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Effect of vermicompost on changes in the bacterial community in maize rhizosphere

Vplyv vermikompostu na zmeny bakteriálneho spoločenstva v rizosfére kukurice

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Abstract

The aim of the study was to observe changes in the diversity of bacterial community in maize rhizosphere influenced by organic and mineral fertilization. Four variants of fertilization were tested - vermicompost (VC) at recommended annual dose 40t*ha^{-1}, doubled annual dose of VC, recommended dose of ammonium saltpeter with dolomite (LAD 27) and combination of VC and LAD 27. Experiment was conducted with potted maize plants in controlled conditions of greenhouse during 74 days. Using PCR-DGGE method, we investigated differences in total bacteria community as well as in community of ammonia oxidizing bacteria. Based on occurrence of operative taxonomic units (OTU) we found differences in bacterial species spectra among fertilization variants. The highest Shannon´s biodiversity index was observed in variant with VC addition in dose 80 t*ha^{-1}.The fertilizers effect on diversity of ammonia oxidizing bacteria was not significant however in each variant with vermicompost addition was the occurrence of new specific OTU observed. This OTU was identified as *Nitrosospira* sp. It was proven that some bacterial species introduced to soil with vermicompost addition can survive for at least 74 days and these bacteria can influence basic functions of soil microbiocenosis in nitrogen cycle.

Keywords: bacterial community, denaturing gradient gel electrophoresis, rhizosphere, vermicompost

Abstrakt

Cieľom štúdie bolo sledovať zmeny diverzity bakteriálneho spoločenstva v rizosfére kukurice vplyvom organického a minerálneho hnojenia. Boli testované štyri varianty hnojenia – vermikompost (VC) v odporúčanej ročnej dávke 40t*ha^{-1}, dvojitá ročná dávka VC, odporúčaná dávka liadku amónneho s dolomitom (LAD 27) a kombinácia VC a LAD 27. Experiment bol realizovaný na nádobách s rastlinami kukurice v kontrolovaných podmienkach skleníka počas 74 dní. Využitím metódy PCR – DGGE sme skúmali rozdiely v celkovej komunite baktérií ako aj komunite nitrifikačných baktérií. Na základe prítomnosti operatívnych taxonomických jednotiek (OTU) sme zistili rozdiely v spektrách bakteriálnych druhov v závislosti od variantov hnojenia. Najvyšší Shannonov index biodiverzity bol zaznamenaný vo variante s prídavkom VC v dávke 80 t*ha^{-1}. Vplyv hnojenia na diverzitu nitrifikačných baktérií nebol preukazný, avšak v každom variante s prídavkom vermikompostu bol zaznamenaný výskyt nového špecifického OTU. Tento OTU bol identifikovaný ako *Nitrosospira* sp. Dokázalo sa, že niektoré druhy baktérií dodané do pôdy prídavkom vermikompostu sú schopné prežívať po dobu najmenej 74 dní a môžu ovplyvňovať základné funkcie pôdnej mikrobiocenózy v cykle dusíka.

Kľúčové slová: bakteriálne spoločenstvo, denaturovaná gradientová gélová elektroforéza, rizosféra, vermikompost

Introduction

Soil is one of the most important environments for biosphere and for cycles of elements essential for life. Strong industrial progress in last years influenced changes in the structure and composition of soil, resulting in changes in the diversity of indigenous microbial communities (Chaparro et al., 2012). High quality and healthy soils contain high amount of organic matter and large community of microorganisms. Microbial community plays an important role in terrestrial ecosystems because of its role in biogeochemical nutrient processes regulation (Li et al., 2015; Zeng et al., 2016). Numerous studies showed that soil microbial properties and its composition can be significantly affected by fertilization (Mäder et al., 2002, Enwall et al., 2005, Gu et al., 2009, Feng et al., 2015).

Mineral fertilizers are generally used for plant nutrition, while the role of soil organic matter as a basic determinant of fertility and soil stability is often underestimated (Singh and Ryan, 2015). According to Li et al. (2015), overuse of mineral fertilizers not only causes low fertilizer effectivity and the rapid depletion of P-deposits and nutrients, but it also leads to degradation of the environment and biodiversity loss. Resolution according to Sradnick et al. (2013) is to reduce using of mineral fertilizers and replace them with organic fertilizers, which are made from agricultural residues. One of the mostly used organic fertilizers is compost (Lazcano et al., 2008). Composting and vermicomposting are two best-known processes of biological stabilization of organic waste (Lung et al., 2001). Vermicomposting involves the bio-oxidation and stabilization of organic material by the joint action of earthworms and microorganisms (Lazcano et al., 2008). This type of fertilizer improves the condition

of plants and thus increases the resistance of plants to unfavorable factors, promotes their rooting, growth and flowering. The excess of vermicompost that soaks to the plant roots increases the amount of soil bacteria and can prevent fungal infections, ensures better absorption of nutrients and cause higher resistance of plants (Adhikary, 2012). One of important part of bacterial community is ammonia-oxidizing bacteria (AOB) which is responsible for the rate limiting step in nitrification. Nitrification is the main nitrogen (N) loss pathway in agricultural systems (Banning, 2015).

This evidence demonstrates that fertilization with different types of fertilizers could have significant impact on changes in soil biodiversity as well as on bacterial community in rhizosphere. Study of the effect that have organic and inorganic fertilizers and combined application of them on soil quality can lead to help to contribute and develop more sustainable and stable ecosystems with high biodiversity while preventing soil degradation.

The objective of this study is to determine how bacterial diversity from maize rhizosphere can be influenced by adding organic (vermicompost) and inorganic (ammonium saltpeter with dolomite- LAD 27) fertilizers into arable soil in controlled greenhouse conditions. We hypothesize that application of organic fertilizer in form of vermicompost encourages the increase of bacterial diversity (including AOB) more than inorganic fertilizer (LAD 27).

Materials and methods

Arable soil used in this experiment characterized (Table 1) as a soil type fluvisol, sandy- loam class, was collected in area Banín (49°39´S. 16°27´V, height above sea level: 454 m) municipality located in the protection zone of underground source of drinking water Březová nad Svitavou (Pardubice Region, district Svitavy, Czech Republic).

Soil was homogenized and then pre-incubated for 20 days under controlled laboratory conditions to achieve identical humidity in the whole volume of sample. Three containers (height = 550 mm; width = 200 mm; thickness = 100 mm) for each fertilization variant were filled with 9 kg of subsoil and 5.6 kg of topsoil (Figure 1). Then all containers were planted with indicator plant *Zea mays* L. (one plant per experimental container) and were kept in a greenhouse (Mendel university in Brno, Czech Republic) at 26 °C (day temperature), 20 °C (night temperature) and 65% humidity (24h) with a day length of 11 h (light intensity 3 000 lx; day period from 8:00 a.m. until 7:00 p.m. and nigh period 7:00 p.m. until 8:00 a.m.). Plants were removed from containers and topsoil was analyzed after 74 days of cultivation.

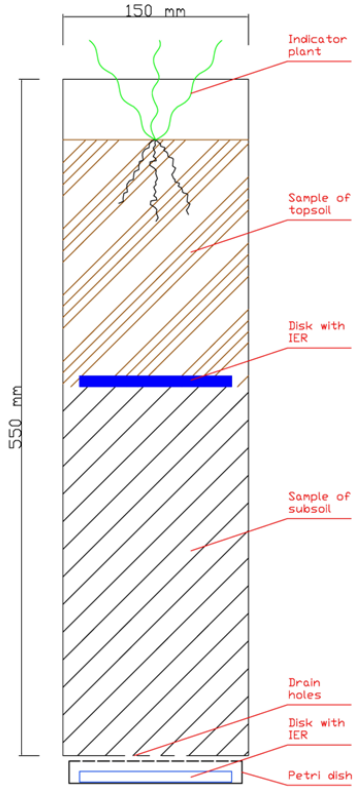


Figure 1. Characteristics of used experimental containers

Figure 1. Charakteristika použitých experimentálnych kontajnerov

For this analysis two types of fertilizers (vermicompost and LAD 27) were used. We applied vermicompost (Table 1) which consisted of compost (41%), cattle manure (20%), peatbog, forest litter (2%), fluvisol (10%), biodegradable waste - grass, old straw, hay etc. (6%) and vermicompost backfilling (21%). There was high content of the basic parameters (C_{ox}, N_{tot}, C_{hwe}) in vermicompost observed. As the mineral fertilizer we chose ammonium saltpeter with dolomite 27 (LAD 27) often used in agriculture, produced by Duslo, a.s. which technically is NH_4NO_3 and contains 27% of nitrogen.

Table 1. Basic characteristics of tested soil and vermicompost

Table 1. Základná charakteristika testovanej pôdy a vermikompostu

Evaluated parameters	Units	Soil	Vermicompost
Dry mass	%	92.73	65.40
C_{ox}	%	1.1689	13.2561
N_{tot}	$mg*kg^{-1}$	1840	14859
C_{hwe}	$mg*kg^{-1}$	0.6010	12.2091
pH H_2O		6.74	7.91
pH KCl		5.74	7.53
P	$mg*kg^{-1}$	139.2	180.61
K	$mg*kg^{-1}$	366.3	167.80
Ca	$mg*kg^{-1}$	3683	1449
Mg	$mg*kg^{-1}$	189.6	52.50

C_{ox} = organic carbon content; N_{tot} = total nitrogen; C_{hwe} = hot water extractable carbon; pH = soil reaction; KCl = potassium chloride

C_{ox} = obsah organického uhlíka; N_{tot} = celkový dusk; C_{hwe} = horúcou vodou extrahovateľný uhlík; pH = pôdna reakcia; KCl = chlorid draselný

In this experiment, five variants of samples were prepared (table 2). We collected the samples from each variant at the beginning (a) and at the end of the experiment (b).

Table 2. Applied doses of fertilizers in experimental variants

Table 2. Aplikované dávky hnojív v experimentálnych variantoch

Variant	Variant composition	Vermicompost	LAD 27
V1	S	0	0
V2	S+40VC	40 t*ha^{-1}	0
V3	S+20VC+30LAD 27	20 t*ha^{-1}	30 kg N*ha^{-1}
V4	S+60LAD 27	0	60 kg N*ha^{-1}
V5	S+80VC	80 t*ha^{-1}	0

V1 = variant no.1; V2 = variant no.2; V3 = variant no.3; V4 = variant no.4; V5 = variant no.5; S = soil; 40VC = addition of vermicompost (40t*ha^{-1}); 20VC = addition of vermicompost (20t*ha^{-1}); 30 LAD 27= addition of LAD 27 (30t*ha^{-1}); 60LAD 27 = addition of LAD 27 (60t*ha^{-1}); 80VC = addition of vermicompost (80t*ha^{-1}); LAD 27 = ammonium saltpeter with dolomite; VC = vermicompost

V1 = variant č.1; V2 = variant č.2; V3 = variant č.3; V4 = variant č.4; V5 = variant č.5; S = pôda; 40VC = prídavok vermikompostu (40t*ha^{-1}); 20VC = prídavok vermikompostu (20t*ha^{-1}); 30 LAD 27 = prídavok LAD 27 (30t*ha^{-1}); 60LAD 27 = prídavok LAD 27 (60t*ha^{-1}); 80VC = prídavok vermikompostu (80t*ha^{-1}); LAD 27= liadok amónny s dolomitom; VC = vermikompost

Genetic analysis

DNA was extracted using Mobio PowerSoil isolation kit (MoBio Laboratories) from each sample (0.25 g of soil). For analysis of total bacterial diversity in maize rhizosphere as well as for diversity analysis of nitrifying bacteria we used PCR-DGGE method. Bacterial 16S rDNA was amplified by using two primers F984GC and 1401R (Brons and Van Elsas, 2008). Total volume of amplified PCR fragment (approximately 433 bp) was 50 µl. The PCR mixture was composed of 1 µl template DNA (approximately 20 ng),1 × PCR DreamTaqTM Green Buffer, 0.2 mM dNTP, 3.5 mM $MgCl_2$, 2 % dimethyl sulfoxide, 10 µg*ml^{-1} bovine serum albumin (BSA), 0.4 µM of each primer (F984GC a 1401R), 30 U/ml DreamTaqTM polymerase (Fermentas). PCR amplifications were carried out with a Biometra TPersonal thermal cycler under following conditions: initial denaturation at 95 °C for 4 min, followed by 10 (touchdown) cycles with decreasing hybridization temperature by 0.5 °C each cycle, the following temperature - time profiles: 30s at 94 °C; 30s at 60 °C; at 72 ºC for 1min followed with annealing: 25 cycles at 94 °C for 30 s, 30 s at 55 °C, and 1 min at 72 °C and a final extension at 72 °C for 30 min ended with cooling at 4°C.

To observe changes in diversity of AOB we used a set of primers 27f, 1492r, followed by nested PCR with primers CTO189f and CTO654r specific for ammonia oxidizers from β - *proteobacteria* group (Kowalchuk et al. 1997; Mahmood et al., 2006).

PCR conditions for the first stage of nested PCR were identical as above. Cycling conditions were: initial denaturation at 95 °C for 3 min, followed by annealing of 35 cycles at 94 °C for 30 s, 30 s at 55 °C, and 1 min at 72 °C, and a final extension at 72

°C for 30 min. The product of the first step was then enzymatically purified using EXOI/FastAP (Fermentas). After this process the mixture was diluted to 1/10 and 2 µl of it was used as a template for the second round of nested PCR. PCR mixture and conditions were the same as in the first step.

Analysis of PCR products by DGGE

DGGE analysis of total bacterial community was performed in Ingeny PhorU2 apparatus (Ingeny). For bacterial (433 bp) PCR product we used 6 % concentration of acrylamide gel (acrylamide: N,N´-methylenebisacrylamide, w/w, 37.5:1) with 45–65 % gradient (Garbeva et al., 2004). For the analysis of specific AOB was also used 6% polyacrylamide gel but the gradient was 30 – 35% (Mahmood et al., 2006). DGGE analysis was performed in 1×TAE buffer (40 mM Tris- acetate, 1mM EDTA) at a constant temperature of 60 °C at 90 V for 17 hours. Bands on gel was visualized after GelRed staining. Pictures were captured with GelLogic 212 PRO Imaging System (Carestream Health, Inc., USA). For identification of ammonia oxidizers, specific bands which were cut out and put in 200 µl of H_2O. DNA from each band was eluted to water during next 24 hrs. PCR was performed with specific primers used in the second round of nested PCR using 1 µl of eluted DNA as template. Products were again put on gel and analyzed by DGGE to confirm identity of band. In case of single band, product were purified by EXO I/FastAP. Pure DNA was diluted to concentration 500 ng*µl. Five µl of prepared DNA was mixed with 5 µl of primer and sent to sequencing (Macrogen Inc., Korea).

Statistical analysis

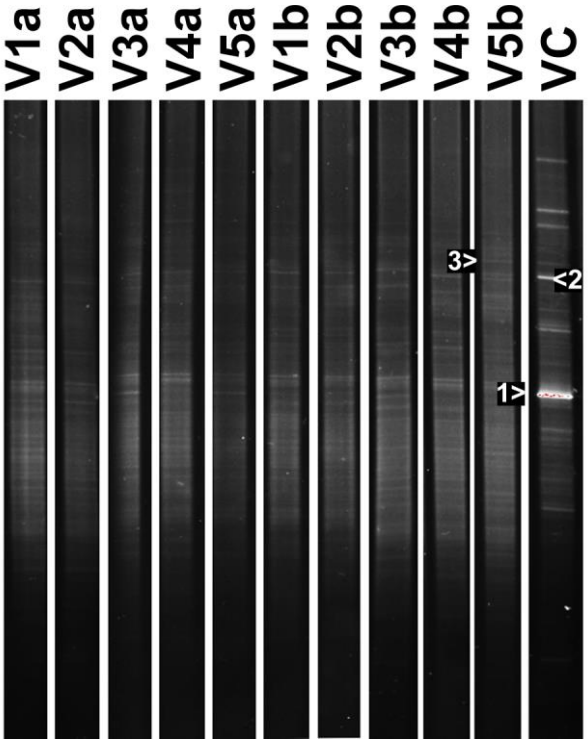
Sequences were transformed by Finch TV program to FASTA format. We compared each sequence to Genbank database by BLAST software. Quantity of bands from PCR-DGGE was analyzed by CARESTREEM program. Principal component analysis (PCA) was figured out in MVSP program (Multivariate statistical package; Kovach computing, Inc.) based on occurrence and relative intensity of bands (OTU). Shannon and Simpson diversity index were based on relative intensity of OTUs given by PCR-DGGE. Diversity indexes and the values of samples in all the PCA components were compared by variance analysis in Statgraphics program (Statpoint Technologies, Inc.)

Results and discussion

Fertilizers influence on bacterial community

The results of PCR-DGGE method showed that the addition of fertilizer influenced the structure of the soil bacterial community. According to Singh and Ryan (2015), fertilizer use can have positive or negative effects on soil health and microbiota. It can affect reduction in some soil organisms, but these effects are relatively short-lived and occur only at the site of the fertilizer application band. Significant increases in microbial biomass are observed by long-term application of fertilizers.

Number of differentiated OTUs varied from 19 to 37 per one variant (Figure 2). In vermicompost (VC) we detected 19 OTUs quite different from source soil (variant V1a). Number of detectable bands rose during incubation. The highest number of OTUs (32 – 37) was found in variant with double annual dose of vermicompost (V5b variants, Figure 2 and 3), OTUs counts in variants V4b (33 – 36 OTUs) and V3b (34 – 36 OTUs) were similar. In comparison, number of OTUs in control variant varied between 27 and 28 OTUs, which was the lowest diversity at the end of experiment.



V1 = soil (control); V2 = soil+vermicompost (40 t*ha^{-1}); V3 = soil+vermicopost (20 t*ha^{-1})+ LAD 27 (30 kg N*ha^{-1}); V4 = soil+LAD 27 (60 t*ha^{-1}); V5 = soil+vermicompost (80 t*ha^{-1}); a = samples taken at the beginning of the experiment; b = samples taken at the end of experiment; VC = vermicompost

V1 = pôda (kontrola); V2 = pôda +vermikompost (40 t*ha^{-1}); V3 = pôda +vermikopost (20 t*ha^{-1})+ LAD 27 (30 kg N*ha^{-1}); V4 = pôda+LAD 27 (60 t*ha^{-1}); V5 = pôda +vermikompost (80 t*ha^{-1}); a = vzorky odobraté na začiatku experimentu; b = vzorky odbraté na konci experimentu; VC = vermikompost

Figure 2. PCR-DGGE profiles of bacterial community in soil, vermicompost and maize rhizosphere

Figure 2. PCR - DGGE profily bakteriálnej komunity v pôde, vo vermikomposte a v rizosfére kukurice

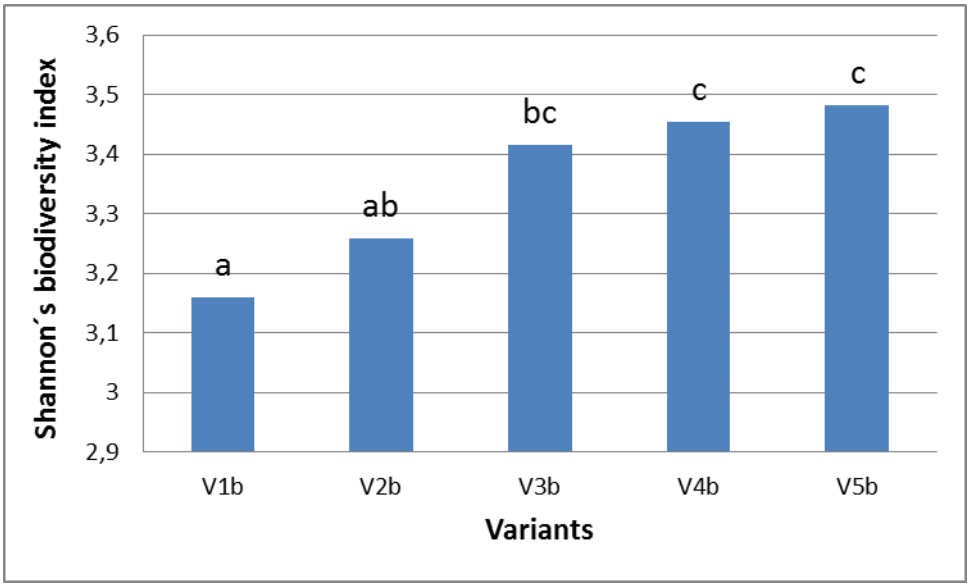
As mentioned, PCR-DGGE bacterial community of vermicompost differs from soil community. Most visible band of VC marked as 1 (identified as *Pseudomonas* sp.) was not found in fertilization variants neither in variant with double annual dose of vermicompost (V5a, V5b). Probably there were not favorable conditions for the survival of this OTU. Similar situation was observed in many other bands. Only bands marked as 2 (*Pirellula* sp.) and 3 (*Flavobacterium* sp.) were detectable after 74 days

of cultivation but just in variant V5b. This can be explained by deficiency of organic matter, which they utilized.

Statistical values of soil bacteria diversity set Shannon's index were at the end of the experiment significantly influenced by fertilization. The highest diversity index was found in variants V3b, V4b and V5b. Whereas the lowest index we encountered in the control treatment without fertilization (V1b) (Figure 3). High diversity in V5b variant may be caused by fact that vermicompost by itself contains its own microbial community, which was added to soil in double dose and included to analysis of bacterial community. As studies showed, the worm cast contains thousands of bacteria, enzymes, and remnants of plant materials that were not digested by the worms. In fact, the bacterial population of it is much greater than the bacterial population of either ingested soil. Microbial activity of microorganisms in worm castings is ten to twenty times higher than that of in the soil and other organic matter (Adhikary, 2012).

Similar results were also reported by Zeng et al. (2007), where addition of organic compounds were not only richer in nutrients, but compared to chemical fertilizers they were also able to persist longer in the soil and provide the desired amount of carbon, nitrogen and energy for growth and multiplication of microorganisms in the soil. According to Zhang et al. (2012), organic fertilizers addition can affect the increased diversity of the bacterial community in the soil directly by transferring specific organisms contained in.

In comparison to control (V1b) three other variants (V3b, V4b and V5b) were significantly different. Overall diversity was increasing more in variants with higher nitrogen content. This shows the relation of bacterial development to amount of available nitrogen. Recently, Geiseller and Scow (2014) published a meta-analysis from 64 long-term experiments from around the world and revealed that application of mineral fertilizer led to a significant increase (15.1%) in the microbial biomass above levels in the unfertilized control treatments.



V1 = soil (control); V2 = soil+vermicompost (40 t*ha^{-1}); V3 = soil+vermicopost (20 t*ha^{-1})+ LAD 27 (30 kg N*ha^{-1}); V4 = soil+LAD 27 (60 t*ha^{-1}); V5 = soil+vermicompost (80 t*ha^{-1}); b = samples taken at the end of experiment; means marked by the same letter are not statistically significant different (ANOVA, Tukey test, α=0.05)

V1 = pôda (kontrola); V2 = pôda +vermikompost (40 t*ha^{-1}); V3 = pôda +vermikopost (20 t*ha^{-1})+ LAD 27 (30 kg N*ha^{-1}); V4 = pôda+LAD 27 (60 t*ha^{-1}); V5 = pôda +vermikompost (80 t*ha^{-1}); b = vzorky odbraté na konci experimentu; hodnoty, ktoré nie sú štatisticky preukazne odlišné sú označené rovnakým písmenom

Figure 3. Differeces in fertilizers variants effect on changes of bacterial community diversity at the end of the experiment (ANOVA, Tukey test; α = 0.05)

Figure 3. Rozdiely vo vplyve variantov hnojenia na zmeny diverzity komunity baktérií na konci pokusu (ANOVA, Tukey test; α = 0.05)

Profiles similarity was compared by PCA where two main components explained 69,6% of variability. It is clear from the graph (Figure 4) that there were significant differences between same samples at the beginning of the experiment and after cultivation. It is seen that microbial community in sample marked VC was significantly different in compare to all other samples. Sample marked as V1b was the control after cultivation and it is the most different in comparison to other cultivated variants. It can be explained by fact, that there was no fertilizer added in this variant and the bacterial community had fewer nutrients to utilize what caused diversity and probably the abundance decline of bacteria. It is also seen that the least effect of fertilizer was found in variant V2 with annual dose of VC addition.

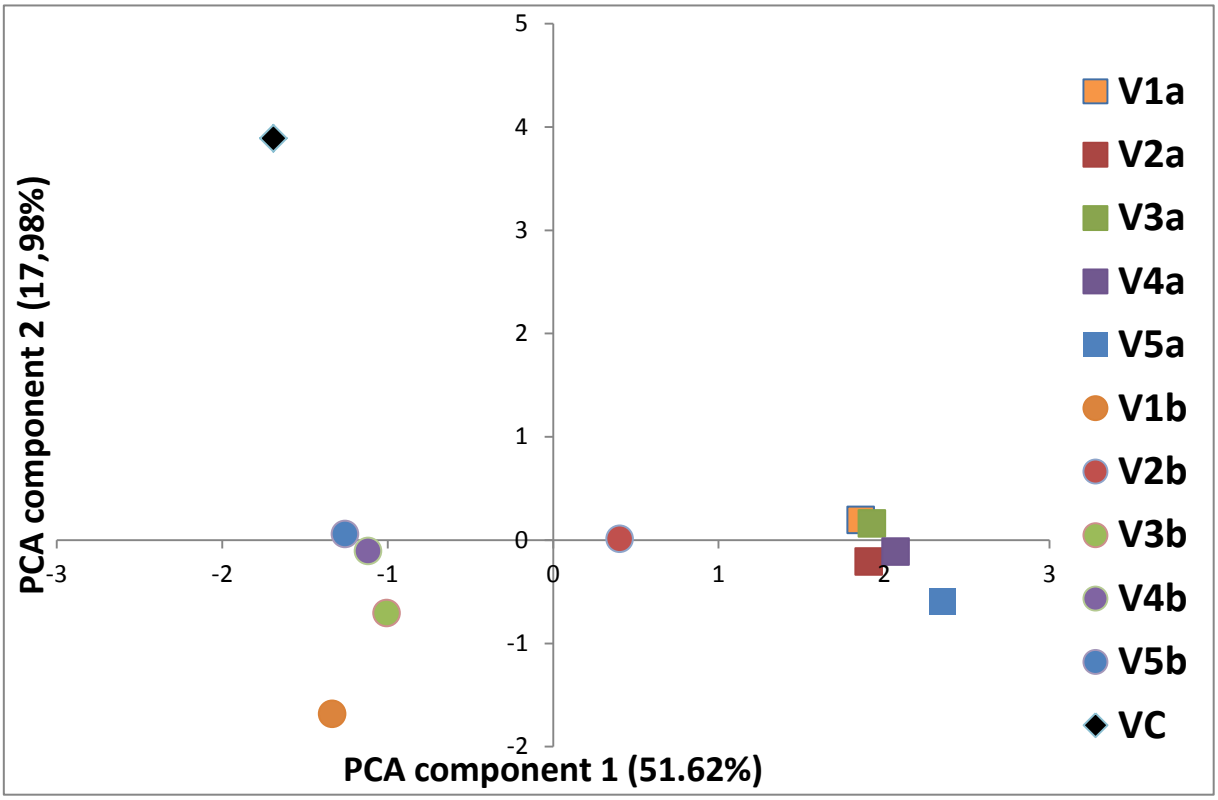


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Figure 4. Principal component analysis (PCA) of main components for different fertilization variants and vermicompost based on PCR – DGGE analysis of total bacteria community

Figure 4. Graf analýzy hlavných komponentov pre rôzne varianty hnojenia a vermikompost na základe PCR - DGGE analýzy celkovej komunity baktérií

Fertilizers influence on AOB community

In AOB community we have seen just 16 OTUs (Figure 5). Number of differentiated bands varied from 2 to 16 per one sample. Differences between variants have not been as significant as in case of bacterial community. The highest diversity was found in variant V3b with combination of vermicompost and LAD 27. Number of OTUs varied from 8 to 12 per sample. The lowest diversity was again found in variant V2b where annual dose of vermicompost was applied (2 – 16 OTUs). In vermicompost the number of OTUs was 9.

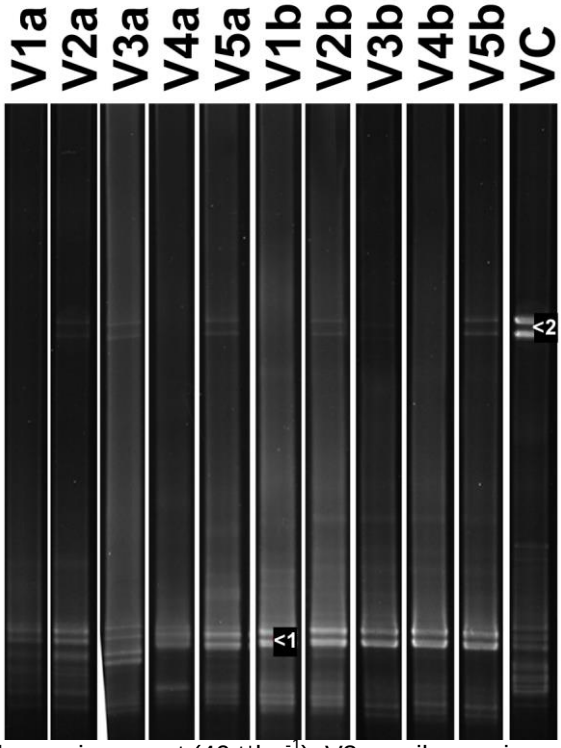

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Figure 5. DGGE gel illustrating diversity of AOB (group *β- proteobacteria)* in vermicompost and maize rhizosphere

Figure 5. DGGE gél zobrazujúci diverzitu AOB (skupina *β- proteobacteria*) vo vermikomposte a v rizosfére kukurice

The sharpest band represented OTU from AOB community marked as 1 (Figure 5) identified as *Nitrosomonas* sp. was found in all samples except vermicompost, at the beginning of the experiment as well as after cultivation. In vermicompost the dominant OTU (marked as 2) identified as *Nitrosospira* sp. was found. Its presence is visible even at the beginning of the experiment in all samples with VC addition and it is still occurred even after the cultivation.

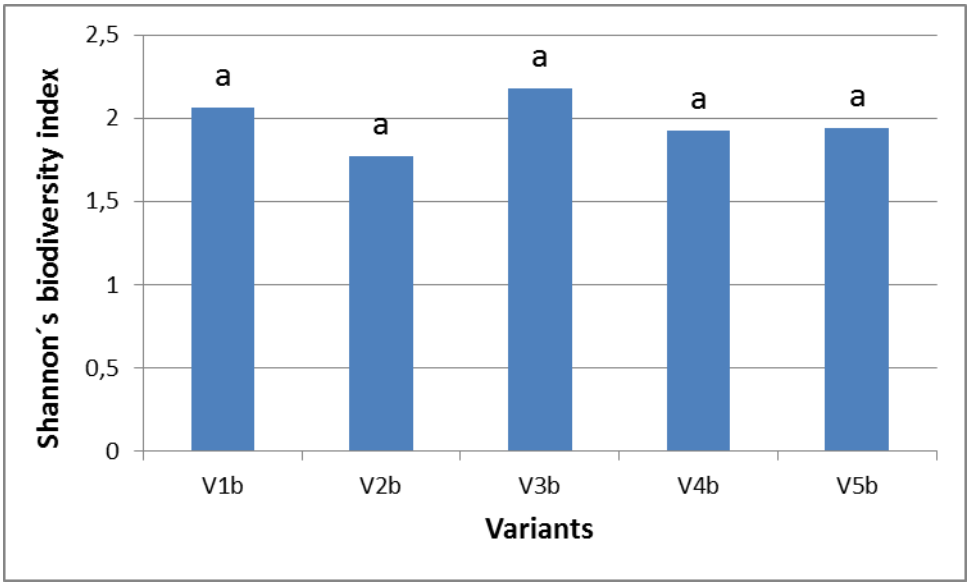

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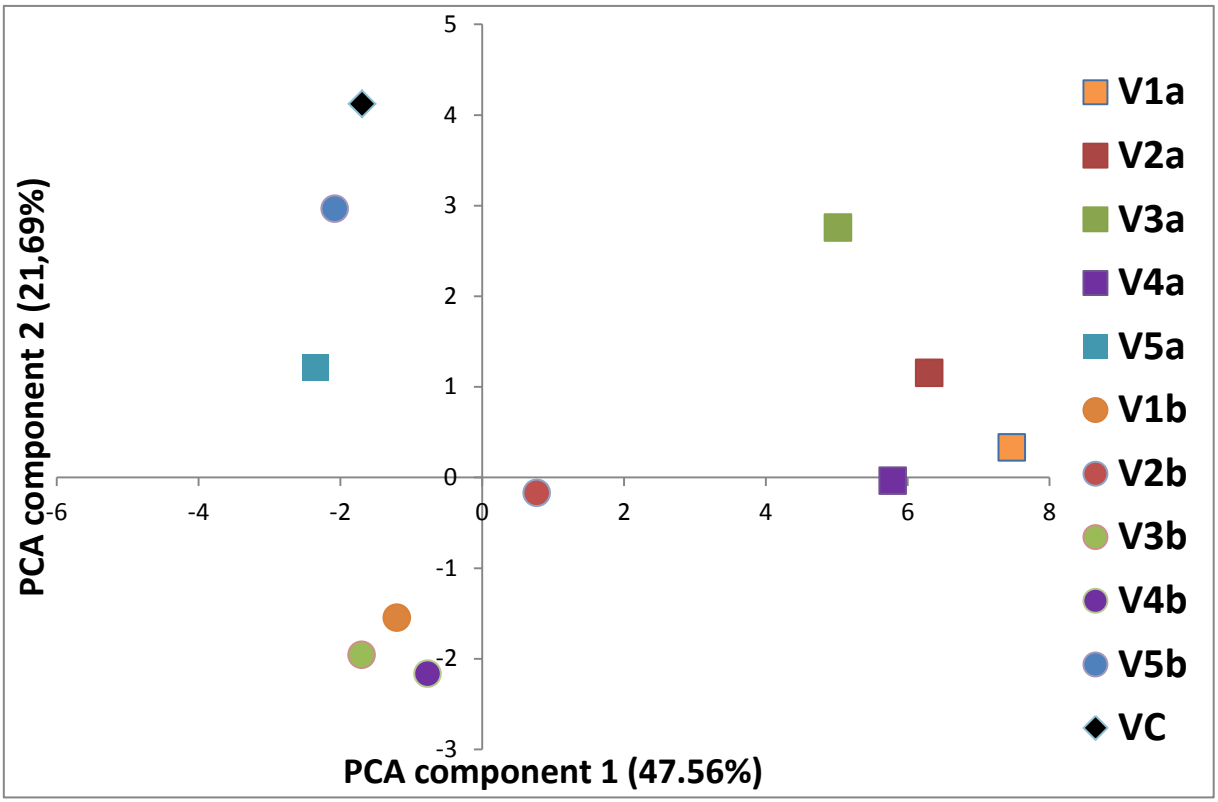
Figure 6. Effect of fertilization on AOB diversity changes at the end of the experiment (ANOVA, Tukey test; α = 0.05)

Figure 6. Vplyv hnojenia na zmeny diverzity AOB na konci pokusu (ANOVA, Tukey test; α = 0.05)

According to statistical values of biodiversity of AOB community set Shannon's index were not influenced by fertilization (Figure 6). The highest number of AOB community OTUs was found in variant V3b with VC and LAD 27 addition. In terms of effects on soil health, crop production or the environment, there is no conflict between mineral fertilizers and organic nutrient sources; quite the contrary, their use is complimentary (Singh and Ryan, 2015). This may be caused by fact that vermicompost by itself contains its own microbial community, which was added to soil in double dose and included to AOB community analysis.



V1 = soil (control); V2 = soil+vermicompost (40 t*ha^{-1}); V3 = soil+vermicopost (20 t*ha^{-1})+ LAD 27 (30 kg N*ha^{-1}); V4 = soil+LAD 27 (60 t*ha^{-1}); V5 = soil+vermicompost (80 t*ha^{-1}); a = samples taken at the beginning of the experiment; b = samples taken at the end of experiment; VC = vermicompost.

V1 = pôda (kontrola); V2 = pôda +vermikompost (40 t*ha^{-1}); V3 = pôda +vermikopost (20 t*ha^{-1})+ LAD 27 (30 kg N*ha^{-1}); V4 = pôda+LAD 27 (60 t*ha^{-1}); V5 = pôda +vermikompost (80 t*ha^{-1}); a = vzorky odobraté na začiatku experimentu; b = vzorky odbraté na konci experimentu; VC = vermikompost.

Figure 7. PCA of main components of four different fertilization variants and vermicompost based on PCR – DGGE analysis of AOB community

Figure 7. Graf analýzy hlavných komponentov pre štyri rôzne varianty hnojenia a vermikompost na základe PCR-DGGE analýzy komunity AOB

Principal component analysis extracted two components explaining 69,25% diversity. Changes in community of AOB illustrated in graph (Figure 7) were different in comparison to total bacterial community diversity (Figure 4). The clusters are not as clear as it was in total bacterial community and the differences between fertilizers variants are not significant. In the graph there is seen that community of sample V5b is similar to community of VC analogous to sample V5a. Addition of vermicompost in this variant was 80 t*ha^{-1}. Based on this picture it is clear that the relevant part of AOB community in these samples was added by the application of vermicompost and the bacteria were able to survive the cultivation conditions. Sample V2b is quite different in compare to other variants as it was also in previous case. In this graph, the interesting fact can be seen. The difference in cluster of variants from the beginning of the experiment is greater than the difference at the end of experiment. It can be explained by fact that AOB could be added to the samples directly in

vermicompost or supported by LAD 27 at the beginning but they were not able to survive the cultivation or they had nothing to utilize during it.

Conclusions

There were found significant differences in fertilizers effect on total bacterial community. Increase of diversity was observed in three variants (V3b, V4b and V5b). The most significant influence on bacterial community was found in variant (V5b) where double annual dose of vermicompost was added. Although community of ammonia oxidizing bacteria did not changed significantly, introduced *Nitrosospira* genus was found in all variants with addition of vermicompost. Nevertheless, in general the fertilizers had positive influence on bacterial community. The difference in the impact of the application of fertilizers and mineral fertilizers consisted mainly of the emergence of new dominant species occurring after addition of organic fertilizers. In conclusion it should be emphasized that wide species spectrum of bacteria is important for maintaining soil health and adequate food crop production and its increase can be supported by fertilizers addition even if they are primarily used to increase nutrient availability to plants.

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