) was performed by the fumigation–extraction method according to ISO 14240-2 (1997) with three replicates. Soil (10 g on dry basis) was preincubated 1 day at 25°C, and the water content was adjusted to 35% of water holding capacity (WHC) if the natural water content was lower than 30% of WHC. Soil was fumigated with ethanol-free chloroform for 24 h prior to being extracted with 40 ml 0.5 M K_2SO_4 . Unfumigated soils were also extracted with 0.5 M K_2SO_4 . Organic C in the extracts was estimated in fumigated and nonfumigated samples photometrically by the dichromate-oxidation method (Yakovchenko and Sikora 1998). The content of organic C of the nonfumigated soil (C_{ex}) was considered the labile fraction of soil C. Nitrogen in the extracts was estimated as nitrate after alkaline persulfate oxidation (Cabrera and Beare 1993; Kandeler 1993). Coefficients $k_{\text{C}}=0.38$ and $k_{\text{N}}=0.45$ were used to calculate microbial

biomass C and N (Joergensen 1995). The percentage of C_{org} presents as microbial biomass C was calculated. Basal respiration (R_{B}) and substrate-induced respiration (R_{S}) were estimated by incubating soil samples (15 g dry weight) in 350 ml flasks. At the beginning of the 6-day pre-incubation period, soil samples were adjusted to a water content of 60% of WHC for the R_{B} experiment or to 40% WHC for the R_{S} experiment when soil moisture was below these values. This allowed the effective mixing of the soil samples with substrate, which was added at a rate of 10 mg g⁻¹ (8.42 g of glucose, 1.37 g of ammonium sulfate, and 0.21 g of potassium dihydrogen phosphate). A preliminary experiment was performed to find the amount of added substrate giving the maximum response. Samples were incubated for 3 days (R_{B}) and for 10 h (R_{S}) at 22°C, and CO_2 was absorbed in 0.1 M NaOH and quantified by titration (ISO 16072, 2002a). The metabolic quotient $q\text{CO}_2$, the specific respiration of the microbial biomass (R_{B}/MBC), was calculated.

Oxitop®-C measuring heads Wissenschaftlich-Technische Werkstätten (WTW) were used to measure respiration curves. This system determines the pressure decrease corresponding to the consumed oxygen during respiration while released CO_2 is trapped into a solution of NaOH. Estimation was performed in flasks (500 ml) by incubating 15 g of soil (on dry basis) in two replicates for 96 h. Temperature, water content, the amount of substrate, and the period of preincubation were the same as for measurement of R_{S} . The decrease of pressure was recorded every 15 min, and the equation of state of ideal gas was used for calculating the consumed oxygen (ISO 16072, 2002a). The specific growth rate (μ) and the time to reach the maximum respiration rate (t_{peakmax}) after the substrate addition were calculated from the plot of the logarithm to the base 2 of the respiration rates according to ISO 17155 (2002b).

Activity of β -glucosidase was measured according to Alef and Nannipieri (1995) with three replicates. Moist soil (1 g) was weighed in 100 ml Erlenmeyer flasks and treated with 0.25 ml toluene, 4 ml modified universal buffer (MUB), and 1 ml of *p*-nitrophenyl- β -D-glucopyranoside (PNG). The content was mixed, and samples were incubated for 1 h at 37°C. In the control, the PNG was added immediately after incubation. Immediately after incubation, 1 ml 0.5 M CaCl_2 and 4 ml 0.1 M Tris buffer (pH 12) were added to the soil suspension, which was filtered before reading the absorbance at 400 nm. The calibration curve was prepared by using *p*-nitrophenol. The MUB buffer was prepared by diluting 100 ml of MUB stock solution with water to 500 ml and adjusting the pH value of the solution at 6.0 with 0.1 M HCl; 500 ml of MUB stock solution contains 6.05 g Tris, 5.8 g of maleic acid, 7.66 g citric acid monohydrate, and 3.15 g of boric acid.

Statistical analysis

Data were evaluated by the Statistica 6.0 (StatSoft, Inc., Tulsa, USA) software, and analysis of variance (treatment, block, year) analysis was performed. If a significant difference among treatments was detected, the Tukey test for multiple comparison was performed. The comparisons among the treatments C, PK, 80 N, 160 N, and between the C and NF treatments were performed separately. The correlation coefficients were computed as Pearson's product-moment correlation. Only significant correlation coefficients at $p < 0.05$ are discussed. The linear trend analysis was calculated according to Quinn and Keough (2005) by the GenStat (VS International Ltd., UK) software.

Results and discussion

Chemical analysis and plant cover data

The P content increased in PK, 80 N, and 160 N plots, in comparison with C treatment (Table 1). The significantly increased content of available K in PK soil in comparison with 80 N and 160 N treatments was caused by lower plant biomass which resulted in lower uptake of K. The pH values and both Ca and Mg contents did not differ in control plots and in fertilized plots (Table 1). The content of both C_{org} and N_{tot} was not influenced by fertilization. Similar results were described by Dolan et al. (2006). On the contrary, Zanatta et al. (2007) showed an increase in the C_{org} in fertilized plots. We did not observe any increase in C_{org} of soil because plant residues were removed, and the only C input by rhizodeposition (Lugato et al. 2006) was not high enough. Both plots fertilized with N, but not soil fertilized with PK, were characterized by significantly lower C_{ex} (Table 1), which may be the result of higher C mineralization after N addition to soil. Mazzarrino et al. (1993) and Majumder et al. (2007) suggested that application of readily available N could enhance consumption of water-soluble C.

A decreasing trend ($p < 0.05$) in water holding capacity in row $C > PK > 80 N > 160 N$ was detected (Table 1). Aboveground plant biomass at the first harvest was significantly higher on the plots with the highest levels of fertilizers (80 N, 160 N), while the positive effects of fertilization on the yield of the second harvest detected in the 160 N treatment were due to the second application (80 kg N ha⁻¹) after the first harvest. Both ammonium and nitrate were significantly higher in 160 N soil than in the other treatments as the result of the second mineral fertilization.

The content of available P, nitrates, and C_{ex} increased in NF soil in comparison with the C treatment (Table 2). No difference was found between both treatments in pH values.

Table 1 Soil chemical properties and plant aboveground biomass

Treatment	Moisture (%)	WHC (%)	pH	Yield 1 (t _{dw} ha ⁻¹)	Yield 2 (t _{dw} ha ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Ca (mg kg ⁻¹)	C_{org} (%)	C_{ex} (μg g ⁻¹)	N_{tot} (%)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
C	25.9 a (8.6)	69.5 a (3.4)	3.9 (0.1)	1.76 a (0.47)	1.19 a (0.56)	24.5 a (12.0)	59.8 a (3.1)	107.0 (36.7)	1171 (245)	2.86 (0.21)	104 a (18)	0.22 (0.02)	8.27 a (4.38)	0.30 a (0.18)
PK	21.5 b (6.3)	67.4 ab (2.9)	3.8 (0.1)	2.43 a (0.98)	1.31 a (0.29)	178.0 b (45.3)	149.8 b (38.2)	80.8 (15.8)	1245 (213)	2.43 (0.36)	106 a (16)	0.21 (0.02)	7.22 a (2.36)	1.34 a (0.82)
80 N	22.6 b (7.4)	64.6 bc (2.2)	4.0 (0.1)	3.80 b (0.57)	1.07 a (0.33)	174.5 b (49.4)	88.0 a (9.6)	98.8 (21.4)	1318 (70)	2.46 (0.23)	92 b (11)	0.21 (0.01)	7.66 a (2.46)	1.70 a (1.30)
160 N	21.8 b (6.9)	62.7 c (3.5)	4.0 (0.2)	3.36 b (0.85)	2.09 b (0.43)	145.3 b (24.3)	61.3 a (11.4)	87.5 (18.9)	1410 (294)	2.39 (0.27)	89 b (13)	0.21 (0.01)	17.8 b (7.2)	8.97 b (6.85)

Data represent means, and values in brackets are standard deviations ($N=4$ for pH, P, K, Mg, Ca, C_{org} , N_{tot} , $N=12$ for the other properties). Different letters indicate statistically different values (Tukey honestly significant difference (HSD), $p < 0.05$). The C treatment is no fertilizers, PK is no N fertilizer but only PK fertilizers, 80 N is PK+80 kg N ha⁻¹ year⁻¹, 160 N is PK+160 kg N ha⁻¹ year⁻¹

C_{org} organic carbon, C_{ex} extractable carbon, N_{tot} total nitrogen, Yield 1 plant aboveground biomass at the first harvest, Yield 2 plant aboveground biomass at the second harvest

Microbial analysis

Both microbial biomass C (MBC) and N (MBN) decreased by increasing rate of applied fertilizer (Table 3). Indeed, this effect was highest in the 160 N treatment, where the decrease in MBC was 20.5% compared to the control treatment. According to Kautz et al. (2004), these lower values probably depend on the lack of microbial available substrate, nutrients despite the soils did not differ in the content of C_{org} , and the yield was significantly higher in the 160 N plots than in the control plots at both harvests. Such a decrease may occur after an initial boost in mineralization activity.

Dilly (2006) suggested that a high input of fresh and easily decomposable organic matter to soil can stimulate copiotrophic (zymogenous) microorganisms, that is, the r -strategists. On the contrary, K -strategists prevail in nutrient-poor soils, and they use recalcitrant organic matter. The type of respiration curves show the prevalence of one of two strategists. The specific growth rate μ was lower in the C soil than in the fertilized soils, and the parameter $t_{peakmax}$ decreased in the fertilized soils. Microbial communities of soils with lower $t_{peakmax}$ and higher μ were probably able to react faster to available substrate and can be considered r -strategists. A correlation analysis showed that μ and $t_{peakmax}$ were significantly influenced by the available P ($r=0.64$, $p<0.01$ for μ ; $r=-0.78$, $p<0.0001$ for $t_{peakmax}$) and by the crop yield at the first harvest ($r=0.51$, $p<0.05$ for μ ; $r=-0.72$, $p<0.001$ for $t_{peakmax}$), indicating that P fertilization and higher rhizodeposition favored r -strategists. This confirms what was reported by Enwall et al. (2007), who found that a lower proportion of r -strategists was associated with a low rhizodeposition. Because P content and the crop yield at the first harvest were correlated ($r=0.70$, $p<0.001$), we have carried out a partial correlation analysis with each of the two parameters with μ and $t_{peakmax}$ while holding constant the value of the other. This analysis revealed that a significant fraction of variance of μ and $t_{peakmax}$ was explained by P ($r=0.46$, $p<0.05$ for μ ; $r=-0.55$, $p<0.05$ for $t_{peakmax}$) after the effect of the plant biomass at the first harvest was taken into account. On the contrary, no relationship between the crop yield at the first harvest and the parameters of the growth respiration curves was found if the effect of P on these relationships was controlled.

Soil respiration, which reflects the activity of a major part of the soil microbial community, was steadily decreasing by the increasing rate of applied fertilizer ($p<0.05$), and the lowest values of respiration were found in the 160 N soil (Table 3). This difference was probably caused by different microbial biomass content, since respiration significantly correlated with MBC ($r=0.75$, $p<0.001$) and MBN ($r=0.90$, $p<0.00001$). The respiration values during

Table 2 Chemical properties of the NF soil fertilized with PK+320 kg N ha⁻¹ year⁻¹ from 1969 to 1989 and plant aboveground biomass at the first (Yield 1) and at the second (Yield 2) harvest

Moisture (%)	WHC (%)	pH	Yield 1 (t _{d.w.} ha ⁻¹)	Yield 2 (t _{d.w.} ha ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Ca (mg kg ⁻¹)	C _{org} (%)	C _{ex} (μg g ⁻¹)	N _{tot} (%)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
23.7 (8.8)	64.2 a (2.5)	4.0 (0.6)	1.62 (0.43)	0.85 a (0.30)	66.5 a (17.9)	54.0 (3.9)	63.3 (9.9)	1090 (117)	2.72 (0.12)	113 a (12)	0.21 (0.01)	9.19 (6.21)	1.88 a (1.67)

Data represent means, and values in brackets represent standard deviations ($N=4$ for pH, P, K, Mg, Ca, C_{org} , N_{tot} , $N=12$ for the other properties). Values marked with the letter a are significantly different from the values measured in the unfertilized soils ($p<0.05$)

C_{org} organic carbon, C_{ex} extractable carbon, N_{tot} total nitrogen

Table 3 Microbial soil properties

Treatment	MBC ($\mu\text{g g}^{-1}$)	MBN ($\mu\text{g g}^{-1}$)	R_B ($\mu\text{g CO}_2$ $-\text{C g}^{-1}\text{ h}^{-1}$)	R_S	Percentage of C_{org} present as MBC	$q\text{CO}_2$ ($\mu\text{g CO}_2$ $\text{C h}^{-1}\text{ mg MBC}^{-1}$)	R_S/MBC	μ (h^{-1})	t_{peakmax} (h)	β -Glucosidase ($\mu\text{g PNF gh}^{-1}$)	MBC/ MBN
C	424 a (94)	109 a (14)	1.38 a (0.14)	8.48 (2.27)	1.48 (0.30)	3.40 (0.79)	21.6 (10.0)	0.131 a (0.035)	31.3 a (1.8)	258 (37)	3.87 (0.62)
PK	405 ab (76)	95 ab (13)	1.26 a (0.15)	9.04 (1.43)	1.68 (0.31)	3.18 (0.51)	23.0 (5.8)	0.196 b (0.019)	27.3 b (1.5)	265 (39)	4.27 (0.57)
80 N	382 ab (88)	91 bc (16)	1.25 a (0.18)	9.09 (1.65)	1.56 (0.36)	3.44 (1.05)	24.8 (6.5)	0.190 b (0.014)	26.4 b (0.9)	267 (40)	4.24 (0.88)
160 N	337 b (78)	77 c (16)	1.07 b (0.14)	8.01 (2.20)	1.41 (0.31)	3.32 (0.79)	25.1 (9.2)	0.196 b (0.025)	27.4 b (1.4)	249 (55)	4.43 (0.88)

Data represent means and values in brackets are standard deviations ($N=12$). Different letters indicate statistically different results (Tukey HSD, $p<0.05$). The C treatment is no fertilizers, PK is no N fertilizer but only PK fertilizers, 80 N is PK+80 kg N $\text{ha}^{-1}\text{ year}^{-1}$, 160 N is PK+160 kg N $\text{ha}^{-1}\text{ year}^{-1}$

MBC microbial biomass carbon, MBN microbial biomass nitrogen, R_B basal respiration, R_S substrate-induced respiration, μ specific growth rate, t_{peakmax} the time to reach the maximum respiration rate after addition of substrate to soil

Table 4 Microbial soil properties of the NF soil fertilized with PK+320 kg N $\text{ha}^{-1}\text{ year}^{-1}$ from 1969 to 1989

MBC ($\mu\text{g g}^{-1}$)	MBN ($\mu\text{g g}^{-1}$)	R_B ($\mu\text{g CO}_2$ $-\text{C g}^{-1}\text{ h}^{-1}$)	R_S	Percentage of C_{org} present as MBC	$q\text{CO}_2$ ($\mu\text{g CO}_2$ $-\text{C h}^{-1}\text{ mg MBC}^{-1}$)	R_S/MBC	μ (h^{-1})	t_{peakmax} (h)	β - Glucosidase ($\mu\text{g PNF gh}^{-1}$)	MBC/ MBN
373 (67)	92 a (14)	1.23 a (0.14)	6.99 (2.12)	1.37 (0.23)	3.39 (0.57)	19.3 (6.5)	0.188 a (0.026)	28.7 a (1.6)	223 (47)	4.05 (0.49)

Data represent means and values in brackets are standard deviations ($N=12$). Values marked with the letter a are significantly different from the values measured in the unfertilized soils ($p<0.05$)

MBC microbial biomass carbon, MBN microbial biomass nitrogen, R_B basal respiration, R_S substrate-induced respiration, μ specific growth rate, t_{peakmax} the time from addition of growth substrate to the maximum respiration rate

the growing season may have been affected by a lack of nutrients due to competition with plants (Böhme and Böhme 2006), but this was probably not the cause since no differences in qCO_2 were found, and this may mean that no differences in microbial stress among investigated soils occurred. However, according to Wardle and Ghani (1995), data in qCO_2 should be interpreted with caution because it can be affected by factors other than microbial stress. The low content of C_{ex} was related to the high values of R_S/MBC ($r=0.64$, $p<0.01$), indicating that the labile organic matter of soils fertilized with N was mostly mineralized by r -strategists. According to Dilly et al. (2003), the ratio R_S/MBC can be an indicator of the proportion of the active biomass since high values of R_S/MBC are typical for r -strategists (Dilly 2006). The negative relationship between C_{ex} and the crop yield at both the first ($r=0.54$, $p<0.05$) and the second ($r=0.57$, $p<0.05$) harvest are probably due to fast mineralization of the available substrate occurring in these soils. Despite the narrow range of pH values (from 3.6 to 4.1), the pH value was significantly correlated by the ratio R_S/MBC ($r=0.58$, $p<0.01$). It suggests faster utilization of labile organic matter by soil microflora in less acid soils, which may explain the lower content of C_{ex} of these soils ($r=0.92$, $p<0.00001$). Slightly higher pH values in the 80 N and 160 N plots were due to the application of limestone with N fertilizer. A positive correlation between P and the ratio MBC/MBN ($r=0.49$, $p<0.05$) may indicate more fungal dominated biomass in soils with the higher content of available P (Nannipieri et al. 2003). The β -glucosidase activity was not influenced by fertilization, and it was positively correlated with R_S ($r=0.85$, $p<0.00001$), probably because this enzyme activity is affected by active microbial biomass, as reported by Dodor and Tabatabai (2005). The percentage of C_{org} present as MBC was correlated with the content of available K ($r=0.73$, $p<0.001$), but no difference between the control soil and the fertilized soil was detected.

A comparison of soil properties between the NF and C treatments revealed that the effects of the mineral fertilizer on soil microflora may be detected 15 years after cessation of fertilization. The increased content of available P in the NF soil (Table 2) probably explained the higher values of μ and the lower values of $t_{peakmax}$ in this soil (Table 4), which indicated a shift of the soil microflora to the r -strategy. The slightly higher content of labile organic matter in the NF soil suggests rather lower mineralization activity of this soil. The lower values of MBN and R_B in the NF soil than those of C plots may be caused by a lower crop yield at the second harvest in the NF plots. However, it is difficult to explain the lower yields in the NF than C plots since the contents of available nutrients, except that for P, are not different between C and NF treatments.

Conclusions

Long-term mineral fertilization increased the proportion of r -strategists in soil, probably due to increases in available P and rhizodepositions. A positive effect of mineral N on decomposition of the labile soil organic matter probably occurred. The effects of mineral fertilization were detected 15 years after cessation of the application of mineral fertilizers. Future research should investigate microbial diversity so as to assess if the changes in the population of r - K strategists are linked to either changes in the composition of microbial communities or by transition between the r and K states.

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