

Effect of Soil Tillage Practices on Dynamic of Bacterial Communities in Soil

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Summary

Several studies have indicated that intensive tillage has notable effect on properties of the soil microbiota that may influence numerous important soils functions, e.g. mobilization of nutrients or change of the overall emission rates of greenhouse gases. Therefore, the aim of our study was to investigate dynamic of microbial communities in soil planted with soybean under different tillage systems. Moreover, abundance of populations harboring the nitrous- oxide reductase gene (*nosZ*) as an indicator for potential shifts in N₂O emission rates was studied. The study was established at chernozem soil of Northern Baranja region in Republic of Croatia as completely randomized block design of four replicate plots for each tillage system in three years experiment. The soil was managed as followed: CT - conventional tillage (moldboard ploughing at 25-30 cm depth), DH - multiple discs harrowing (10-15 cm depth), and NT – no-tillage system. Soil samples were collected in summer and autumn in year 2003. Our results suggested that the reduction of tillage had no effects on the bacterial community structure. This might be a result of the very dry climatic conditions at the investigated site and /or a result of plant species effect (soybean). Slight effects of the tillage management became visible at least when samples were taken in autumn for microbes harboring the N₂O reductase gene, indicating that there might be shifts in denitrification pattern in response to changes in tillage practice.

Key words

soil microbiota, tillage, soybean, denitrification

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Introduction

The microbiome of soils acts as driver for major ecosystem services of soils including the production of plants, the sequestration of carbon or the degradation of xenobiotics (van Elsas et al., 2002; Larkin, 2003; Feng et al., 2003). Besides site specific conditions like soil type and climate, one of the major factors that influences the structure and function of the soil microbiome is the type of land use and the corresponding soil management (Nannipieri and Badalucco, 2003). Therefore, an in depth understanding of microbial diversity and activity as a part of below-ground processes is crucial in order to predict the long-term response of ecosystems to particular management practice. Many studies have been performed to understand the influence of soil management on functional traits of bacteria, fungi and archaea, with the aim to develop management forms which are more sustainable than existing ones (Geisseler and Scow, 2014; Mrkonjić Fuka et al., 2008; Shiwei et al., 2015). The response pattern of the soil microbiome towards changes in management is also of high value for development of new indicators for soil quality, as microbes react most rapidly to changes in the external environment (Lalande et al., 2000). Different indicators such as microbial biomass, microbial diversity or activity could be used to monitor the microbial response to different soil treatments and finally, to determine the influence of those anthropogenic interventions on the entire soil ecosystem (Schloter et al., 2003).

Tillage is one of the most frequently used forms of management in traditional agriculture. Despite the big advantages of this form of agricultural practice, including high yields and reduced pressure of weeds, soil tillage is also known to reduce the carbon sequestration potential of soils and strongly interferes with the development of stable food web structures in soil, which often leads to reduced nutrient mobilization potential. Several studies demonstrated that intensive tillage has notable effects on the microbial structure, causing decreases in biodiversity, with increased selection of some species (Adl et al., 2006; Haynes et al., 1996). *Vice versa* the increased soil organic matter content as a result of reduced tillage serves as nutrient reservoirs for plants and microorganisms such having positive affect not only on plant growth but also on entire soil processes (Feng et al., 2003). Moreover, as a result of the improved internal nutrient mobilization from dead biomass by soil microbes, reduced tillage may decrease the production and emission of greenhouse gasses from soils such as N_2O , that has 300 times higher global warming potential than CO_2 and can cause serial destruction of ozone in stratosphere. However, the so far published results strongly differ mainly when the benefits of reduced tillage towards the reduction of greenhouse gas emissions from soils are considered (Zhang et al., 2015).

Therefore, in the present study we investigated the influence of reduced tillage on the overall structure of bacterial communities in soil using a molecular fingerprinting approach based on the 16S rRNA gene. As the emission pattern of N_2O is strongly linked to the potential of microbes to reduce the formed N_2O to N_2 during denitrification, we also investigated the effect of reduced tillage on the abundance of N_2O reducing microbes using the *nosZ* gene (N_2O reductase) as a marker. We hypothesized that tillage type would have influence on microbial community structure reducing tillage would increase bacterial diversity and the abundance of *nosZ* genes at the sites under investigation.

Material and methods

Field sites and soil sampling

The study was performed in the Northern Baranja region in Republic of Croatia on a chernozem soil as a plot trial, with three replicates for each tillage system under investigation. The tillage practice started at the end of 2001 and it was annually repeated until the 2004. The forms of tillage used can be defined as follows: CT - conventional tillage (moldboard ploughing at 25 - 30 cm depth), DH - multiple disks harrowing (10 - 15 cm depth), and NT- no-tillage. The plots were cultivated with wheat from autumn to spring and soybean from spring to autumn. Soil samples (up to 20 cm in depth) were collected in triplicates during the cultivation of soybean in summer (flowering) and autumn (post harvest) in 2003 from each plot and treated as true replicates. Fertilizer and herbicides recommendations were based on standard soil testing recommendations and included less than 40 kg N/ha during the cultivation of soybean and 100 kg N/ha when wheat was grown on the plots. Soil samples were homogenized by sieving (2 mm mesh) and stored at $-20^{\circ}C$.

DNA extraction

Soil DNA was extracted and purified using the FastDNA SPIN Kit for Soil (Bio 101, Vista, USA) as recommended by manufacturer. Five hundred mg of soil sample was weight in "MULTIMIX 2 Matrix Tubes" for each of the three replicate of soil samples and per each of management system. The quality of DNA was evaluated in 1% agarose gel followed by 20 min staining with ethidium bromide. The amount of isolated DNA was estimated using the Nanodrop (NanoDrop Technologies, Wilmington, USA).

Fingerprinting of the 16S rRNA gene

A part of the 16S rRNA gene was amplified using a primer set designed by Nübel et al. (1996) (968 forward – 5' AACGCGAAGAACCTTAC 3'/1401 reverse – 5' CGGTGTGTA CAAGACCC 3'). A GC clamp was attached to forward primer (Muyzer et al., 1996). The reaction mixture contained 1xbuffer, 2 mM $MgCl_2$, 0.3% BSA (Sigma-Aldrich, Germany), 0.2 mM dNTPmix (MBI Fermentas, Lithuania), 20 pmol of each primer (Thermo Hybaid, Germany), 5% DMSO, 2.5 U of *Taq* polymerase (Gibco BRL, Germany) and 1 μL of DNA template, respectively, in a final volume of 50 μL . The PCR reaction was carried out as follows: initial denaturation step at $95^{\circ}C$ for 10 min followed by 35 cycles at $94^{\circ}C$ for 1 min, $54^{\circ}C$ for 1 min, $72^{\circ}C$ for 1 min and a final extension at $72^{\circ}C$ for 10 min (Jontofsohn et al., 2002b). PCR products were visualized on 1.5% agarose gel after staining with ethidium bromide. Purification of amplicons were carried out by Qiaquick PCR purification Kit (Qiagen, Hilden, Germany) as recommended by manufacturer.

Fingerprints were performed using denaturing gradient gel electrophoresis (DGGE) (D-Code system; Bio-Rad Laboratories, Germany) as described by Muyzer et al. (1996). For analyzing the amplicons 6% (w/v) polyacrylamide gels (ratio of acrylamide and bisacrylamide 37:1) with a denaturant gradient from 45% to 62% were used. The gels were run at $60^{\circ}C$ and 60 V for 16 h.

DGGE gels were stained with SyberGreen. The DGGE-PCR profiles obtained were analyzed by clustering via the unweighted pair group method with mathematical averages (UPGMA; Pearson coefficient of similarity) using GelCompar II Software

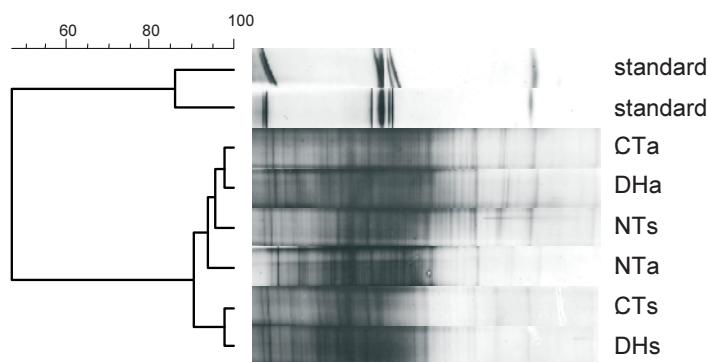


Figure 1. GelCompare analysis of DGGE fingerprints from 16S rRNA amplicons from total DNA extracted from soil samples in summer and autumn. The scale represents the percentage of similarity of the samples. CT = conventional tillage, DH = multiple disks harrowing, NT = no-tillage. s = summer, a = autumn.

(Applied Maths, Kortrijk, Belgium). The position tolerance was set at 1% and background subtraction was applied. Both strong and weak bands were included in the analysis, thus taking into account only the presence and absence of bands at specific positions. Cophenetic correlations were calculated using the same software.

Prior to the analysis of the different treatments on one DGGE gel (one treatment per lane), additional DGGE were run to test the homology of samples taken from replicate plots.

Quantification of the *nosZ* gene

Real-Time PCR was preformed to amplify gene fragments of the N_2O reductase gene (*nosZ*). Amplification and monitoring was carried out on the ABI 7300 Sequence Detection System (Perkin Elmer, USA). The Real-Time PCR reaction mixture was done in 25 μ l, and the assay contained 12.5 μ l of Power SybrGreen PCR Master Mix (Applied Biosystem, Germany), 0.5 μ l of 3% BSA, 0.35 μ l of 10 pmol of each of the *nosZ* primers (*nosZ2F*- 5' CGCRACGGCAASAAGGTSMSGT 3' or *nosZ2R*- 5' CAKRTGCAKSGCRTCAGAA 3' (Henry et al., 2006), 0.5 μ l of 5% DMSO and 50 ng of DNA template. Thermal cycling conditions was as followed: an initial cycle of 95°C for 15 min; six cycles of 95°C for 15 s, 65°C for 30 s, with a touchdown of -1°C by cycle, 72°C for 30 s, and 80°C for 15 s (acquisition data step); 40 cycles of 95°C for 15 s and 60°C for 15 s, 72 °C for 30 s, and 80°C for 15 s. The plasmid containing *nosZ* gene fragments amplified from *Pseudomonas fluorescens* was used as external standards. The preparation of standards was done as described by Henry et al. (2006). All samples and standards were analyzed in triplicates and additionally several negative controls were included. In order to avoid inhibition of the PCR reaction an experiment with dilution series of all samples was performed in advance. For confirming specificity of the amplicons after each PCR run a melting curve and a 2 % agarose gel were conducted. The amplification efficiencies were calculated with the equation $Eff = [10^{(-1/\text{slope})} - 1] \times 100$.

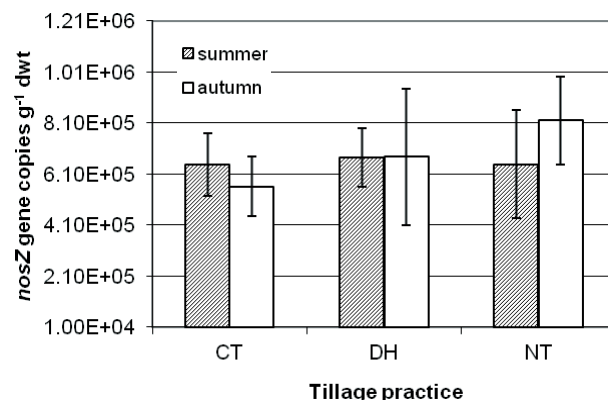


Figure 2. Copy number of *nosZ* gene per g dwt soil collected in summer and autumn. CT = conventional tillage, DH = multiple disks harrowing, NT = no-tillage. Error bars represent the standard deviation.

Results

Influence of different forms of tillage management on the bacterial community structure

DGGE profiling of PCR-amplified 16S rRNA genes was used to assess management-induced changes in the bacterial community structure. PCR products of expected size (470 bp) were obtained from all samples collected in summer and autumn 2003. The obtained fingerprints were highly complex, indicating a highly complex structure of bacterial communities in the investigated soil samples. The average number of bands was above 35 for all samples. Fingerprints obtained from soil samples from replicated plots were highly reproducible, with similarity values of more than 98% between replicates (data not shown). Surprisingly, the type of tillage management did not influence the overall number of bands obtained, which might be an indication that bacterial biodiversity was not positively influenced by reducing the tillage intensity. Also the similarity between the profiles of differently managed soils was very high and in all cases similarity values above 93 % were found, indicating that none of the bacterial groups present in soil benefited or suffered from the change in tillage management. Consequently, the performed cluster analysis did not show a consistent variation in the profiles that could be attributed to soil management (Figure 1), indicating no detectable shifts in the total bacterial community structure under different tillage conditions irrespective of sampling time.

Abundance of *nosZ* harboring microbes

Tenfold serial dilutions of the plasmids ranging from 10¹ to 10⁶ gene copies μ l⁻¹ was used as a template to determine the calibration curves. The standard curves had a linear range over six orders of magnitude and the reaction efficiency of the assay was 85%. *NosZ* gene copy numbers of the samples analyzed ranged between 5.6 × 10⁵ and 8.2 × 10⁵ g⁻¹ dwt (Figure 2). The *nosZ* gene copies number remained constant under different tillage practices in summer. However, in autumn, the *nosZ* gene copies number increased in those plots where reduced or no-tillage management had been applied and reached maximum under no tillage practice (8.2 × 10⁵ copies g⁻¹ dwt).

Discussion

In the last decades, agricultural production has been faced with an ever increasing demand for food for a growing world population. On the other hand, it is burdened by continuing reduction of agricultural land caused by the different types of land damage and urbanization (Chen, 2007). Thus there is a need to implement agricultural management forms that take into account these social developments. As the soil microbiome can be considered as the major driver for soil quality and fertility, modern forms of management must take into account shifts in the diversity and activity of bacteria, fungi and archaea to protect the soil sustainability (Feng et al., 2003).

The primary purpose of tillage is to physically disturb the soil profile (Young and Ritz, 2000), but it can also induce soil compaction that could negatively impact crop productivity due to restriction in root growth. It has also been suggested that compaction may affect soil microbial populations, slowing the decomposition of plant materials and the subsequent cycling of nutrients required for plant growth (Dick et al., 1988). In our trial, bacterial community structure and abundance of N_2O reducing microbes was assessed in summer and autumn using soil samples from an experiment where different forms of tillage management were applied. Although much information exists regarding effect of tillage practice on biomass and numbers of culturable microorganisms, characterization of soil microbial communities and diversity using culture-independent methods that avoid the biases of microbial isolation techniques, are rare (Feng et al., 2003). Lupwayi et al. (1998) reported that soil microbial diversity under wheat was affected by rotation and tillage system. Merilas et al. (2009) evaluated the effect of different soybean management practices on soil microbial community. They showed that no-till helped to increase biodiversity and activity of microbial communities in soil. In our study, tillage system had no effect on bacterial communities' diversity assessed by 16S rRNA gene fingerprinting approach. This is opposite to study of Runion et al. (2004), in which soil water content, microbial biomass N and PLFA amount were all consistently higher in no-till compared to conventional tillage plots. They suggested that conventional tillage results in a lower, more static, possibly more mature community of microbes, while the microbial community under no-till appears to be younger and more viable growing population. Therefore, the stability of microbial community noticed in our study could be mainly due to the extremely dry season in 2003. As such, water availability was probably the most important factor influencing microbial diversity. Furthermore, soybean forms symbiotic interactions with rhizobia, which might influence the stability of the soil microflora per se, despite the differences in tillage management. It has also to be taken into account that the fingerprinting approach, mainly when universal primers are used, is not very sensitive towards changes. This becomes obvious when considering that 1 g of soil may harbor more than 10^5 different species and the number of bands detected was below 40 (which is typical for this type of technique) suggesting that there is a huge degree of hidden diversity behind each band.

When targeting a specific group of microbes (N_2O reducers), the effects of the different forms of tillage management were more pronounced. Results from this study suggested that

the abundance of *nosZ* gene increased by reducing tillage practices and the effect is much stronger in autumn than in summer period. No-tillage has mostly positive influence on the number of denitrifiers in soil due to the, as suggested, generally higher moisture content of no-till soils (Giambiagi, 1995; Rice and Smith, 1982). In Vertisols under fertilizer regime and legume - crop rotation, the abundance of denitrifiers and the respective potential denitrification rates was higher under NT compared to CT during the vegetation period, but not after harvesting (Melero et al., 2011). However, differences in denitrifier numbers were within the same order of magnitude emphasizing that NT has a limited impact on denitrification (Melero et al., 2011). Again, it becomes obvious that beside the type of management site, specific conditions like climate or soil type have also a high impact on microbial communities in soil that could be even more pronounced than the investigated effect of management.

Conclusion

Our results suggested that the reduction of tillage had no effects on the overall bacterial community structure that may be mainly due to the extremely dry season at the site present and the crop plant (soybean). Slight effects of the tillage management became visible for microbes harboring the N_2O reductase gene, indicating that there might be shifts in denitrification pattern during the season in response to changes in tillage practice. However, it is important to investigate if the differences observed in the abundance of a functional group of microbes influence also functional pattern and fluxes of elements in soil. Here, additional studies using activity based approaches based on the transcriptome or proteome have to be implemented.

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