Agriculture, Ecosystems and Environment 224 (2016) 12–21

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium)

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 1 February 2016
Accepted 10 March 2016
Available online 22 March 2016

Keywords:
Tillage
Crop residue management
454 pyrosequencing
Microbial diversity
Indicator taxa

ABSTRACT

Among the soil management practices used to promote sustainable agriculture, reduced tillage and retention of residues from the previous crop are reported to enhance significantly both soil fertility and crop productivity. Here, high-throughput sequencing (454 technology) was used to see how the *tillage regime* (conventional vs. reduced tillage) and the *fate of crop residues* (retention or removal) affect microbial communities at two sampling *depths* (top soil: 0–5 cm and deeper soil: 15–20 cm) in a fertile silty loam soil in Belgium. All combinations of these three factors were studied. After 6 years of conversion from conventional to reduced tillage, *depth* emerged as the main factor responsible for variation in microbial diversity, *tillage regime* ranked second, and finally, *crop residue fate* had no influence on microbial diversity. For both bacteria and fungi, the diversity appeared higher in the top soil than in the deeper soil, and surprisingly, higher under conventional than under reduced tillage. These differences are explained by changes in community composition due to taxon loss rather than taxon replacement. The specific local set of environmental conditions (a loess-derived soil and an oceanic temperate climate) may explain these results. These observations raise the question: does impoverishment in indicator taxa influence soil processes, and thus crop production? To answer this question, we discuss how the presence of certain indicator taxa liable to play an ecological role might relate to crop productivity.

1. Introduction

Agricultural practices, such as the degree of soil disturbance by tillage and the manner in which crop residues are managed, are recognized to influence soil parameters such as water content, temperature, aeration, and the degree of contact between organic matter and mineral soil particles (Kladivko, 2001). Variations in such parameters notably have an impact on soil microbial communities. Soil microbes play essential roles in soil processes (Nannipieri et al., 2003; Nannipieri and Badalucco, 2003), providing ecosystem services such as carbon transformation, participation in soil organic matter dynamics, nutrient capture and cycling, and soil structure maintenance (Kibblewhite et al., 2008).

Particular soil management practices, such as reduced tillage, soil protection by means of crop residue retention or soil mulching, crop rotation, and intercropping can significantly enhance both soil fertility and crop productivity in agroecosystems (Scopel et al., 2012). Conservation agriculture based on such practices is recognized as an economically sound, sustainable, and environmentally friendly alternative to conventional agriculture (Hobbs et al., 2008).

Alternative soil management practices are expected to increase soil biodiversity (Clapperton, 2003), thereby improving soil resistance and resilience so as to ensure agroecosystem stability and productivity. Yet agronomists are still far from understanding the impacts of specific practices on microbial communities, their ecological functions, and their ultimate effects on agroecosystems.

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http://dx.doi.org/10.1016/j.agee.2016.03.017
0167-8809/

Massive parallel DNA sequencing technology is becoming an important tool in microbial ecology (Poisot et al., 2013) for understanding patterns and processes linked to species richness. The high resolution of metagenomic approaches offers insights into the structures of complex microbial assemblages at the level of individual microbial taxa (Cardenas and Tiedje, 2008).

To date, only a few studies have applied metagenomics to investigate the influence of different tillage regimes and types of crop residue management on soil microbial communities (Dorr de Quadros et al., 2012; Lienhard et al., 2013; Navarro-Noya et al., 2013; Sengupta and Dick, 2015). These studies were conducted under specific climates on soils characterized by particular land-use histories. Sengupta and Dick (2015), for example, focused on the native North American prairies, which have a particularly high biological richness, an excellent soil structure, and a very high organic content and microbial biomass. Knops and Tilman (2000) observed that the recent conversion of native grassland to cultivated soil has had detrimental effects on the soil ecosystem, causing loss of some 50–80% of the original soil organic matter. As organic matter dynamics is closely related to microbial activity, the conversion of native grassland to cultivated soil has profoundly changed the diversity and composition of microbial communities (Fierer et al., 2013).

A very different biogeographical and ecological context is to be found in certain areas of Western Europe, such as central Belgium, whose loess-derived soils are among the most fertile in the world and have long been used for intensive agriculture. As pointed out by Rhoton (2000), the context is important, as the influence of a particular tillage regime on soil physical, chemical, and biological properties depends on site characteristics such as soil type, climate, and the number of years since implementation of the tillage system.

To date, no metagenomic study conducted in a sustainable agriculture perspective has yet investigated the response of microbial communities to particular tillage regimes in a loess-derived soil under a temperate oceanic climate. The aim here was to perform such a study on a soil in central Belgium. Specific objectives were to determine diversity levels (based on OTU levels) and changes in microbial community composition (based on taxonomic level, i.e. OTUs are aggregated into taxa) under different combinations of tillage regime (conventional vs. reduced) and crop residue fate (residue removal vs. residues left on the field). As reduced tillage results in two contrasting zones (the first centimeters of soil are mixed each year, while the soil below remains unperturbed), we chose to perform the analysis at two depths: 0–5 cm and 15–20 cm.

2. Materials and methods

2.1. Site description

The experimental field is located on the experimental farm of Gembloux Agro-Bio Tech (University of Liège, Gembloux, Belgium) characterized by an oceanic temperate climate. According to the World Reference Base (WRB), the soil type is classified as Cutanic Luvisol and is considered one of the most fertile soils in the world: the soil texture is silt loam (FAO) inherited from the loess deposit, with 18–22% clay, 70–80% silt, and 5–10% sand particles, and the organic matter is characterized by a C:N ratio between 10 and 12.

2.2. Experimental design and soil treatments

The experimental design and different soil treatments have been applied since autumn, 2008. Before 2008, the site was under conventional tillage. The design of the experimental field consisted of a Latin square arrangement with 16 plots: four soil treatments replicated four times. Each plot is 40 m long and 15 m wide. The different soil treatments were as follows: conventional tillage with residue removal (CT/R-, the agricultural practice most commonly used in Belgium for cereals), conventional tillage with residue retention (CT/R+), reduced tillage with residue retention (RT/R+), and reduced tillage with residue removal (RT/R-). The residues removed consisted of harvestable straw, while stubbles and chaffs were left on field in both R+ and R-. In all plots, stubble breaking at a depth of 10 cm was performed to bury the residues. After stubble breaking, plowing to a depth of 25 cm was applied only to the CT plots, with a moldboard plow. Seedbed preparation was identical on all plots and performed at a depth of 7 cm.

Fertilizer, fungicide, and weedkiller treatments were applied equally to each plot (See supplementary data Table S1 in the online version at DOI: 10.1016/j.agee.2016.03.017). Crops are rotated on the studied field and crop history is as follows: *Brassica napus* (2009), *Triticum aestivum* (2010, 2011 and 2012), *Vicia faba* (2013), and *Triticum aestivum* (2014).

2.3. Soil sampling

Soil samples were collected from each of the 16 plots in July 2014, at the grain-filling stage. From each plot, six cores were obtained, and from each core two sub-cores were removed: 0–5 cm and 15–20 cm. The six sub-cores corresponding to the same depth range were pooled and mixed, constituting a composite sample to be used for DNA isolation and soil parameter determinations.

2.4. Physico-chemical analysis

In each composite sample, soil physical and chemical properties were determined. Water content was measured by weighing the sample before and after drying it at 105 °C. Soil pH was measured in 1 M KCl (2:5 w:v) after two hours of equilibration. Water-extractable elements were quantified by flame absorption (Ca, Mg), flame emission (P, Na), or colorimetry (P) after extraction of 20 g of 8-mm-sieved fresh soil in 100 ml H_2O for 1 h at room temperature and filtration on 602H 1/2. Total organic carbon was quantified by the Walkley-Black method (Nelson and Sommers, 1982) and total nitrogen was quantified by the Kjehldahl method as described by (Bremner and Mulvaney, 1982).

2.5. DNA extraction and 454 pyrosequencing of bacterial and fungal genes

DNA was isolated from the soil samples (8 g wet weight) with the PowerMax® soil DNA isolation kit (MO BIO Laboratories, Solana Beach, CA) according to the manufacturer's recommendations. The V1–V3 region of the 16S rRNA (~512 pb) and the D1-D2 region of the 28S rRNA (~680pb) genes were amplified with the help of the following primers: E9-29: 5′- GAGAGTTTGATCATGGCT-CAG-3′ and E530-541: 5′-ACCGCGGCTGCTGGCAC-3′ (Brosius et al., 1981) and NL-1; 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and NL-4; 5′-GGTCCGTGTTTCAAGACGG-3′ (Kurtzman and Robnett, 1997). The reaction mixture contained 5 U FastStart High Fidelity DNA polymerase (Roche Diagnostics, Vilvoorde, Belgium), 1x enzyme reaction buffer, 200 μM dNTPs (Eurogentec, Liège, Belgium), each primer at 0.2 μM, and 100 ng genomic DNA in a final volume of 100 μl. The thermocycling conditions were: denaturation at 94 °C for 15 min followed by 25 cycles of 94 °C for 40 s, 56 °C for 40 s, 72 °C for 1 min, and a final 7 min elongation step at 72 °C. Finally, the 454 pyrosequencing technology (Roche) was used to sequence the PCR products. To maximize the number of bacterial or fungal sequences per run, the top-soil and deeper-soil samples were run separately.