

# Impact of Monoculture Practices and Fertilization Regimes on the Diversity of the Bacterial Communities in Soil

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## Introduction

Soil is a complex biological system in intimate interplay with agricultural plants. Details of this interplay however, are poorly understood. Today there is a consensus that not only chemical quality of soils but also its biological "health" has an important impact on the results of agricultural practice and *vice versa*. Better insight into those interaction shall lead us to more sustainable agriculture.

The aim of this study was to compare bacterial and archaeal communities in agricultural soil and see whether their characteristics depend on any of the variables under controlled conditions. We had an opportunity to study a long-term monoculture field trial where number of variable criteria was brought to a minimum. Calcaric cambisol soil samples were collected from controlled monoculture test field situation where we could study two variables, crop species and level of fertilizer use. Extracted total DNA was amplified with broad-range bacterial and archaeal 16S rDNA targeting primers and pyrosequenced using a 454@ platform.

## Results

Taxonomic richness (at species level - 97% operational taxonomic unit (OTU) threshold) in **bacterial communities** had the tendency to decrease with the increase in fertilization level (Fig 1) ( $t=-7.87$ ;  $P=0.016$ ). There was no significant effect of fertilization to the OTU richness in **archaeal communities** detected ( $t=-1.21$ ;  $P=0.350$ ) (Fig 1.). Additionally, the richness of OTUs was affected by crop species in bacterial and archaeal communities (Fig 1).

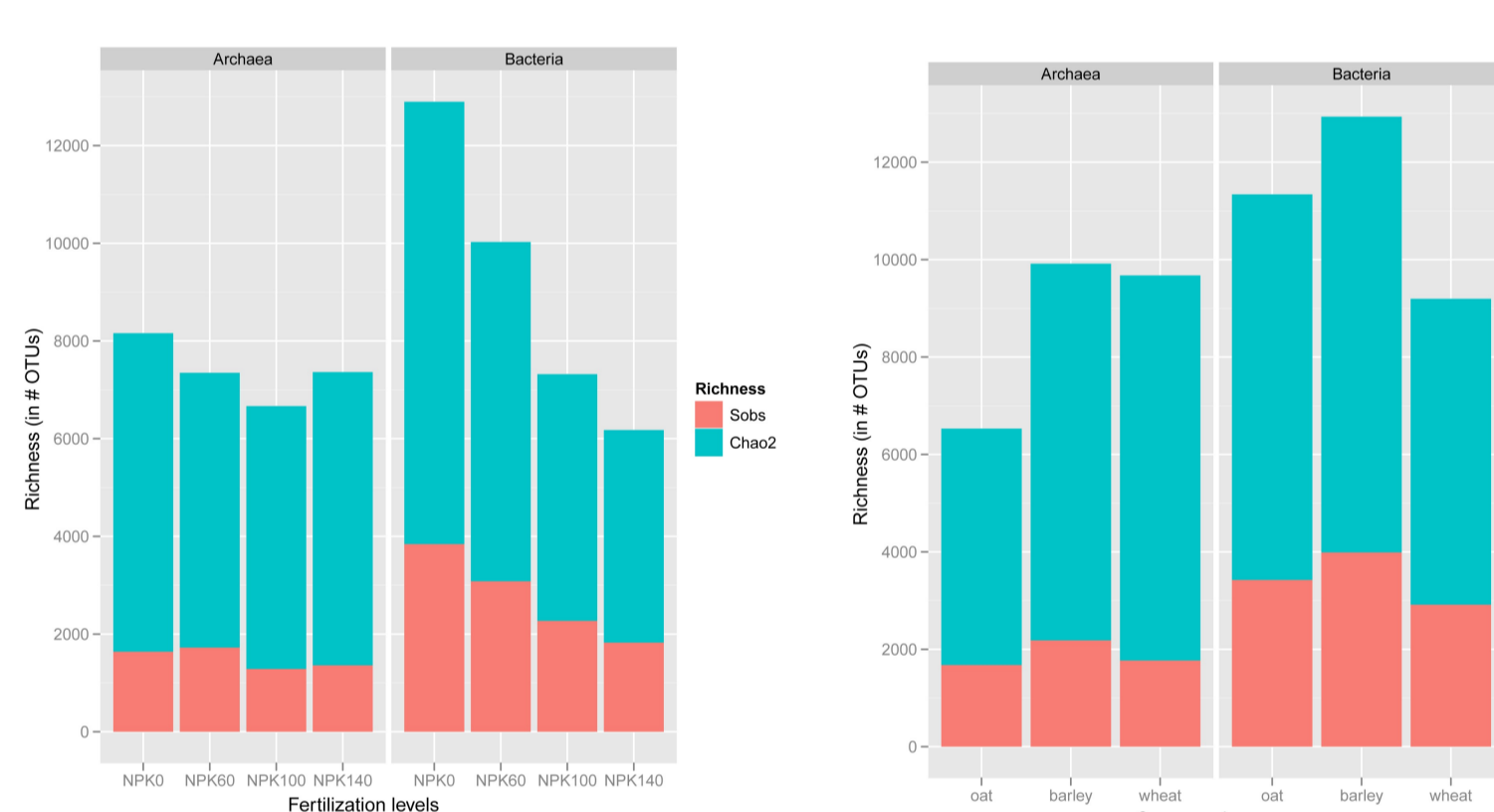
Diversity in **bacterial communities** as shown by the Shannon diversity index decreased with the increase in fertilization level (Fig 2) ( $t=-3.51$ ;  $P=0.001$ ), whereby the number of OTUs was not affected by the crop type (Table 1). No significant effect of fertilization level ( $t=-1.64$ ;  $P=0.113$ ) or crop species on the diversity in **archaeal communities** (Fig 2, Table 1) could be detected.

However, level of fertilizer use affects both bacterial and archaeal community structure more than cultivated species covered in this analysis (Fig 3.).

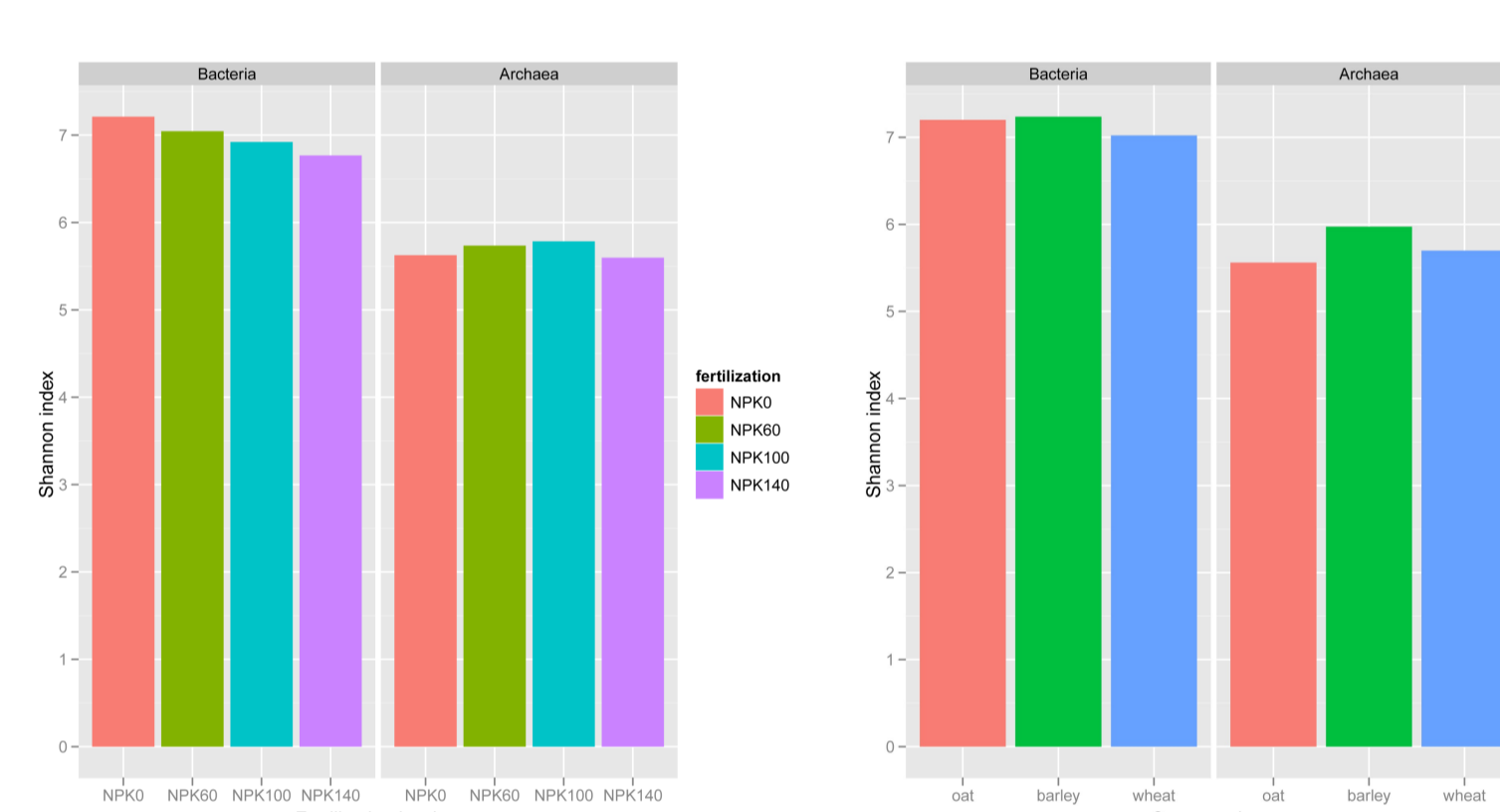
The most abundant OTUs assessed from the samples were from phyla *Proteobacter*, *Bacteroidetes* and *Acidobacteria* (Fig 4).

**TABLE 1.** Regression models for Shannon diversity measure using fertilization level and crop species as explanatory variables. For bacterial communities  $n=34$  and for archaeal communities  $n=33$ .

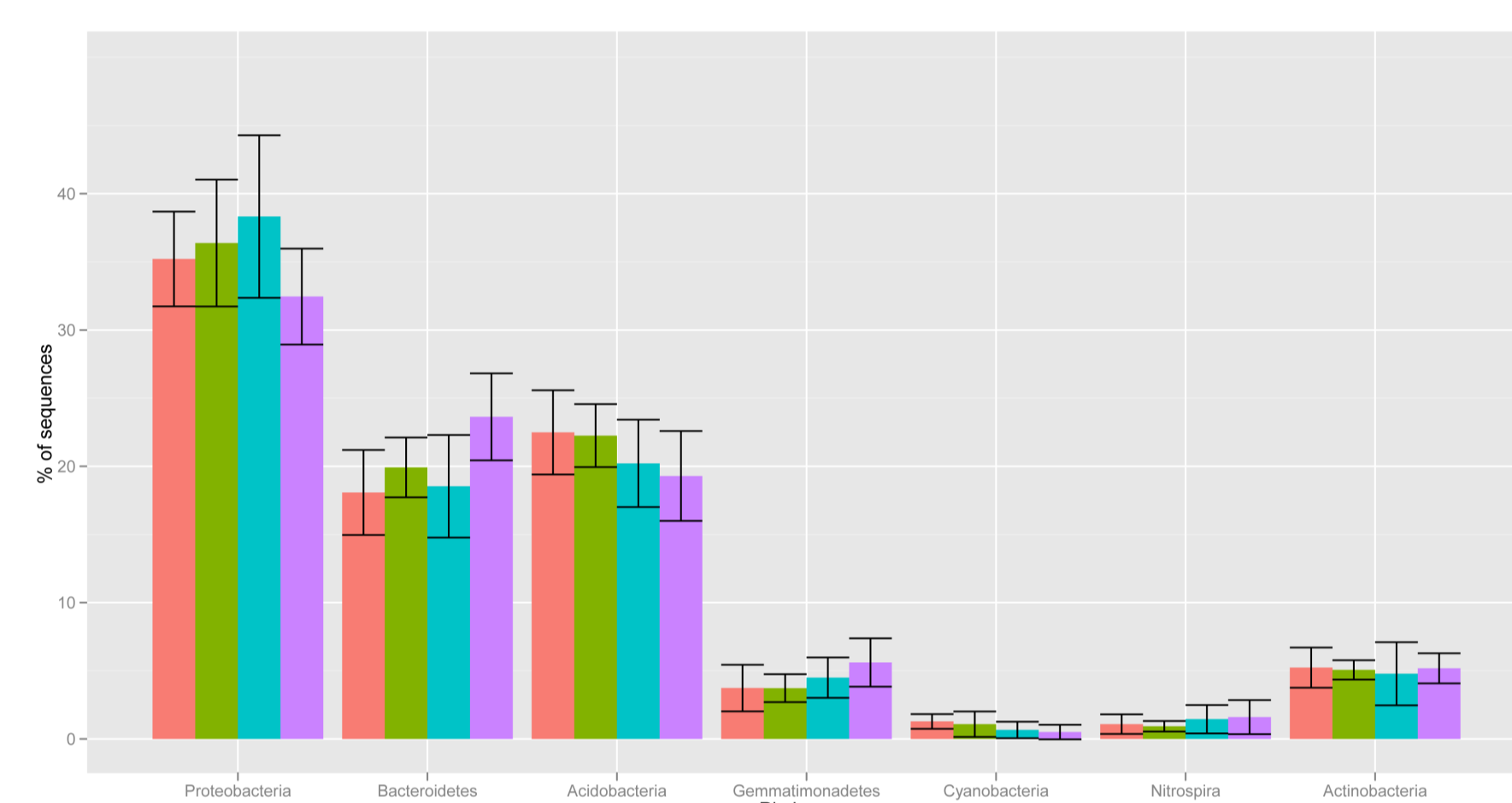
Independent variable	Fertilization level	Barley	Wheat
Shannon measure for bacterial communities	<b>P = 0.001</b> <b>t = -3.51</b>	P = 0.240 t = 1.20	P = 0.568 t = -0.58
Shannon measure for archaeal communities	P = 0.113 t = -1.64	P = 0.101 t = 1.70	P = 0.796 t = 0.26



**FIG. 1.** The summarized Chao2 index estimates the number of unobserved species based on the distribution of species among samples. On the chart Chao2 richness index is split into observed richness (Sobs) and estimated unobserved richness (Chao2) of OTUs in soil bacterial and archaeal communities grouped by fertilizer usage and cultivated crop.

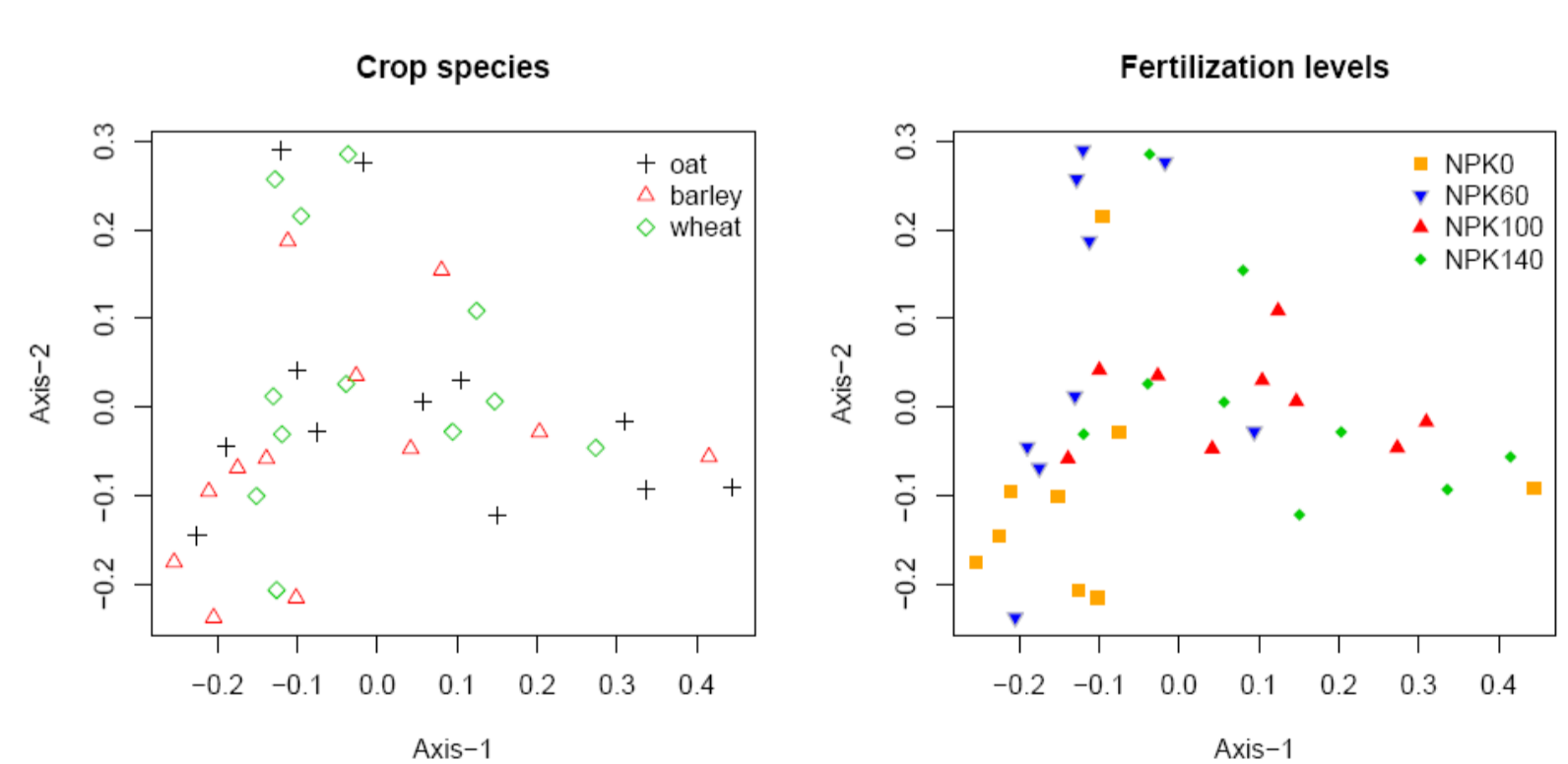


**FIG. 2.** Shannon diversity index for soil bacterial and archaeal communities grouped by fertilizer usage ( $n=9$  for each group) and crop species ( $n=12$  for each group).

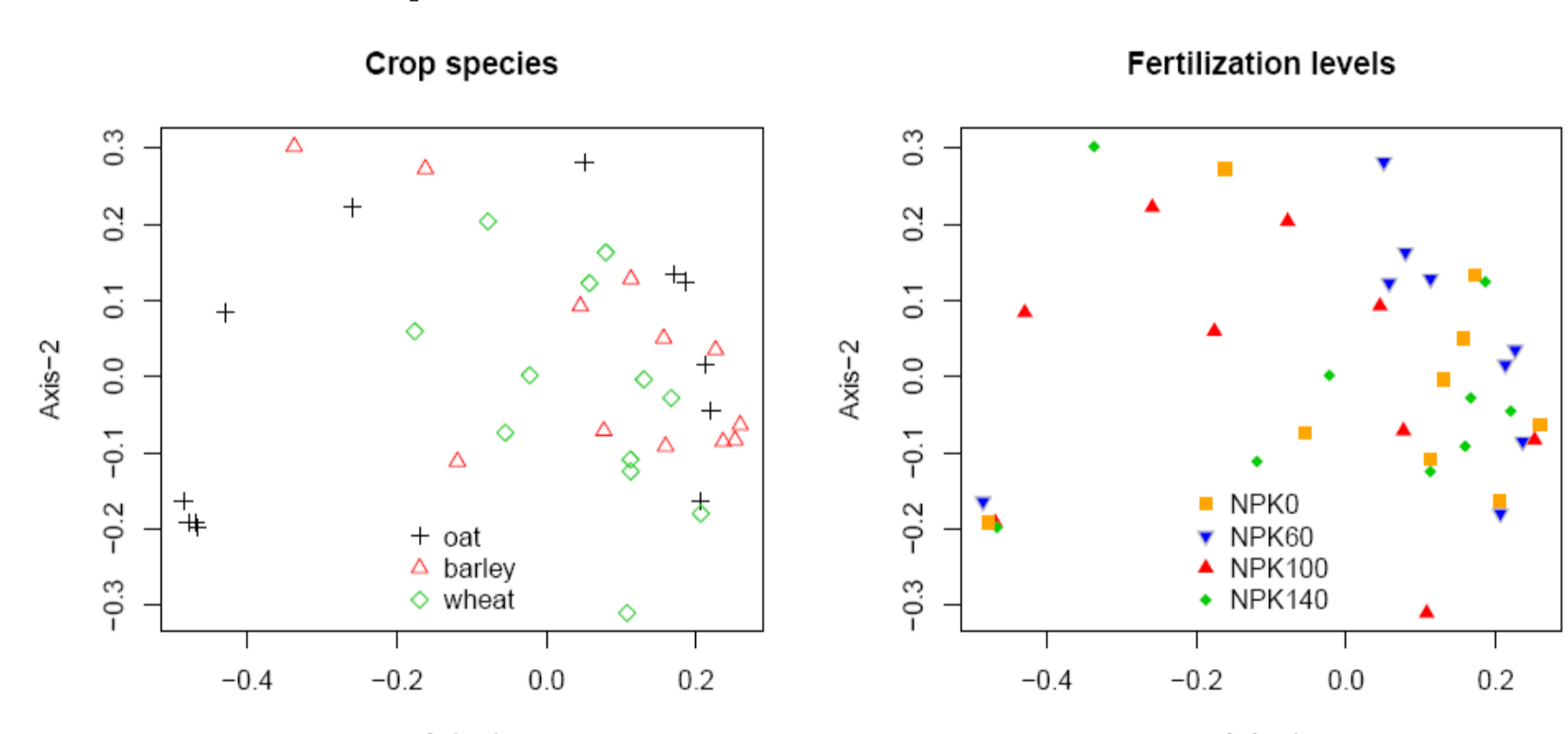


**FIG. 4.** Relative abundance (mean and 95% confidence interval) of the bacterial taxa in the different treatment groups ( $n=9$  for each fertilization level and  $n=12$  for each crop species) shown as percentage of sequences.

## PCoA plot for bacterial communities in soil



## PCoA plot for archaeal communities in soil



**FIG 3.** Principal coordinates analysis (PCoA) plot for bacterial and archaeal communities in samples of normalized unpooled sequences, based on Yue & Clayton (YC) measure of similarity at 97% OTU threshold. The axes for bacterial communities represented 14.8% variance of YC similarity measure. The axes for archaeal communities represented 25.3% variance of YC similarity measure.

## Conclusions

Level of fertilizer use had negative effect on both bacterial diversity and richness in agricultural soil.

The crop species covered in this analysis had no significant effect on the diversity of bacterial and archaeal communities.

Reason and functions for showed dependencies need further investigation in controlled trials as well as in regular agriculture.

Also more detailed taxonomic background of these differences in community structure should be further analyzed.

## Material and methods

Samples were collected post-harvest and before tillage from one location, monoculture fertilizer used test field, test initiated at 1993. Altogether 12 patches were sampled including 3 crops (barley "Anni", oat "Villu", wheat "Vinjett") and 4 fertilizer use practices (NPK0 = untreated control N0P0K0 kg ha<sup>-1</sup>; NPK60 = N60P13K23; NPK100= N100P22K39; NPK140= N140P31K54). Fertilizer was applied to the soil before sowing using a complex fertilizer Kemira Power (N18P4K7). All other treatments to test field were equal.

DNA was extracted from 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 (archaeal primers arch934R 5'-GTCTCCGACTCAGNNNNNNNGYGASCAGKCGMGAAW-3', arch349F 5'-TTGGCAGTCTCAGNNNNNNNGTGTCTCCCGCAATTCCT-3', bacterial primers 8F 5'-TTGGCAGTCTCAGNNNNNNNAGTTTGATCCTGGCTCAG-3', 357R 5'-GTCTCCGACTCAGNNNNNNNCTGCTGCCTYCCGTA-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'-CCTATCCCCTGTGTGCCTGGCAGTCTCAG-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 1.17.2 (Schloss et al. 2009) and its embedded tools for aligning sequences, chimeraSlayer, precluster, cluster,  $\alpha$ - and  $\beta$ -diversity tools, Libshuff. 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):7537-41

Sebastianelli A., Sen T., and Bruce I.J., Extraction of DNA from soil using nanoparticles by magnetic bioseparation. Letters in Applied Microbiology, 2008. 46(4):488-491

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