Serbian Society of Soil Science
University of Belgrade, Faculty of Agriculture

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3rd International and 15th National Congress

SOILS FOR FUTURE UNDER GLOBAL CHALLENGES

21–24 September 2021
Sokobanja, Serbia

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BACTERIAL COMMUNITIES IN ACIDIC SOIL

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Abstract

Acidification is one of the main types of soil degradation in Serbia, as a result of excessive use of mineral fertilization, pollution, as well as reduction of soil organic matter. Increased soil acidity directly affects plant nutrition and food productivity, at the same time leading to biodiversity changes. Bacterial diversity in soil is recognized as the main pillar of soil quality, ecosystem stability, climate change resilience, and represents an important element of sustainable agriculture. The diversity and abundance of bacteria in soil are strongly related to various abiotic factors, particularly to soil pH as one of the major determinants shaping their community structure.

The main objective of the research was to access the bacterial community in agricultural acid soils using metagenomic approach. Soil samples were taken at three locations: cornfield near Zaječar (Eastern Serbia), apple and raspberry orchards near Čačak (Central Serbia). The representative samples were subjected to sequencing of V3 and V4 regions of 16S rRNA gene using Illumina® MiSeq™. Besides microbiome, physico-chemical analyses were performed, including mechanical composition, adsorptive complex properties, and basic parameters of soil fertility.

Soil samples from Čačak have strongly acidic reaction, belonging to class of clay loam with a significant share of powder fraction. Soil from Zaječar is heavy clay with 48.80% of the clay fraction, and middle acidic reaction. Cation exchange capacity (CEC) is the middle level, and saturation of the adsorptive complex with base cations is weak in raspberry orchard soil, while soil under apple and corn showed high levels of CEC and middle saturation of adsorptive complex with base cations. Soil from apple orchard is characterized by low humus content and low content of available phosphorous (P), and middle P content in the raspberry orchard and cornfield. Available K content was similar for three analyzed fields (35.60-37.90 mg $100g^{-1}$).

In all of the studied soils, the most abundant phyla were *Firmicutes*, *Proteobacteria*, and *Actinobacteria* (each above 20%), which are the usual predominant phyla in the fertile soil. *Proteobacteria* composition showed differences between the soil samples, with higher share of *Enterobacteriales* in cornfield soil. Acid soil from Zaječar had 4.89% abundance of *Chloroflexi*, while the soils from Čačak included six additional phyla besides *Chloroflexi* (with more than 1% abundance), indicating significantly higher biodiversity. After the three most common phyla, *Acidobacteria* were predominantly abundant, and the presence of these oligotrophic taxa is characteristic of less fertile soil. *Actinobacteria* are mainly related to neutral or alkaline soil, but in recent decade acidotolerant *Actinobacteria* are being highlighted in terms of maintaining ecosystem balance, and raise of pH.

The composition of bacterial community showed some similarities between tested soils, with differences within their microbiome that can be attributed to mechanical composition and agronomic practice.

Keywords: acidic soil, bacterial community, metagenomic analysis, 16S DNA

INTRODUCTION

The need to provide food for the growing population leads to the intensification of agricultural production, and makes the soil quality and biodiversity decrease a global issue. Soil acidification is one of the main types of soil degradation in Serbia, where acidic soils make more than 60% (Ličina et al., 2011). Among the main reasons for soil acidification is excessive use of mineral fertilization, pollution, as well as reduction of soil organic matter content. Most of the cultivated plants require a weak acid, neutral or weak alkaline reaction. Increased soil acidity directly affects plant nutrition through nutrient solubility (Rousk et al., 2020), which seriously limits productivity, and directly leads to food production decrease. Besides the effects on plant nutrition, soil acidity leads to changes in biodiversity. Also, the mechanical composition has a great influence on the water-air, thermal, biological, and nutritional regime of the soil (Gajić, 2006).

Bacterial diversity in soil is recognized as the main pillar of soil quality, ecosystem stability, climate change resilience, nutrient cycling, and important element of sustainable crop production. The diversity and abundance of bacteria in soil are strongly related to various abiotic factors, particularly to soil pH as one of the major determinants and predictors of their community structure. Over time, environmental factors like pH, inevitably shape bacterial diversity and promote microbial groups that are well adapted to the changing surrounding. Bacterial diversity is particularly related to soil acidity and decreases with pH drop (Wu et al., 2017). Wan et al. (2020) confirmed that soil pH predicts and directly influences bacterial diversity and community function of rhizosphere bacteria.

Traditionally, cultivation-based techniques have been used to access microbial diversity in the environment, but those methodologies are restricted to only 0.1 to 1% of bacterial communities, so that the result obtained by cultivation techniques do not represent overall bacterial community (Torsvik et al., 1990). Cultivation-based techniques are irreplaceable in obtaining valuable microbial strains, but in diversity research, they do not provide a complete picture of community composition (Smit et al., 2001).

The main objective of the presented research is to access bacterial community in agricultural acid soil using a metagenomic approach and to relate those results with physic-chemical soil properties. Soil samples were taken at three locations: cornfield (near Zaječar, Eastern Serbia), and apple and raspberry orchard (near Čačak, Central Serbia). In addition to 16S ribosomal RNA gene targeted sequencing, those soils with acidic reactions are analyzed through their bacterial community as well as physico-chemical characteristics, such as pH, the concentration of ions, cation exchange capacity (CEC), and heavy metal concentration, were measured concurrently.

MATERIALS AND METHODS

Soil sampling

The surface layer of soil (0-30 cm) was sampled at three locations: corn field Halovo (near Zaječar, Eastern Serbia), apple orchard in Trnava, and raspberry orchard in Grab (both near Čačak, Central Serbia).

Soil samples were used for physico-chemical analysis, and one portion of the soil was prepared for microbiome analysis by mixing with DNA/RNA Shield (Zymo Research, Irvine, CA) in ratio 1:10 in order to preserve soil DNA prior to analysis.

Physico-chemical analysis

Examination of the mechanical composition, physical-chemical parameters, and basic soil fertility was performed in the laboratory of the Institute of Fruit Growing, Čačak. The aggregate composition of the soils was determined by sieving procedures (Bošnjak et al., 1997), and physico-chemical analyzes included the determination of the sum of exchangeable adsorbed alkaline cations (S meq 100 g^{-1}) (Kappen method), determination of hydrolytic soil acidity (H meq 100 g^{-1}), cation exchange capacity (CEC (T) meq 100g^{-1}), saturation with adsorbed bases (V%) (Gajić and Dugalić, 2005). Basic soil fertility included testing the pH value in H_2O and 1N KCl (potentiometrically); humus (the method by Kotzman); total nitrogen (the method by Kjeldahl); easily accessible phosphorous and potassium (AL method, P_2O_5 colorimetrically, K_2O flame photometrically).

Microbiome analysis

Soil samples are analyzed by the ZymoBIOMICS® Targeted Sequencing Service for Microbiome Analysis (Zymo Research, Irvine, CA). DNA was extracted, and 16S ribosomal RNA gene sequencing was performed by ZymoBIOMICS®-96 MagBead DNA Kit (Zymo Research, Irvine, CA). PCR inhibitors were removed from DNA using OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA). DNA samples were prepared for targeted sequencing with the *Quick*-16S™ NGS Library Prep Kit (Zymo Research, Irvine, CA). Primer set used for amplification was: *Quick*-16S™ Primer Set V3-V4 and (Zymo Research, Irvine, CA). The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles). The sequencing was performed with 10% PhiX spike-in. Afterward, unique amplicon sequences were inferred from raw reads using the Dada2 pipeline (Callahan et al., 2016). Chimeric sequences were also removed with the Dada2 pipeline. Uclust from Qiime v.1.9.1 (Caporaso et al., 2010) and internally designed Zymo Research 16S Database were used for taxonomy assignment. Where applicable, a taxonomy that has significant abundance among different groups was identified by LEfSe (Segata et al., 2011).

RESULTS AND DISCUSSION

Soils of the shallow profile, low content of clay with low capacity of absorption of cations and acid reaction, have limited possibilities for the cultivation of certain crop types. The clay fraction content of 20-30% enables optimum potential soil fertility, given that other suitable agro-ecological conditions of fruit cultivation must be met. Plots in apple and raspberries plantations are in the class of clay loam with 59.30 to 67.30% of the physical clay and a high proportion of the powder (41.20 to 43.80%). The soil under corn contains 48.80% of colloidal clay and 79.50% of physical clay, which classifies it in the class of heavy clays (Table 1).

Table 1. Mechanical composition of soil (%)

Samples	2-0.2 mm	0.2-0.02 mm	0.02-0.002 mm	<0.002 mm	>0.02 mm	<0.02 mm	Soil texture
Apple orchard	8.35	24.35	43.80	23.50	32.70	67.30	Clay loam
Raspberry orchard	15.36	25.34	41.20	18.10	40.70	59.30	Clay loam
Cornfield	2.36	18.14	30.70	48.80	20.50	79.50	Heavy clay

Physico-chemical characteristics of the soil (Table 2) show that the values of hydrolytic acidity are very high (> 8 meq 100 g^{-1}) in all of the three plantations. The highest values are in the raspberry orchard, followed by cornfield and apple orchard. The sum of exchangeable adsorbed base cations is lowest in raspberries (7.22 meq 100 g^{-1}), higher in apples (12.76 meq 100 g^{-1}), and highest in maize (28.98 meq 100 g^{-1}). The previously mentioned results are in accordance with the degree of saturation with adsorbed bases, so that the soil in the raspberry orchard is poorly saturated, and in the apple orchard and cornfield it is moderately saturated (Baize, 1993). Based on the classification of Culman et al. (2019) soil in the apple orchard is in the class of silt loams with values of CEC 22.48 meq 100g $^{-1}$, and in the soil under raspberries and corn in the class of clay and clay loam with values of 27.53 meq 100 g $^{-1}$ and 40.03 meq 100 g^{-1}.

Table 2. Hydrolytics and adsorptive complex

Samples	H	S	CEC-S	CEC	V
	meq 100 g^{-1}				%
Apple orchard	14.95	12.76	9.72	22.48	56.76
Raspberry orchard	31.25	7.22	20.31	27.53	26.23
Cornfield	17.00	28.98	11.05	40.03	72.40

Soil acidity is one of the most important chemical properties and affects nutrient uptake and microbiological activity (Liu and Hanlon, 2012). Most fruit species are grown in soil pH ranging from 5.5 to 7.0. Large deviations from the stated soil pH values are not suitable for nutrient uptake and achieving optimal yields (Liu et al., 2014). In production practice, the pH value of the soil is often not in the optimal range, so one of the following alternatives should be considered: using only suitable soils with appropriate pH values; selection of fruit species and/or varieties that are tolerant to existing soil pH values; application of lime fertilizer for bringing the soil into the range of optimal acidity

(Milinković et al., 2017). The basic fertility of the soil in an apple orchard showed a strongly acidic reaction with a low content of humus, a medium content of total N, low content of easily accessible phosphorus and high content of easily accessible potassium. Similar fertility results of the soil of the Čačak region were determined by Milinković et al. (2016). The uptake of phosphorus is related to the reaction of the soil solution. When soil pH is lower than 5.5 or higher than 7.5, iron and aluminium or calcium and magnesium can bind phosphorus ion and phosphorus becomes unavailable for adoption by fruit trees (Lui et al., 2014). In the raspberry plantation, in addition to the strongly acidic reaction, there is medium humus content, a high content of total N and an optimal content of easily accessible phosphorus and potassium. The examined soil in the Zaječar area is in the class of degraded vertisol, with acid reaction, medium humus content, high total N content, medium content of easily accessible phosphorus and high content of easily accessible potassium (Table 3).

Table 3. Basic soil fertility

Samples	pH H_2O	pH KCl	$CaCO_3$ (%)	humus (%)	total N (%)	P_2O_5 mg 100 g^{-1}	K_2O mg 100 g^{-1}
Apple orchard	5.29	4.21	0.56	2.86	0.14	6.41	37.50
Raspberry orchard	4.61	3.84	0.28	4.63	0.23	15.98	35.60
Cornfield	5.86	4.90	0.56	4.21	0.21	11.21	37.90

Lauber et al. (2009) analyzed bacterial communities in a wide variety of locations in America, and they concluded that community composition depends on soil pH, while geographical location is not crucial for its composition. Later, Cho et al. (2016) obtained results indicating that pH is not so crucial for shaping bacterial community, since its effect is combined with other environmental factors. Anyhow, the effects of various environmental and anthropogenic factors on soil microbiome are not well understood, and their synergistic effect makes them challenging to understand.

The obtained microbiome results showed that soil from cornfield (Zaječar) have significantly lower bacterial diversity compared to two soils collected in the Čačak region, which are similar to each other to some level (Figure 1, Table 4). In all of the studied soils, the most abundant phyla were *Firmicutes*, *Proteobacteria*, and *Actinobacteria* (each above 20%, Fig. 1), which are the usual predominant phyla in the agricultural soil (Qi et al., 2018). Acid soil from Zaječar had 4.89% abundance of *Chloroflexi*, while the soils from Čačak included six additional phyla besides *Chloroflexi* (with more than 1% abundance), indicating significantly higher biodiversity.

After the three most common phyla, *Acidobacteria* were predominantly abundant in soil from Čačak region, and the presence of these oligotrophic taxa is characteristic of less fertile soil (Qi et al., 2018). Although metagenomic analysis showed a significant abundance of those microbes in soil, their reluctance to cultivation led to unfamiliarity with their functions (Kalam et al., 2020). They have an important role in the transformation of organic matter in soil (de Chaves et al., 2019). Their presence in soil is strongly connected to nutrient inputs, as well as organic matter content, and *Acidobacteria* could have a role in soil recovering after drastic ecosystem disturbances (Kielak et al., 2016). *Actinobacteria* are mainly related to neutral or alkaline soil, but in the recent decade acidotolerant *Actinobacteria* are being highlighted in terms of maintaining ecosystem balance, and raise of pH (Tamreihao et al., 2018a; Tamreihao et al., 2018b). The abundance of *Actinobacteria* in soil is usually lower compared to some other phyla such as *Firmicutes*, but their carbohydrate-active enzymes make a significantly higher portion in soil indicating their important role in plant residue decomposition (Bao et al., 2021). Besides their plant growth promoting and biocontrol activities provide multiple benefits for sustainable agriculture (Tamreihao et al., 2018b).

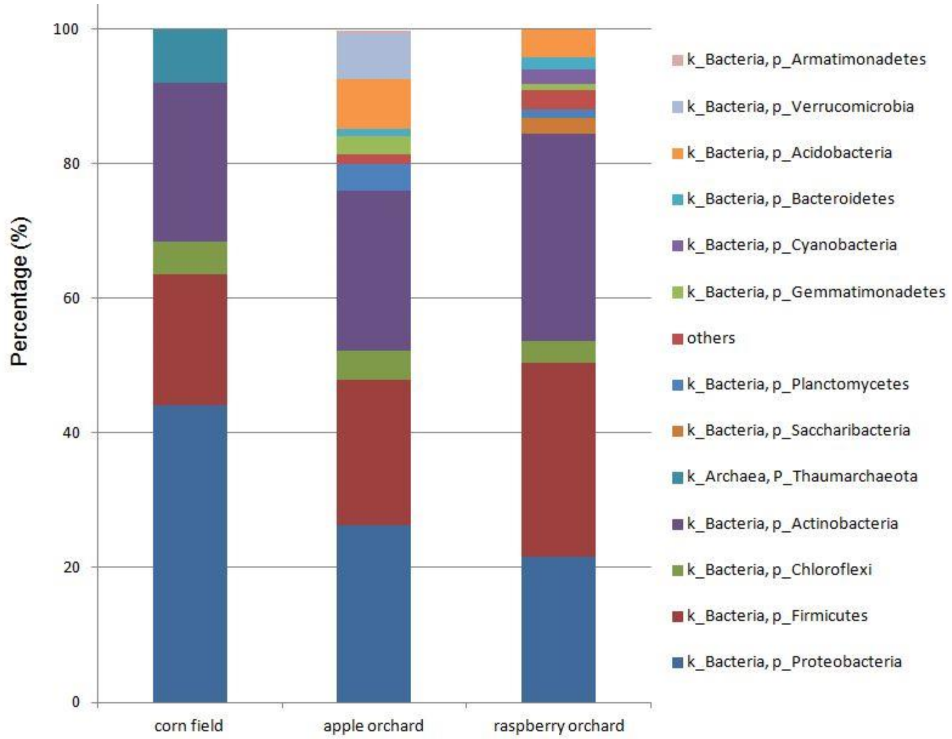

Figure 1. Microbial community composition at phylum level, based on 16S RNA sequences (relative abundance)

Proteobacteria are commonly abundant soil microbes, but their composition showed differences between the soil samples, with a significantly higher share of *Enterobacteriales* in cornfield soil (Table 5). *Enterobacteriales* are ubiquitous order in nature, capable to survive in soil, and including numerous foodborne pathogens (Iwu et al., 2020). Although they could have a beneficial effect on organic matter transformation in anoxic zones (Degelmann et al., 2009), and some of them even exhibit plant growth promoting characteristics (de Souza, et al., 2015; Khalifa et al., 2016), their presence in soil represents a risk for food production safety. One of the possible sources of *Enterobacteriales* in cornfield could be soil fertilization with inappropriately prepared manure.

Soil from cornfield generally showed significantly lower bacterial diversity that is evident at all of the taxonomy levels. Only five orders belonging to *Proteobacteria* are detected in that soil, which is almost twice lower diversity compared to the acidic soil from apple orchard (Table 5). The most abundant representatives in the soil from apple orchard were *Rhizobiales* (6.72%), and *Rhodospirillales* were the most abundant for the soil from raspberry orchard (Table 5). The abundance of those orders in soil, and rhizosphere particularly is of importance because of the nitrogen availability, especially in N-deficient conditions (Wasaki et al., 2018).

Table 4. Classes-level relative abundance of the three most abundant phyla: *Firmicutes*, *Proteobacteria*, and *Actinobacteria*

Taxonomy	Relative abundance (%)		
	Soil from corn field	Soil from apple orchard	Soil from raspberry orchard
p_Proteobacteria, c_Gammaproteobacteria	24.23	2.52	9.72
p_Proteobacteria, c_Deltaproteobacteria	9.79	4.61	0.2
p_Proteobacteria, c_Betaproteobacteria	7.92	4.69	0.9
p_Proteobacteria, c_Alphaproteobacteria	2.28	14.54	10.72
p_Firmicutes, c_Bacilli	19.42	11.38	9.21
p_Actinobacteria, c_ Thermoleophilia	17.69	6.23	5.2
p_Actinobacteria, c_ Actinobacteria	5.78	15.96	21.59
p_Firmicutes, c_NA	/	1.01	1.08
p_Firmicutes, c_Erysipelotrichia	/	0.32	2.42
p_Firmicutes, c_Clostridia	/	8.83	16.13
p_Actinobacteria, c_Acidimicrobia	/	0.42	2.89
p_Actinobacteria, c_NA	/	1.26	/
Classes belonging to other phyla	12.89	28.23	19.94

According to Xue et al. (2018), the basic soil fertility properties are connected to the absolute abundance of microbial groups, however, other factors such as pH and texture had higher impact on their relative proportion. In our study, it seems that soil mechanical composition significantly affects bacterial diversity, making it scarcer in heavy clay compared to clay loam. The other factor contributing to bacterial community differences could be applied agricultural practices, which demand their upgrade through application of bioferilizers and corresponding base cations directed to the improved microbial diversity and ecosystem stability.

Tab. 5. Proteobacteria community composition

Taxonomy	Relative abundance (%)		
	Soil from corn field	Soil from apple orchard	Soil from raspberry orchard
c_Alphaproteobacteria, o_Rhizobiales	2.23	6.72	3.25
c_Alphaproteobacteria,o_Sphingomonadales	/	3.64	2.14
c_Alphaproteobacteria, o_Rhodospirillales	/	3.92	4.74
c_Alphaproteobacteria, o_Caulobacteriales	/	/	0.59
c_Betaproteobacteria, o_Burholderiales	7.92	1.47	0.86
c_Betaproteobacteria, o_Nitrosomonadales	/	1.2	/
other Betaproteobacteria	/	1.79	/
c_Gammaproteobacteria,o_Xantomonadales	4.19	1.57	9.72
c_Gammaproteobacteria, o_Enterobacteriales	20.04	/	/
other Gammaproteobacteria	/	0.96	/
c_Deltaproteobacteria, o_Myxococcales	9.79	4.13	/

CONCLUSION

Analyzed soils with acidic reaction showed significant differences in the composition of their bacterial communities. Based on physico-chemical analysis, soil from cornfield showed high potential fertility, but significantly high content of the colloid clay affects chemical and microbiological properties, including bacterial community composition. The results indicate that other environmental factors besides pH acting on different geographical locations have additional effects on bacterial diversity in soil. Future extensive research is needed to evaluate the particular contribution of soil acidity to microbial communities, since our findings indicate that other factors such as mechanical composition are more important determinant.

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