Assessing soil bacterial community along vertical sampling gradient of the non-fertilized barley field in Estonia

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INTRODUCTION

The composition and diversity of microbial communities in agricultural soils influence directly the processes like nutrient cycling, nutrient capacity, aggregate stability etc. that are in high correlation with the health and productivity of valuable crop plants. Soil quality in agricultural farming depends also on management and land-use decisions. Understanding the impact of agricultural practices on soil microbial communities and their dynamics has become more tangible since the development of new metagenomic approaches.

It is known that the majority of bacteria in soils are found in the top 1 meter of soil (*e.g.* Whitman *et al.*, 1998), therefore it is important to determine the vertical dynamics of microorganism communities to make the soil sampling for broader research more effective.

Hereby, we investigated vertical changes in bacterial community structures with sampling depth in soil of zero fertilized patch of barley field. This barley field is part of a long-term monoculture field trial where number of variable criteria was brought to a minimum. The soil of the experimental site was Calcaric (Eutric) Cambisol (FAO classification). Bacterial 16S rDNA was analysed by 454 Life Sciences HTP pyrosequencing platform.

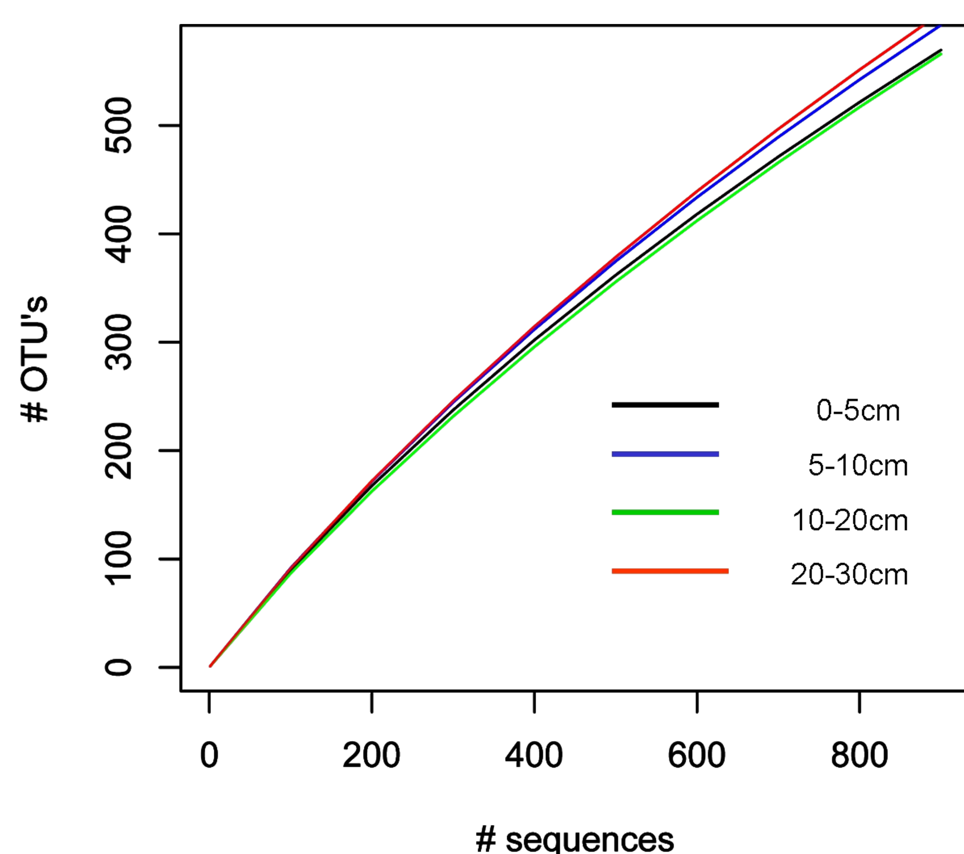

FIG. 1. Rarefaction curves for estimating the number of operational taxonomic units (OTUs) (assigned by RDP Classifier) at 3% dissimilarity level in four different soil sampling depths (average of 9 individual samples for each depth curve).

TABLE 1. T-test for comparing Shannon diversity index and number of observed OTUs of soil bacterial communities in 0-5 cm column to the other sampled depths. Total N=36 (9 in each depth grouping).

Sampling depth as factor	5-10 cm	10-20 cm	20-30 cm
T-test for comparing Shannon diversity index	P=0.43	P=0.67	P=0.82
	t=-0.79	t=-0.42	t=0.23
T-test for comparing number of observed OTUs	P=0.86	P=0.73	P=0.39
	t=0.18	t=-0.35	t=0.87

RESULTS

Neither bacterial diversity nor richness of soil community differed along vertical sampling gradient as revealed in the regression model, where sampling depth was included as factor (TABLE 1).

Sequences forming OTUs at the 3% dissimilarity level showed highly similar patterns according to the rarefaction curves (FIG. 1).

The most abundant OTUs assessed from the samples were from phyla *Proteobacteria*, *Bacteroidetes* and *Acidobacteria* (FIG. 2). There were significantly higher amount of bacteria from phylum *Bacteroidetes* (t=3.9, P=0.0005), tendency of higher amount of *Cyanobacteria* (t=2.1, P=0.047) and significantly lower amount of representatives belonging to the phylum *Nitrospira* (t=-4.0, P=0.0003) in the columns of deeper samples (TABLE 2.).

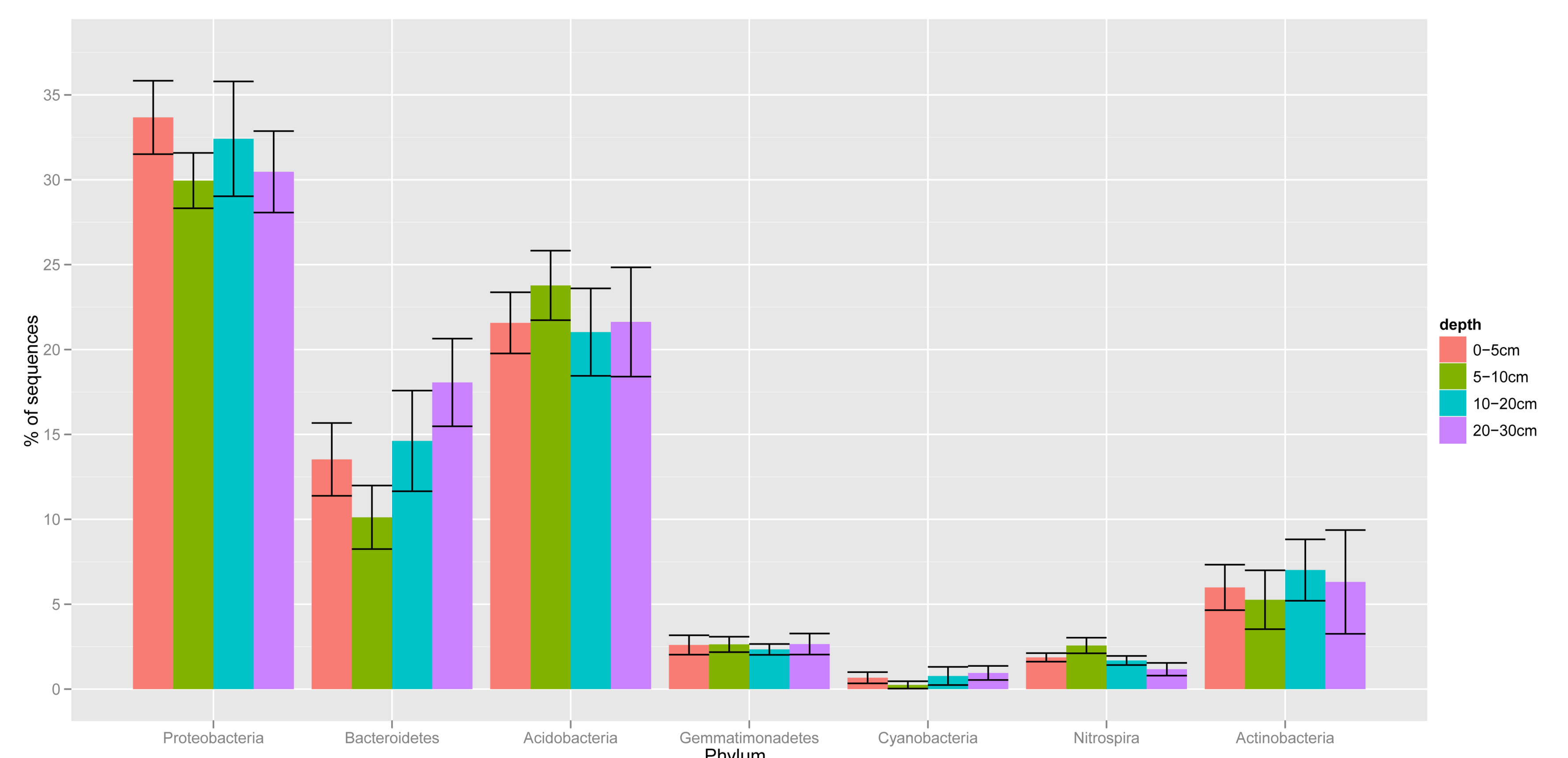

FIG. 2. Relative abundance (mean and 95% confidence interval) of the top seven bacterial taxa in the different sampling depths (n=9 for each depth) shown as percentage of acquired sequences.

TABLE 2. Linear regression for testing the relationships between the particular bacteria abundance and different sampling depths. Model DF=32.

Phylum	P(t)
Proteobacteria	0.239(-1.2)
Bacteroidetes	**0.0005**(3.9)
Acidobacteria	0.468(-0.7)
Gemmatimonadetes	0.895(-0.1)
Cyanobacteria	0.047(2.1)
Nitrospira	**0.0003**(-4.0)
Actinobacteria	0.439(0.8)

CONCLUSIONS

Bacterial community parameters were highly similar along the vertical soil sampling gradient.

There existed significant differences in the abundance of *Bacteroidetes* and *Nitrospira* bacteria in different sampling depth columns.

Further analysis for estimating the microbial community structure and dynamics along the vertical sampling gradient in the monoculture farming soils are needed.

MATERIAL AND METHODS

Samples were collected post-harvest and before tillage from one location, monoculture fertilizer used test field, test initiated at 1993. In this study 36 samples from 3 patches and 4 different depths (0-5 cm, 5-10 cm, 10-20 cm and 20-30 cm) of NPK0 = untreated control cultivated for barley ("Anni") were included into the pyrosequencing and bioinformatical analysis. Samples were collected using 30 cm steel probe that was washed thoroughly with 1% sodium hypochlorite solution and dH2O in-between samples. Soil samples were kept on ice for transport and frozen in 6 hours from sampling. DNA was extracted form 0.25 g (wet weight) soil according to procedure described by Sebastianelli *et al.* (2008) on Thermo Scientific KingFisher Flex system with added mechanical disruption step with ceramic beads in lysis solution (300 rpm for 6 min on Qiagen TissueLyser bead beater instrument).

DNA was amplified, 8nt barcode (marked as NNNNNNNN) and partial sequencing adapter sequences added to amplicons in PCR reaction (primers 8F 5'-TTGGCAGTCTCAGNNNNNNNNAGTTTGATCCTGGCTCAG-3',
357R 5'-GTCTCCGACTCAGNNNNNNNNCTGCTGCCTYCCGTA-3').

Reaction was run with Smart-Taq Hot Red 2X PCR Mix (Naxo, Estonia), 1 µl of extracted DNA and 0.2 µM each primer; initial heating 95°C for 15 min, 5 cycles (42°C 30 sec, 72°C 90 sec, 92°C 45 sec), 35 cycles (65°C 30 sec, 72°C 90 sec, 92°C 45 sec).

Full sequencing adapters were added in second PCR reaction
(Primers A 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3' and B 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3').

Reaction was run with Smart-Taq Hot Red 2X PCR Mix (Naxo, Estonia), 1 µl of 10x diluted amplicon and 0.2 µM each primer; initial heating 95°C for 15 min, 5 cycles (42°C 30 sec, 72°C 90 sec, 92°C 45 sec), 20 cycles (65°C 30 sec, 72°C 90 sec, 92°C 45 sec).

Amplicons were sequenced on Roche GS Junior instrument in BiotaP LLC laboratory according to manufacturer protocols. Sequences were processed and data analysed using mothur 1.19.1 (Schloss *et al.* 2009) and its embedded tools for aligning sequences, precluster, cluster, α- and β-diversity tools. Single-sequence OTUs were discarded from community comparisons to down-weight the effects of rare "species". Statistical analysis and charts were processed by R 2.12.1.

REFERENCES

Schloss P.D., *et al.*, Introducing mothur: Open-source, platform independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol, 2009. 75(23):7537-7541

Sebastianelli A., *et al.*, Extraction of DNA from soil using nanoparticles by magnetic bioseparation. Letters in Applied Microbiology, 2008. 46(4):488-491

Whitman W.B., *et al.* Prokaryotes: The unseen majority. Proc. Natl. Acad. Sci., 1998. 95:6578–6583

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