Soil biological characteristics and microbial community structure in a field experiment

Abstract: The influence of different fertilization treatments on soil biological characteristics and microbial community structure was investigated. Soil samples were taken from a long-term field experiment which was conducted to explore the effect of eight treatments: control (non-fertilized), NPK (nitrogen, phosphorus and potassium), FYM (farmyard manure), FYM + NPK, CSI + St (cattle slurry + straw), CSI + St + NPK, CSI, CSI + NPK. The highest values of invertase activity were found in treatment fertilized with farmyard manure combined mineral fertilizers, similarly to total N content, activity of urease, and C of microbial biomass. Dehydrogenase activity was lower in all treatments with mineral fertilization. Bacterial and actinobacterial T-RFLP profiles of 16S rRNA genes showed similar patterns in response to eight fertilization treatments. In both, the communities formed distinct groups, which were separated by organic fertilization i.e. cattle slurry and straw amendments along the x axes and by NPK amendments along the y axes using the Sammon’s method of multidimensional scaling. Significant correlations were determined in several situations related to diversity: between invertase and 1/Db (dominance, bacteria), 1/Da (dominance, actinobacteria) and between T-RFLP profiles of actinobacteria, nitrogen and organic carbon content and bacterial E (evenness).

Keywords: Microbial community; long-term experiment; soil enzymes

1 Introduction

One of the basic functions of soil is supporting microbial activities and diversity that are essential for plant growth. Mineral and organic fertilization, primarily with nitrogen, not only increases soil fertility but also influences its chemical, physical and biological properties. It can affect diversity and function of soil microorganisms, and thus also availability and recycling of nutrients not only their actual content [1,2,3].

Soil quality is developed slowly as a consequence of changing mineral or organic fertilization systems. The effect of fertilizer amendment on soil microbial communities seems to be much stronger than that of land use or season [1]. Gu et al. [2] demonstrated that the combination of mineral fertilizers with farmyard manure increased the diversity of the soil bacterial community more than that of mineral fertilizers alone. On the contrary, Wei et al. [3] found that the structure and function of the bacterial community were similar in the manure amended soil and the control treatment, suggesting that the application of manure increased microbial populations, but had little effect on bacterial community structure. Jangid et al. [1] ascribed the lower bacterial diversity in the inorganic fertilizer amended soils to decreased evenness.

Microbial community composition is controlled by soil type and it is associated with the particle size fractions [4]. Nevertheless, as the long-term fertilization cannot change the soil type, it affects the structure of the soil bacterial community through changes of soil nutrients [2]. Since it was documented that even members of bacterial clades classified at high taxonomic ranks possess ecological and therefore also functional coherence [5], studying the changes in composition and abundance of microbial communities under long-term fertilization systems contributes to better understanding of fertilizers’ impact on soil quality.

It is well known that long-term fertilization management practices are connected to the biological activity of soil and soil organic matter (SOM) content. Organic matter (OM) can be added either directly by incorporating manure, slurry or...
straw or indirectly via increased plant production. Content of OM in soil predominantly affects microbial biomass and diversity of soil biota [6]. Generally, organic matter addition supports microbial biomass but also activity of various degrading enzymes. In particular, enzymatic activities are considered to be good indicators of soil quality because they control the release of nutrients for plants and the growth of microorganisms. Determining specific enzymatic activity can also encompass certain processes occurring in the soil. For example, Nayak et al. [7] reported that the nitrogen cycle processes can be suitably characterized by urease activity. The activity of invertase was chosen for its critical role in releasing low molecular weight sugars that are important as energy sources for microorganisms. Finally, the use of microbial biomass and enzyme activity as indicators of soil quality is advantageous not only because of their relationship to soil biology, but also for the ease of measurement, rapid response, and the high sensitivity to changes caused by both management and environmental factors [8,9].

However, Marschner et al. [10] revealed that changes in enzyme activities are not necessarily accompanied by changes in community composition. The assessment of soil biodiversity is relatively complicated because no method can capture a complete list of present microorganisms. Therefore, microbial diversity is mostly studied by comparison of microbial community fingerprints. The set of methods applied for determination of microbial biomass, enzyme activities and microbial community in this study were found sensitive to site differences and fertilization effects on the structure, size and activity of microbial communities in soils of a long-term fertilization experiment [11].

The aim of our work was to determine the effects of soil treatments on some biological parameters and microbial community structure. Our results were obtained from long-term field experiments in Prague. Each change in land use, cropping, tillage or fertilization starts long-term processes that tend to reach a new equilibrium. Long-term field experiments are, therefore, indispensable means for the study of agro-ecosystems and their biological and ecological functions. The biological activity and microbial community structure of a soil are connected to soil quality and fertility.

2 Experimental Procedures

2.1 Site description and soil sampling

The long-term field experiment in Prague-Ruzyne (50°05′14″N and 14°17′27″E) is one of the longest running studies in the Czech Republic. It was established in 1955 with the aim to investigate the effect of various fertilization systems on the yields, nutrient uptake and soil quality. The altitude of the site is about 352 m above sea level, average annual temperature is 8.1°C and average annual precipitation 450 mm. Soil type is Orthic Luvisol, clay-loam, developed on diluvial sediments mixed with loess. Individual blocs were split according to organic fertilization and each part was further randomly split into treatments of mineral fertilization. The plot size was 12 m × 12 m (144 m²). Each of the 24 treatments had four replicates. Eight fertilization treatments were selected in this study: 1. Nil – without fertilizer, 1a. NPK – mineral fertilization, 2. FYM – farmyard manure, 2a. FYM + NPK – farmyard manure with mineral fertilization, 3. CSI + St – cattle slurry + straw, 3a. CSI + St + NPK – cattle slurry + straw with mineral fertilization, 4. CSI – cattle slurry, 4a. CSI + NPK – cattle slurry with mineral fertilization. The selected fertilizing treatments were similar to the recommended fertilization profile in the region. Organic fertilizers have been applied under root crops, while mineral fertilizers have been applied annually, except for the years when Lucerne was grown. P and K fertilizers have been applied in the autumn, N fertilizers in spring. The crop rotation has been classical 9-year rotation (45% cereals, 33% root crops, 22% fodder crops) since the beginning of the experiment. Soil reaction is neutral (pH KCl is 6.8-7.1) in the whole profile. Table 1 shows average doses of nitrogen fertilizers in kg of N per year in individual treatments.

Soil sampling was carried out annually in the early spring (end of March, beginning of April), before mineral fertilization and other agronomic measures, over the time period from 2007 to 2010 (four samplings). Soil samples were taken from the topsoil layer 0–0.2 m, at three samples from each individual plot. The fresh soil samples from each plot were mixed together (about 1 kg composite sample per plot), and passed through a 2 mm mesh sieve. The fresh soil samples were then stored in a refrigerator at 4°C before analyses. Determination of microbial biomass C, enzyme activities and extraction of the soil DNA proceeded within a few days from fresh soil samples. The remaining samples were air dried at room temperature. Hot water soluble C and total N contents were determined later on from air dried samples.

2.2 Chemical and biological analyses

Microbial biomass carbon (C-biomass) was determined by the chloroform fumigation extraction method [12]. 25g of fresh soil samples were weighed into Petri dishes and inserted a dessicator containing chloroform. The dessicator was then evacuated and the soil samples were...
fumigated for 24 hours at 25°C. After the fumigation, the soil samples were extracted simultaneously with the same no fumigated samples with 0.5 M K₂SO₄ for 30 min in an oscillating shaker at 200 rev min⁻¹ and then were filtered through a folded filter.

Hot water soluble carbon (Chwl) content in the soil samples was determined according to Schulz [13]. This characteristic represents an active part of soil organic matter. Total nitrogen (Ntot) was determined on a VARIO MAX analyzer on air-dried soil samples. Dehydrogenase activity was measured in soil samples of 6 g that had been incubated at 37°C for 24 h in the presence of 3% triphenyltetrazoliumchloride. The red colored product triphenylformazan (µg TPF/g/h) was extracted with ethanol, and measured in a spectrophotometer at 485 nm [14]. For the measurement of invertase activity, 5 g of fresh soil was incubated for 2 h at 37°C, and measured at 508 nm. The determination is based on the reaction of sodium salicylate with NH₃ in the presence of sodium dichloroisocyanurate which forms a green-colored complex under alkaline pH conditions (µg N/g/h). Sodium nitroprusside was used as a catalyst [16]. All analyses were made in triplicate and average values were further processed.

### 2.3 DNA extraction

Soil DNA was extracted from all replicated samples by the SK method according to Sagova-Mareckova et al. [17] as follows: soil (0.5 g) was homogenized in a Mini Bead Beater (BioSpec Products, Bartlesville, OK) for 90 s, at 2500 rpm with 600 µl of extraction buffer (50 mM Na-phosphate buffer, pH 8, 50 mM NaCl, 500 mM Tris-HCl, pH 8, 5% SDS) and 300 µl of phenol/chloroform/isoamyl alcohol (25:24:1) and 0.5 g sterile glass beads (0.25 mg of 0.1 mm and 0.25 mg of 0.5 mm diameter). The homogenate was centrifuged at 16000 × g for 2 min. The supernatant was mixed with the same volume of phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged at 6000 × g for 5 min. Then, NaCl was added to the supernatant to the final concentration of 1.5 M and CTAB (cetrimonium bromide) to 1%, and the mixture was incubated at 65°C for 30 min. The solution was cooled, mixed with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 3400 × g for 20 min. The DNA was precipitated with isopropanol, dissolved in water, incubated with the same volume of 1 M CaCl₂ in 1 M HEPES-NaOH, pH 7, and purified with the GeneClean Turbo DNA kit (Q-biogene, Irvine, CA).

### 2.4 Terminal restriction fragment length polymorphism analysis.

Primers used for amplification of bacterial and actinobacteria16SrDNA genes for T-RFLP were:forward bacteria, forward 16Seu 27f (5’-AGAGTTTGATCMTGGCKCAG; Lane [18]; modified by Cermak et al. [19]) labelled with HEX on the 5’ end, and reverse 783r (equimolar mix of 5’CTACCAGGATCTAATCGT, 5’CTACCGGTTATCTAATCCCG and 5’CTACCGGGGTATCTAATCCGG; Sakai et al. [20]) and for actinobacteria, same forward primer and reverse act1114r (GAGTTGACCCCGGCRGT; Kyselkova et al. [21]).

All PCRs were performed on a BioRad C 1000 Thermal Cycler. Before adding to the PCR mix, 50 ng (in approx.
1-3 ml) of template DNA was preheated with 3 μl BSA (10 mg ml⁻¹) and 6 ml TE⁻¹ (pH 8) at 90°C for 1 min [22]. PCR amplification consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 90 s with Taq Purple polymerase (Top-Bio, Prague, Czech Republic). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and cleaved by AluI for 4 h at 37°C. After inactivation at 65°C for 20 min the cleaved DNA fragments were purified with Sigma SpinTM Post-Reaction Clean-Up Columns (Sigma-Aldrich, St. Louis, MI). The fragment analysis was performed at the Genomac International (Prague, Czech Republic, with a 96-capillary sequencer (Applied Biosystems, Foster City, CA).

### 2.5 Statistical analysis

The results were then analyzed by ANOVA. Statistical analyses were carried out with the stat soft Statistica Cz program (Tukey HSD). The columns (which are designated by the same letter) did not significantly differ statistically (P = 0.5).

The T-RFLP projection was done using Sammon’s Multidimensional Scaling. The Manhattan metric (sum of absolute differences) was used to calculate the distance matrices. Diversity of bacterial and actinobacterial communities was calculated using Simpson’s 1/D and E indices, in which T-RF lengths were used as OTUs and their signal intensities as quantity estimates. The correlations between soil characteristics, T-RFLP profiles of bacteria and actinobacteria, and diversity indices were tested using Spearman’s rank correlation coefficient on the distance matrices. Mantel test (or null correlation of the Spearman’s correlation coefficient) was used to make a formal test of null correlation against positive correlation. All the computations were conducted with R (http://www.r-project.org).

### 3 Results and Discussion

In order to detect long-term effects of organic and mineral fertilization on the soil organic matter, enzyme activities, soil microorganisms and soil microbial community structure, we decided for early spring soil sampling. In this time period, there is the least effect of growing plant roots, post-harvest residues and organic fertilizers on the soil microbial community. Thanks to usually long and warm autumns, the decomposition of the post-harvest residues is advanced and slow, the readily decomposable components of the organic manures are decomposed and the effect of winter cereals is negligible. This is the best time to determine the effect of more than 50 years of different organic and mineral fertilizers on soil microorganisms, which may be hidden by the effects growing crops, later in the vegetation period.

#### 3.1 Total organic N content, hot water soluble C content and biomass C content in soil

#### 3.1.1 Total organic N content

Organic fertilization slightly increased total organic N content (Fig. 1) in topsoil compared to non-fertilized control in all treatments.

![Figure 1: Average values of total organic N content (% N) in soil samples from the selected treatments of the long-term field experiment over the time period 2007 to 2010. Different letters over the columns indicate statistically significant differences in average values.](image-url)
Statistically significant difference, however, was confirmed for fertilization with farmyard manure only (treatment 2). N content in mineral NPK fertilized treatment (1a) was not significantly different from the non-fertilized control (1). Blair et al. [23] found similar results in the oldest long-term field experiment at Broadbalk in Rothamsted, UK. Farmyard manure addition, N fertilizer and straw incorporation increased all C fractions, particularly the labile C fraction. The addition of 35 t ha⁻¹ year⁻¹ of FYM increased total organic C content in soil to 2.5 times that of the control (no fertilizer) treatment and labile C to 5 times that of the control. With the highest N application and straw returned, total C increased by 1.3 times and labile C by 1.5 times that of the control treatment. There were linear relationships between rate of N fertilizer applied and all C fractions, the rate of increase almost doubled with the inclusion of straw.

In our experiment, all treatments of combined organic and mineral fertilization significantly increased total organic N content in topsoil over the non-fertilized control (treatment 1). It is interesting to note that the combined fertilization with farmyard manure and mineral NPK significantly enhanced organic N content in topsoil over single fertilization with farmyard manure only (treatment 2 and 2a).

The increase of total N content as a result of long-term application of both FYM and fertilizers was described in Blair et al. [24] and Mandal et al. [25].

### 3.1.2 Hot water soluble C content

Hot water soluble C content in topsoil in the selected treatments of the long-term field experiment is presented in Fig. 2.

Fertilization with farmyard manure and cattle slurry plus straw significantly increased the hot water soluble C content in topsoil compared to non-fertilized control (Fig. 2). The effect of cattle slurry alone was not statistically significant. The same is true for mineral fertilization. Combined organic and mineral fertilization significantly enhanced hot water soluble C content over organic fertilization alone in treatments 2a and 3a (farmyard manure and cattle slurry plus straw). The effect of combined organic and mineral fertilization in treatment 4a (cattle slurry plus NPK) was also positive, but not statistically significant. The increase in the hot water soluble C content in soil as a result of organic and/or organic and mineral fertilization is likely to be due to the increase in the total soil organic matter content. Similar results were found in the case of the total N content in soil.

A higher input of organic matter, both as organic fertilizers and post-harvest residues, caused a higher soil organic matter accumulation. However, other mechanisms may have taken place, e.g. enhancement of the decomposition processes by mineral fertilizers.

Hot water soluble C content represents the easily decomposable (active) part of soil organic matter. Usually, it accounts for 2 to 5% of total organic C in topsoil of arable land in temperate climate zone [26]. This C fraction includes low molecular weight residues of plants and microorganisms, and also substantial part of the living soil microorganisms [27]. Values of the hot water soluble C content in soil partly reflect those of the total organic N content indicating that the changes in soil organic matter predominantly take place in its labile and decomposable part [24]. The value of the hot water soluble C content in soil are thus a more sensitive indicator of the changes...
in the soil organic matter. It may be a sensitive indicator of the impact of fertilization, crop rotation, tillage and similar measures on the soil organic matter [27].

3.1.3 Biomass C

Microbial biomass (Fig. 2) has been defined as a living part of soil organic matter. Organic fertilization and combined organic and mineral fertilization significantly enhanced microbial-C content in topsoil compared to the non-fertilized treatment (Fig. 2). The highest biomass-C content showed the treatment fertilized with farmyard manure and mineral fertilizers (treatment 2a). Cattle slurry plus straw and cattle slurry, either in combination with mineral fertilizers or without them, have also significantly increased biomass C content. The positive effect of organic fertilizers on the biomass-C content in soil appeared to correspond with a higher content of soil organic matter in fertilization treatments. The effect of mineral fertilizers on the microbial biomass is ambiguous. Mineral fertilization significantly decreased microbial biomass-C content in soils that did not receive organic fertilizers (treatment 1, 1a); however, it significantly increased the biomass-C content in soil that was fertilized with farmyard manure (treatment 2a). This confirmed the observations of Bohme and Bohme [28] and Mandal et al. [25]. Bohme and Bohme [28] studied effects of plant growth on soil microbial biomass C and soil enzyme activities in a pot experiment using spring barley and sugar beet. The soil originated from selected plots of the Static long-term field experiment in Bad Lauchstadt. Content of the microbial C in soil correlated with the total organic C content and it was two to three times higher in the soil from the plots fertilized with farmyard manure and NPK than in the soil from plots that have not received any fertilizers for more than 100 years. Mandal et al. [25] investigated the effect of six long-term (34-year) fertilizer and farmyard manure (FYM) treatments on the microbial biomass carbon in soil. It was found that a balanced application of NPK+FYM gave the highest values for the measured parameters and lowest at the control.

The effect of mineral fertilizers on the biomass-C content in soil that was fertilized with cattle slurry and straw was not statistically significant (treatment 3a).

The negative effect of mineral fertilization on the biomass-C content in topsoil may be due to several reasons. One of them may be an accelerated decomposition of the post-harvest residues that was not limited by lack of mineral nutrients, primarily that of nitrogen. Decomposition of the readily decomposable part of the post-harvest residues proceeds predominantly during autumn and winter in these climate conditions. Lack of mineral nutrients (nitrogen) may have slowed down these processes in the plots without mineral fertilization. In consequence, the decomposition of the post-harvest residues may have been delayed and not yet complete in the next springtime in the soils that have not received mineral fertilizers. Presence of readily available substrate in soil might have enhanced the growth of soil microorganisms and their quantity in soil at the time of soil sampling, i.e. early springtime. This mechanism may have occurred in the plots that have not received any organic fertilizers [29].

Higher organic matter content in plots supplemented by organic fertilizer enhanced the biomass of soil microorganisms. Not surprisingly, plots that were fertilized with farmyard manure had the highest biomass-C content. In those plots, even combined mineral fertilization significantly enhanced microbial-C content in topsoil. This might be due to the introduction of a large amount of a broad variety of microorganisms with the farmyard manure. The application of farmyard manure might have changed the soil macro-ecosystem which finally changed the structure of the soil bacterial community [2]. Hartman et al. [30] reported that application of farmyard manure consistently revealed the strongest influence on bacterial community structures and biomass contents.

3.2 Enzymatic activities

3.2.1 Urease activity

Urease can exist in soil as an extracellular enzyme in a three-dimensional network of organo-mineral complexes [31]. Activity of urease in topsoil in the selected treatments of the long-term field experiment is presented in Fig. 3.

It can be easily observed that urease activity was higher in the treatments with farmyard manure (Fig. 3, treatment 2, 2a). Similarly, Kandeler et al. [32] and Nayak et al. [7] found that urease activity was enhanced by fertilization with farmyard manure. The urease activity showed statistically significant correlation with the total N content in soil (Table 2) since it practically mimics the results of the total N content in individual treatments. A strong correlation between urease activity and total N content was observed previously [31]. The highest urease activity was found in the treatment that was fertilized with farmyard manure and mineral fertilizers (treatment 2a), in which the total N content was the highest. Also, the urease activity was always higher in the NPK treatment. Our results confirm the value of urease activity determination for evaluation of decomposition processes of organic matter in soil and its role in the nitrogen cycle in soil.
3.2.2 Dehydrogenase activity

Soil dehydrogenases are mainly intracellular enzymes associated with soil microbes and they are considered to be an indicator of soil microbial activity [33]. That is why they belong among enzyme activities that are highly sensitive to any impacts that may affect soil, including mineral fertilization. Our results showed a decrease in dehydrogenase activity in all treatments with mineral fertilization (Fig. 4).

This result is consistent with the study of Saha et al. [34] and Simek et al. [35]. They reported that dehydrogenase activity was highly sensitive to the inhibitory effects associated with mineral fertilizer additions [34] and that dehydrogenase activity was inhibited by large amount of fertilizer in a long-term experiment [35]. The microbial C content dropped in these treatments, with the exception of areas fertilized with farmyard manure and mineral fertilizers (treatment 2a). The highest values of dehydrogenase activity were found in treatments 3 and 4 (cattle slurry plus straw and cattle slurry alone).
3.2.3 Invertase activity

Invertase activity plays an important role in the carbon cycle by the hydrolysis of sucrose which is a common constituent of plant tissue. Activity of invertase in topsoil of the long-term field experiment is presented in Fig. 5.

The activities of invertase were found clearly correlated with the organic C and carbohydrate content in soil [36,37]. The highest values of invertase activity were found in treatment that was fertilized with farmyard manure and mineral fertilizers (treatment 2a), similarly to total N content, activity of urease, and C of microbial biomass. In accordance with our results Saha et al. [34] reported that the highest value of invertase activity was found in NPK + FYM treated soil. There was a positive correlation between invertase activity and microbial community structure (Table 2).

![Figure 5: Average values of activity of invertase (mg glucose/g/h) in soil samples from selected treatments of the long-term field experiment over the time period 2007 to 2010. Different letters over the columns indicate statistically significant differences in average values.](image)

Table 2: Relationship between soil characteristics [correlation coefficients (R)].

<table>
<thead>
<tr>
<th></th>
<th>Invertase</th>
<th>Dehydrogenase</th>
<th>Urease</th>
<th>Nt</th>
<th>Corg.</th>
<th>C biomass</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dehydrogenase</td>
<td>0.143</td>
<td>0.071</td>
<td></td>
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</tr>
<tr>
<td>Nt</td>
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<td>0.071</td>
<td>0.690</td>
<td>0.952*</td>
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<tr>
<td>Corg.</td>
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<td>0.571</td>
<td>0.643</td>
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<tr>
<td>C biomass</td>
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<td>-0.132</td>
<td>-0.295</td>
<td>-0.084</td>
<td>0.335</td>
</tr>
<tr>
<td>OTUb</td>
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<td>-0.095</td>
<td>0.309</td>
<td>0.488</td>
<td>0.667</td>
<td>0.429</td>
</tr>
<tr>
<td>1/Db</td>
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<td>0.405</td>
<td>0.854*</td>
<td>0.833*</td>
<td>0.024</td>
</tr>
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<td>Eb</td>
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<td>0.357</td>
<td>-0.119</td>
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<tr>
<td>T-RFLP b</td>
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</table>

OTU operational taxonomic units - number of detected terminal restriction fragments
b actinobacteria
D dominance diversity index
E evenness diversity index
T-RFLP distance matrix of respective terminal restriction fragment length profiles
Note: *Significant at p < 0.05
3.3 Microbial community structure

Bacterial and actinobacterial T-RFLP profiles of 16S rRNA genes showed similar patterns in response to eight fertilization treatments (Fig. 6, 7).

Actinobacteria were studied separately from total bacteria because this group was found most responsive to changes in environmental factors in other studies [38]. Additionally, actinobacteria belong among the dominating groups capable of decomposition of recalcitrant organic matter so their community structure may reflect the major changes in decomposition of added substrates [39].

In both, the communities formed distinct groups, which were separated by organic fertilization i.e. cattle slurry and straw amendments along the x axes and by NPK amendments along the y axes using the Sammon’s method of multidimensional scaling. Also, the separate effect of organic matter and NPK was demonstrated also on PLFA profiles, in which OM and OM + NPK treatments were clearly different from organic manure deficient treatments [40].

Bacterial diversity was also dependent on fertilization. Bacterial E (evenness) was significantly higher, while number of OTUs was significantly lower in treatments with NPK. Finally, a significant correlation was determined in several situations related to diversity: between invertase and 1/Db (dominance, bacteria), 1/Da (dominance, actinobacteria) and T-RFLP profiles of actinobacteria, nitrogen and organic carbon content and bacterial E.

Organic fertilizers had a significantly greater impact (P<0.05) on both biomass C and invertase activity, compared to mineral fertilizers [36]. Consequently, correlations between invertase activity and bacterial community might be a result of a combined effect between increased nutrients, organic matter and bacterial community changes. It was found that functional diversity indices of H’, D, and U were all significantly increased (P<0.05) by balanced fertilization which included organic matter [36]. The missing correlation between nutrient and organic matter content in our results might be explained by the more significant impact of organic matter quality than quantity on invertase activity [41]. Therefore it seems that the increase of nutrients and organic C together with changes of organic matter quality increases activities of invertase on the one hand and changes of bacterial diversity on the other.

4 Conclusions

This paper reports studies on the effect of long-term fertilization on total organic content, hot water soluble C content, biomass C content, enzymatic activities and microbial community structure under crop rotation in Orthic Luvisol. Combined organic and mineral fertilization significantly enhanced hot water soluble C content over organic fertilization alone in farmyard manure and cattle slurry plus straw treatments. The highest value of invertase...
activity was found in treatment that was fertilized with farmyard manure and mineral fertilizers, similarly to total N content, activity of urease, and C of microbial biomass. The total N content in soil showed statistically significant correlation with the urease activity and hot water soluble C content. Our results showed a decrease in dehydrogenase activity in all treatments with mineral fertilization. Bacterial diversity was also dependent on fertilization. Finally, a significant correlation was determined in several situations related to diversity: between invertase and 1/Db (dominance, bacteria), 1/Da (dominance, actinobacteria) and T-RFLP profiles of actinobacteria, nitrogen and organic carbon content and bacterial E.

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Conflict of interest: Authors declare nothing to disclose.

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