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Soil Microbial Communities and Enzyme Activities after Long-Term Application of Inorganic and Organic Fertilizers at Different Depths of the Soil Profile

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Abstract: Fertilization is a key factor for sustaining productivity in agroecosystems. A long-term experiment in cambisol following periodical application of several types of fertilization has been running at the experimental site since 1954. In this study, we determined the impact of applied inorganic and/or organic fertilizers on the activity of soil enzymes and on the structure of microorganisms at depths of 0–30 cm and 30–60 cm. Single-factor comparison showed that use of inorganic and/or organic fertilizer had an insignificant effect on the activities of soil enzymes (at depths 0–30 cm and 30–60 cm) and also on the structure of microbial communities at both depths studied. Only soil respirations exhibited stimulation by combined fertilization. The results, irrespective of sampling depth (0–60 cm), showed that application of combined organic and inorganic fertilization stimulated the activity of glucosidases and use of inorganic fertilizer inhibited the activity of arylsulphatases. Respirations were stimulated by application of organic fertilizer and combined fertilization. Nevertheless, principal component analyses, which calculate with multidimensional data, revealed differences in samples treated by sole mineral fertilizer compared to other variants, especially in the lower layer. In general, our results indicate that use of combined fertilization may improve biological characteristics in deeper parts of soil profile and possibly increase biological activity in agroecosystems.

Keywords: soil microorganisms; phospholipid fatty acids; soil enzyme activities; agricultural soil fertilization

1. Introduction

Agricultural land shows a high level of spatial variability, including variability in physicochemical characteristics of soil and management of agroecosystems [1,2], which can influence activity and composition of soil biota [3–5]. Soil is a very complex system in which plants and microorganisms collaborate to obtain sources of water and nutrients. These forms of life cooperate with one another and, together with soil, they create continually changing conditions [6]. Microbial biomass is the main living part of soil organic material which is responsible for maintaining and improving soil quality [7].

Soil organic mass is an important aspect for the function and quality of soil. High content of soil organic matter (SOM) can increase supply of nutrients [8] and improve physical and biological characteristics of soil [9,10], therefore maintaining SOM is important for preserving the productivity

of agroecosystems. Many ways of farming in agriculture, such as fertilization or cultivation of land, significantly influence SOM [11,12]. Quantity of SOM is mostly estimated by content of soil organic carbon (SOC), and changes in SOC influence the cycle of C and N in terrestrial ecosystem [13,14]. Changes of organic C and N fractions in organic substances are the result of combined effects of chemical and biological characteristics of the soil [15].

Fertilization is an important agricultural practice for improving nutrition of plants, reaching high yield, and also for changes of soil environment—such as the chemistry of soil carbon and nitrogen [16], which can change the activity and diversity of soil microorganisms [17,18]. Former studies have shown that fertilization influences and changes to the microbial community in nitrogenous bacteria [19], methanotrophs [20], and cellulotic bacteria [21]. Microorganisms may react to changing conditions of soil environment by adjustment of composition in microbial community and total quantity of microbial biomass [22]. Moreover, changes in microbial community influence transformation of C and N in the soil ecosystem [14,23]. Diversity of microbial biomass can possibly be a valuable indicator of soil quality and soil health [18,24].

Various microbial communities are responsible for specific functions in decomposition of organic mass. Bacteria dominate in initial phases of decomposition of plant residues, whereas fungi prevail in later phases [25]. Saprophytic fungi are an important source of soil oxidation enzymes [23].

Soil extracellular enzymes are synthesized and secreted by soil microorganisms, and they are agents for creation and decomposition of organic material [26]. Soil enzymes are dominantly hydrolases, which help to acquire carbon, nitrogen, and phosphorus for support of primary metabolism; or oxidoreductases, which contribute to decomposition of organic compounds [27]. Measured activities of such enzymes reflect the intensity and direction of various biochemical processes in soil environment and can be used for evaluation of microbial demands for nutrients and for expressing the response of ecosystem, which reflects changes in the environment as such [28,29].

The goal of our study was to quantify the influence of long-term application of inorganic fertilizer (NPK), organic fertilizer (farmyard manure, FYM), and their combination (NPK + FYM) on the structure of soil microbial communities and activities of representative soil enzymes below vegetation of sugar beet. Our hypothesis was that the combined application of FYM+NPK positively influence the activity of soil enzymes, even in the depth of 30–60 cm.

2. Materials and Methods

2.1. Site Description

The Ruzyně long-term fertilizer experiment was located in the western part of Prague (Central Europe, temperate climate zone, 50°05′15′′ N and 14°17′28′′ E) and was established in 1954. The annual average temperature and precipitation were 9.9 °C and 470 mm, respectively (1954–2015, Prague–Ruzyně weather station). The altitude of the experiment site is 370 m above sea level. The soil type is classified as haplic Luvisol (World Reference Base). The initial soil pH at the beginning of the experiment was 6.5 in the top 20 cm layer.

2.2. Experimental Design

The long-term experiment was made up of five fields (I, II, III, IV, and V). Each field comprised of 96 plots (12 × 12 m), where 24 different fertilizer treatments are tested. Each treatment was replicated fourfold, arranged in a completely randomized design. The subject of this study was field number III. Its 9-year crop rotation consists of alfalfa, alfalfa, winter wheat, sugar beet, spring barley, potatoes, winter wheat, sugar beet, and spring barley. The sugar beet was grown in the 2017 season, and in September 2017 the sampling for microbial analyses and enzymatic activities determinations were carried out. The growing stage of sugar beet at the time of harvesting was BBCH 39 (Biologische Bundesanstalt, Bundessortenamt and CHemical industry), leaves covered more than 90% of the ground.

Out of 24 fertilizer treatments, we analyzed four treatments for the purpose of this paper. The treatments were unfertilized control (Control), mineral fertilizers (NPK), farmyard manure (FYM), and farmyard manure with NPK (FYM + NPK). Fertilization has been carried out periodically every year since 1955 at the same times and established doses. The FYM was applied in October prior to middle tillage at a dose of 21 t ha^{-1}. The content of nutrients in the FYM is 105 kg N, 39.4 kg P, and 123.9 kg K in the dose of 21 t of the FYM. The minerals N, P, and K were applied at the doses 200, 80, and 200 kg ha^{-1}, respectively, as calcium ammonium nitrate (27% N), superphosphate (8.3% P), and potassium chloride (49.8% K), respectively. Nitrogen fertilizers were applied in the spring before sowing and during vegetation according to the state of the stand. P (superphosphate) and K (potassium chloride) fertilizers were applied in the autumn.

2.3. Sampling and Soil Parameters

The soil samples were taken from two different depths, 0–30 cm and 30–60 cm (four samples from each depth and each replication). After withdrawal, the samples were stored in a mobile refrigerator, then sieved through a 2 mm sieve and stored in a refrigerator at 4 °C. Table 1 shows basic chemical parameters, including the concentrations of available nutrients. Soil pH was measured in suspensions of soil—water and soil: 1 M KCl at a ratio of 1:2.5. Concentrations of available nutrients (Ca, K, Mg, and P [30]) of samples were determined by means of an ICP–OES as described previously [31].

Table 1. Basic chemical parameters of the soils, including the content of available nutrients in depth 0–60 cm.

Plot	Ct (%)	N_t (%)	C/N	pH/KCl	pH/H_2O	Available Nutrients (mg/kg) *			
						P	K	Ca	Mg
Control	1.01	0.129	7.82	5.22	6.32	24.2	150	3297	187
NPK	1.21	0.145	8.34	5.12	6.12	71.7	177	2711	133
FYM	1.21	0.143	8.46	5.50	6.62	35.6	149	3483	189
FYM + NPK	1.33	0.166	7.89	5.22	6.28	76.2	207	3094	170

Ct—total carbon, Nt—total nitrogen, * Mehlich extract [30].

2.4. Phosphilipid Fatty Acid Analyses

Phospholipid fatty acid profiles (PLFA), an indicator of living microbial biomass and rough community structure, were determined according to ISO/TS 29843-2 [32]—a method adopted from Zelles et al. [33] used in previous studies [31,34–37]. Briefly, total lipids were extracted from the soil by a mixture of methanol, chloroform and phosphate buffer (0.05 mol/L, pH 7, ratio 2:1:0.8) and separated on polar silica SPE columns to fractions of non-polar lipids, glycolipids, and polar lipids. The last fraction was methanolized by a mixture of methanol and KOH, and fatty acid methyl esters (FAME) were determined by GC–MS. Total PLFA ($PLFA_{tot}$), an indicator of biomass of living microorganisms, was determined as the sum of all FAME of length C_{10}–C_{20}. Biomass of microbial groups were discriminated using indicator fatty acids according to Federici et al. [38], i.e., fungal biomass ($PLFA_{fun}$) by concentrations of 18:2ω6,9; bacterial biomass ($PLFA_{bac}$) by sum of i14:0, i15:0, a15:0, 16:1ω7t, 16:1ω9, 16:1ω7, 18:1ω7, 10Me-16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 10Me-18:0, cy19:0; biomass of gram-positive bacteria ($PLFA_{G+}$) by sum of i14:0, i15:0, a15:0, i17:0, a17:0; biomass of gram-negative bacteria ($PLFA_{G-}$) by sum of cy17:0, cy19:0, 18:1ω7; and biomass of actinobacteria ($PLFA_{Ac}$) by sum of 10Me-16:0, 10Me-17:0, and 10Me-18:0. Stress indicator *trans/cis* was calculated as ratio of concentrations (16:1ω7t + 18:1ω7t)(16:1ω7 + 18:1ω7), and nutrition indicator *cy/pre* [39] was calculated as ratio of concentrations (cy17:0 + cy19:0)/(16:1ω7 + 18:1ω7).

Values were recalculated to dry weight of soil. Soil humidity was determined by drying at 105 °C to a constant mass.

2.5. Soil Enzyme Assays

Activities of selected hydrolases (phosphatases, proteases, glucosidases, arylsulphatases) were determined spectrophotometrically by means of direct incubation of soil with solutions of para-nitrophenol evolving substrates, as described previously [31]. Dehydrogenase activities were determined spectrophotometrically as a ratio of reduction of triphenyltetrazolium chloride to triphenylformazan according to ISO 13859:2014 [40]. Soil respiration was determined by kinetics of adsorption of evolving CO_2 in a solution of NaOH and further reversed titration, as described previously [41]. All activities were determined at 25 °C and ambient soil pH without using buffers.

2.6. Data Analysis

The statistical analysis was performed by Statistica 13.3 (TIBCO Software, Palo Alto, CA, USA). To analyze the effect of the fertilizer treatments on the soil parameters we used a factorial analysis of variance. The Tukey's post hoc HSD test was used in the case of finding of statistically significant differences between analyzed parameters.

The robust analysis of principal components (PCAs) was calculated in statistical software XLSTAT v2016.03 (Long Island City, NY, USA). Spearman correlation was used for the calculation.

3. Results and Discussion

3.1. Structure of Soil Microbial Communities

The PLFA data are summarized in Table 2. The values of total PLFA (14.4 ± 2.9 mg/kg dwt), indicating living microbial biomass, were not high, but comparable to common arable soils [37,42] including contaminated soils [35]. Also, other PLFA characteristics are standard for arable soils (low proportion of fungal PLFA, approximately equal proportion of $PLFA_{G+}$ and $PLFA_{G-}$, low indicator of stress *trans/cis*) [43]. This indicates good soil health (Table 2).

Table 2. Summary data for phospholipid fatty acid profiles (PLFA) analysis at two depths of the soil profile (average ± standard deviation, n = 4).

Plot (Depth cm)	$PLFA_{tot}$ (mg.kg^{-1} dwt)	$PLFA_{fun}$ (mg.kg^{-1} dwt)	$PLFA_{bac}$ (mg.kg^{-1} dwt)	$PLFA_{G+}$ (mg.kg^{-1} dwt)	$PLFA_{G-}$ (mg.kg^{-1} dwt)	$PLFA_{Ac}$ (mg.kg^{-1} dwt)	*cy/pre ()*	*trans/cis ()*
Control U	14.5 ± 1.0	0.51 ± 0.05	9.5 ± 0.7	3.7 ± 0.3	2.9 ± 0.2	1.35 ± 0.08	0.59 ± 0.05	0.00 ± 0.00
Control L	13.3 ± 2.0	0.44 ± 0.08	8.9 ± 1.3	3.2 ± 0.6	2.7 ± 0.4	1.44 ± 0.09	0.77 ± 0.37	0.00 ± 0.00
NPK U	14.1 ± 1.3	0.52 ± 0.05	9.0 ± 0.8	3.6 ± 0.4	2.7 ± 0.2	1.35 ± 0.11	1.06 ± 0.52	0.00 ± 0.00
NPK L	12.1 ± 1.2	0.40 ± 0.03	7.2 ± 0.3	2.8 ± 0.2	2.1 ± 0.1	1.63 ± 0.14	1.73 ± 0.15	0.00 ± 0.00
FYM U	16.3 ± 0.6	0.49 ± 0.06	10.7 ± 0.4	4.0 ± 0.1	3.3 ± 0.2	1.58 ± 0.04	0.64 ± 0.04	0.01 ± 0.03
FYM L	16.4 ± 1.7	0.64 ± 0.08	10.5 ± 1.1	3.9 ± 0.4	3.4 ± 0.4	1.64 ± 0.17	0.81 ± 0.50	0.06 ± 0.11
FYM + NPK U	14.9 ± 0.4	0.44 ± 0.06	9.8 ± 0.3	3.7 ± 0.1	3.0 ± 0.1	1.41 ± 0.03	0.70 ± 0.06	0.00 ± 0.00
FYM + NPK L	13.5 ± 2.0	0.47 ± 0.08	8.7 ± 1.3	3.2 ± 0.5	2.8 ± 0.5	1.61 ± 0.13	1.18 ± 0.63	0.00 ± 0.00

Upper index letters denote significant differences based on Tukey's post hoc HSD test ($p < 0.05$). U = upper layer of the soil (0–30 cm deep), L = lower layer of the soil (30–60 cm), dwt = dry weight of the soil. PLFA indicators are explained in Section 2.4.

3.2. Effects of Fertilizers on Microbial Communities

In our study, we investigated the influence of several variants of fertilization on the activity of selected soil enzymes and the number of microorganisms and fungi at depths of 0–30 cm and 30–60 cm.

Application of fertilizers did not have a statistically significant influence ($p < 0.05$) on the amount of fungal, bacterial, and actinobacterial PLFA (Table 2) in comparison with the control area. To promote differences in variable soils, the comparison was carried out, regardless of sampling depth (Table 3). Differences can be observed in areas with application of NPK and FYM, where FYM had a positive effect on the amount of soil bacteria and the influence of NPK was negative.

Analysis of $PLFA_{tot}$ was used to determine the biomass of soil microbial communities. Our results, which show the influence of several kinds of fertilization on the biomass of microorganisms and fungi in soil, are contrary to results of other authors. For instance, Gong et al. [9] and Blanchet et al. [44] found that application of NPK, FYM, or NPK + FYM increases the amount of bacteria and fungi in soil environment. A possible explanation of such contrasts in the activity of enzymes, including $PLFA_{tot}$, may be that other authors collected samples from lower depths than we did.

Comparison of PLFA profiles, regardless of the depth of sampling structure of fungi, bacteria, $PLFA_{G+}$, $PLFA_{G-}$, and actinobacteria revealed statistically insignificant differences in comparison with control samples. This is contrary to results of other authors, who found out that application of NPK, FYM, or NPK + FYM increased the amount of $PLFA_{tot}$, bacteria, and fungi [9,44,45].

The effect of fertilizers on the activity of enzymes was not statistically significant ($p < 0.05$) as shown in Table 4, which take into account the individual examined depths (0–30 cm, 30–60 cm). Nevertheless, much higher respiration was observed in samples with combined application of FYM+NPK, where slightly higher respiration activity was observed in depth 30–60 cm. The increase was 6.81 ± 0.13 mU g^{-1} dry soil in comparison with control area (1.00 ± 0.21 mU g^{-1} dry soil). In the case of arylsulphatases we can see a difference between application of FYM and NPK, where use of NPK decreased activity of this enzyme to the level of 1.54 μgU g^{-1} dry soil (depth 0–30 cm) and 1.41 μgU g^{-1} dry soil in depth 30–60 cm; whereas FYM stimulated the activity of this enzyme to 2.46 μgU g^{-1} dry soil (depth 0–30 cm) and 2.38 μgU g^{-1} dry soil in depth 30–60 cm (Table 4).

Statistical evaluation of results, which considers individual depths, did not show significant differences between control group and individual variants of fertilization. A statistically significant difference was seen in arylsulphatases when comparing their activities in the case of fertilization by NPK and FYM. Positive effects of the application of FYM on this enzyme was also proved in the results of Zhang et al. [46]—and vice versa, a negative effect of NPK on the activity of arylsulphatases was found by Ai et al. [47] in application of cattle manure, where stimulating influence of combined application of NPK + FYM on soil respirations was observed. This influence applies to both studied depths. Likewise, Bohme and Bohme [48] proved a positive effect of several kinds of fertilization (NPK, FYM, NPK + FYM) on respiration of soils, in which case the highest respiration activity was observed with the application of mineral fertilizers (NPK).

Table 3. Summary data for PLFA analysis without resolution of depth of soil profile (average ± standard deviation, n = 8).

Plot Depth (cm)	$PLFA_{tot}$ (mg.kg^{-1} dwt)	$PLFA_{fun}$ (mg.kg^{-1} dwt)	$PLFA_{bac}$ (mg.kg^{-1} dwt)	$PLFA_{G+}$ (mg.kg^{-1} dwt)	$PLFA_{G-}$ (mg.kg^{-1} dwt)	$PLFA_{Ac}$ (mg.kg^{-1} dwt)	*cy/pre ()*	*trans/cis ()*
Control	13.9 ± 1.1	0.48 ± 0.05	9.2 ± 0.7 ^{ab}	3.5 ± 0.3	2.8 ± 0.2 ^{ab}	1.4 ± 0.1	0.68 ± 0.26	0.00 ± 0.00
NPK	13.1 ± 0.9	0.46 ± 0.04	8.1 ± 0.5 ^{a}	3.2 ± 0.2	2.4 ± 0.2 ^{a}	1.5 ± 0.1	1.39 ± 0.50	0.00 ± 0.00
FYM	16.3 ± 0.9	0.56 ± 0.06	10.6 ± 0.5 ^{b}	3.9 ± 0.2	3.3 ± 0.2 ^{b}	1.6 ± 0.1	0.73 ± 0.34	0.04 ± 0.08
FYM+NPK	14.2 ± 1.0	0.45 ± 0.05	9.3 ± 0.6 ^{ab}	3.4 ± 0.2	2.9 ± 0.2 ^{ab}	1.5 ± 0.1	0.94 ± 0.49	0.00 ± 0.00

Upper index letters denote significant differences based on Tukey's post hoc HSD test ($p < 0.05$). dwt = dry weight of the soil. PLFA indicators are explained in Section 2.4.

Table 4. Soil enzyme activities and soil respiration at two depths of the soil profile (average ± standard deviation, n = 4).

Plot Depth (cm)	Phosphatases µU.g^{-1} Dry Soil	Arylsulphatases µU.g^{-1} Dry Soil	Glucosidases µU.g^{-1} Dry Soil	Proteases µU.g^{-1} Dry Soil	Dehydrogenases µU.g^{-1} Dry Soil	Respiration mU.g^{-1} Dry Soil
Control U	40.9 ± 1.8	2.19 ^{abc} ± 0.23	3.76 ± 0.14	11.3 ± 0.4	0.14 ± 0.09	1.79 ^{a} ± 0.23
Control L	34.2 ± 0.9	2.06 ^{abc} ± 0.23	3.47 ± 0.37	10.0 ± 1.1	0.21 ± 0.21	1.00 ^{a} ± 0.21
NPK U	41.4 ± 2.5	1.54 ^{b} ± 0.10	3.93 ± 0.16	9.9 ± 1.2	0.02 ± 0.02	2.19 ^{a} ± 0.48
NPK L	38.1 ± 3.3	1.4 ^{bc} ± 0.07	3.76 ± 0.13	9.1 ± 0.6	0.01 ± 0.01	1.55 ^{a} ± 0.25
FYM U	33.9 ± 1.4	2.46 ^{a} ± 0.10	3.93 ± 0.22	8.3 ± 0.5	0.17 ± 0.17	2.1 ^{a} ± 0.30
FYM L	33.6 ± 0.5	2.38 ^{a} ± 0.16	4.28 ± 0.09	9.4 ± 0.8	0.06 ± 0.06	2.22 ^{a} ± 0.10
FYM+NPK U	38.8 ± 2.8	1.85 ^{abc} ± 0.17	4.33 ± 0.18	10.1 ± 0.8	0.27 ± 0.16	6.79 ^{b} ± 0.25
FYM + NPK L	43.9 ± 3.9	2.20 ^{ac} ± 0.20	4.24 ± 0.17	11.8 ± 0.9	0.11 ± 0.09	6.8 ^{b} ± 0.13

Upper index letters denote significant differences based on Tukey's post hoc HSD test ($p < 0.05$). U = upper layer of the soil (0–30 cm deep), L = lower layer of the soil (30–60 cm).

Evaluation of the influence of fertilization on microbial activity and activities of soil enzymes, regardless of individual depths, showed the influence of activity on two enzymes. The activity of glucosidases increased in the area with combined application of NPK + FYM. The activity increased from 3.61 μgU g^{-1} dry soil (control) to 4.29 μgU g^{-1} dry soil (Table 5). The second enzyme that was influenced by fertilization was arylsulphatase. Its activity was inhibited by application of NPK. Activity of this enzyme dropped from 2.12 μgU g^{-1} dry soil (control) to 1.48 μgU g^{-1} dry soil (Table 5). In the case of phosphatases, we observed a difference between application of FYM and application of NPK + FYM. The use of FYM decreased activity of phosphatase (33.71 μgU g^{-1} dry soil) while the use of NPK + FYM stimulated the activity of this enzyme (41.29 μgU g^{-1} dry soil). A statistically significant increase was observed ($p < 0.05$), namely, the increase of respiration activity from 1.40 mU g^{-1} dry soil (control) to 2.17 mU g^{-1} dry soil (FYM) and 6.80 mU g^{-1} dry soil (FYM + NPK) (Table 5). Similarly, Wang et al. observed almost doubling of the CO_2 evolution rate in response to FYM application [49], and several other studies observed such increase after application of a combination of mineral and organic fertilizers [49–51]. Multiplication of respiration rate after three years of application of combination of mineral and organic fertilizer was observed by Dhull et al. [52]. This longer study well corresponds with our ~5-times increase.

Table 5. Soil enzyme activities and soil respiration at two depths of the soil profile (average ± standard deviation, n = 8).

Plot Depth (cm)	Phosphatases μU.g^{-1} dwt	Arylsulphatases μU.g^{-1} dwt	Glucosidases μU.g^{-1} dwt	Proteases μU.g^{-1} dwt	Dehydrogenases μU.g^{-1} dwt	Respiration mU.g^{-1} dwt
Control	37.6 ^{ab} ± 1.56	2.12 ^{a} ± 0.15	3.6 ^{a} ± 0.19	10.6 ± 0.6	0.17 ± 0.10	1.40 ^{a} ± 0.21
NPK	39.7 ^{ab} ± 2.02	1.48 ^{b} ± 0.06	3.84 ^{ab} ± 0.10	9.5 ± 0.7	0.06 ± 0.01	1.87 ^{ab} ± 0.28
FYM	33.7 ^{a} ± 0.69	2.42 ^{a} ± 0.09	4.10 ^{ab} ± 0.13	8.8 ± 0.5	0.12 ± 0.09	2.17 ^{b} ± 0.15
FYM+NPK	41.3 ^{b} ± 2.39	2.03 ^{a} ± 0.14	4.29 ^{b} ± 0.11	11.0 ± 0.6	0.19 ± 0.09	6.80 ^{c} ± 0.3

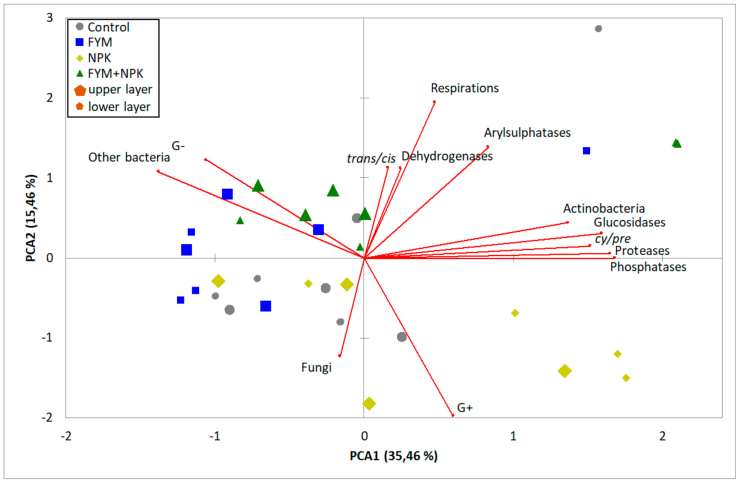
Upper index letters denote significant differences based on Tukey's post hoc HSD test ($p < 0.05$). dwt = dry weight.

Results which assess the activity of enzymes, regardless of individual examined depths, show that fertilization by NPK, FYM, and their combination (NPK + FYM) did not influence the activity of dehydrogenases or proteases. Unlike our work, stimulation of proteases [48,51,53] or proteases and dehydrogenases [54], was detected after application of NPK, FYM, and NPK + FYM. Similarly, Liang et al. [55], in the case of dehydrogenases' activity, discovered a stimulating effect of fertilization on this enzyme. Application of fertilizers had a stimulating effect on the activity of glucosidases (application of NPK + FYM); and an inhibitive influence on the activity of arylsulphatases (application of NPK). A positive effect on the activity of glucosidases after application of NPK + FYM was demonstrated [47]. Other studies realized that higher activity of glucosidases happens in application of FYM rather than NPK or combination of NPK + FYM [46,56,57]. Inhibition of arylsulphatase activity was also discovered in the work of Ai et al. (2012) [47]. Contrary to this, other studies showed that application of NPK as well as FYM stimulated the activity of this enzyme [46,55,58]. Statistically significant difference in phosphatases activities were observed between fertilization by FYM and NPK + FYM, where application of FYM decreased the activity of this enzyme, while application of NPK + FYM stimulated the activity. Similar results have been described [47,53,57,59]. In the above-mentioned studies, these results were statistically significant, similarly to our work, when comparing variants of fertilization. Nevertheless, there were significant differences in comparison with control areas.

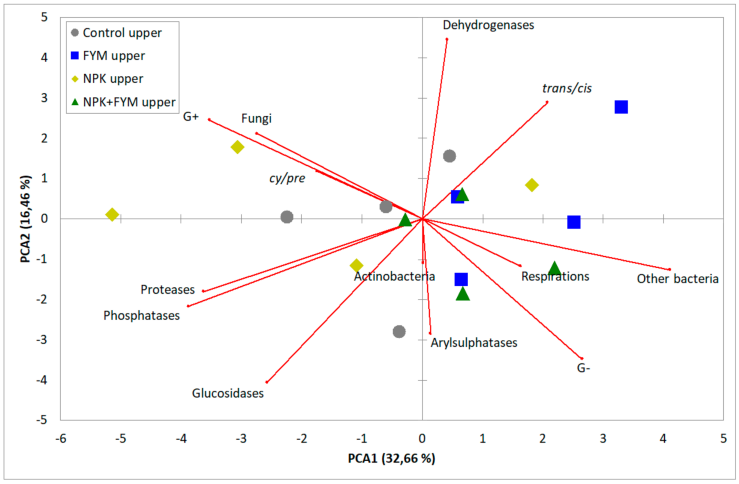
3.3. Analysis of Data Variability

Overall the detected differences were very low and insignificant in direct single-factor comparison. We therefore decided to calculate the principal component analysis (PCA) to help understanding the data variability. The Spearman correlation matrix was used for its robustness to outliers. For better interpretation of results, the Varimax rotation of PCA was applied to produce the final graphs. In our previous studies, this approach contributed to understanding of differences of microbial communities,

e.g., on different localities [37], such as in sandstone caves affected by tourist traffic [31], or in post-military sites during phytomanagement [34]. Considering both soil layers together (Figure 1a), two principal components together explain only 51% of the data variability; together with PC3, the explained variability is 64%. Other PCs are below 10% of variability explanation. This comparison just confirms the fertility of used cambisol, serving as a highly competitive environment for microorganisms. PC1 is highly correlated with activities of hydrolases (glucosidases, proteases, and phosphatases, but not arylsulphatases) as well as actinobacterial PLFA and *cy/pre* indicator. In undisturbed soils, this indicator is usually interpreted to exhibit lack of growth based on nutrient limitations [39,43]. Also, the increased activities of hydrolases are considered to show the microbial effort to gain nutrients under their limitation [26]. Therefore, variability of nutrients can be considered as one possible driver of the microbial variability. PC2 correlates fairly positively with activities of dehydrogenases, arylsulphatases, *trans/cis* indicator, and respiration; and negatively with proportion of $PLFA_{G+}$ and $PLFA_{fun}$. Explanation of variability on this axis can thus be related to variability of environmental conditions. Individual samples are widespread across the PCA chart, thus confirming high natural variability of the data and low effect of the fertilization. Nevertheless, NPK samples that slightly tend to cumulate in fourth quadrant correlated more with PC1 (nutrient insufficiency) and negatively with PCA2 (lower microbial activity). This indicates that from the point of view of soil microorganisms, the NPK presents worse fertilizer compared to the other two tested.

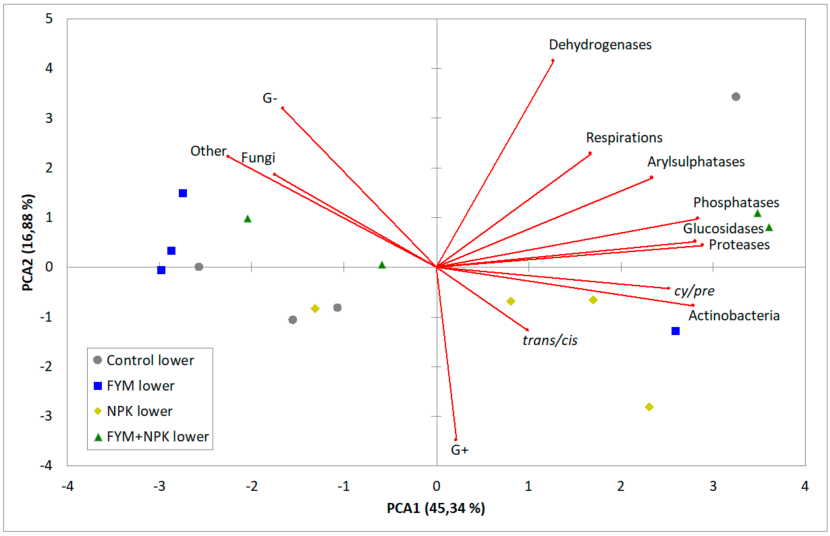


(**a**)



(**b**)

Figure 1. *Cont.*



(**c**)

Figure 1. Principal component analysis (PCA) with varimax rotation of the microbiological data. (**a**) all data; (**b**) upper layer data; (**c**) lower layer data.

The upper layer explained variability is similar (Figure 1b), i.e., 49% for two PC and 64% for three PC. Nevertheless, the two main PC have exchanged, i.e., the nutrient-related x-axis explains less variability. In case of the lower soil layer (Figure 1c) overall variability is higher, i.e., two PC explain 62% and three contribute to 73%. This is consistent with the expectations that fertilization will affect the upper soil layer more compared to the lower soil layer.

4. Conclusions

In our work, we were concerned with the influence of application of NPK, FYM, and NPK + FYM on the soil microbial community (assessed via phospholipid fatty acid profiles) and activity of soil enzymes at depths of 0–30 cm and 30–60 cm. Different fertilization did not significantly influence the activity of soil enzymes at depths of 0–30 cm a 30–60 cm. The same effect was discovered in $PLFA_{tot}$, the biomass of fungi, bacteria, and actinobacteria. The influence of fertilizer application was evident only in respirations, namely, in combined fertilization by NPK + FYM. When evaluating results, regardless of the depths of sampling, we found out that application of NPK + FYM stimulated the activity of glucosidases, and application of NPK inhibited the activity of arylsulphatases. Respirations were stimulated by application of FYM as well as by combination of NPK + FYM. Principal component analyses revealed slightly worse state of microbial communities when only NPK was applied. Our study suggests that use of FYM or combined fertilization by NPK and FYM could be a beneficial strategy for preserving soil productivity, and it could provide a supply of soil ecosystem services in the future.

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