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RESEARCH ARTICLE

A novel sub-phylum method discriminates better the impact of crop management on soil microbial community

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Abstract Soil microorganisms such as mycorrhizae and plant-growth-promoting rhizobacteria have beneficial effects on crop productivity. Agricultural practices are known to impact soil microbial communities, but past studies examining this impact have focused mostly on one or two taxonomic levels, such as phylum and class, thus missing potentially relevant information from lower levels. Therefore, we propose here an original, sub-phylum method for studying how agricultural practices modify microbial communities. This method involves exploiting the available sequence information at the lowest taxonomic level attainable for each operational taxonomic unit. In order to validate this novel method, we assessed microbial community composition using 454 pyrosequencing of 16S and 28S rRNA genes, and then we compared the results with results of a phylum-level analysis. Agricultural practices included conventional tillage, reduced tillage, residue removal, and residue retention. Results show that, at the lowest taxonomic level attainable, tillage is the main factor influencing both bacterial community composition, accounting for 13 % of the variation, and fungal community composition, accounting for 18 % of the variation. On the other hand, phylum-level analysis failed to reveal any effect of soil practice on bacterial community composition and missed the fact that different members of the same phylum responded differently to tillage practice. For instance, the fungal phylum *Chytridiomycota* showed no impact of soil treatment, while sub-phylum-level analysis revealed an impact of tillage practice on the *Chytridiomycota* sub-groups *Gibberella*, which includes a notorious wheat pathogen, and *Trichocomaceae*. This clearly demonstrates the necessity of exploiting the information obtainable at sub-phylum level when assessing the effects of agricultural practice on microbial communities.

Keywords Microbial diversity · Microbial community composition · Taxonomical level · Pyrosequencing · Conservation agriculture

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1 Introduction

Soil microorganisms are abundant and diverse and can have both beneficial and adverse effects on crop growth. Some,

such as plant-growth-promoting rhizobacteria and mycorrhizae, are well known to favor crop productivity and plant health (Siddiqui and Futai 2008; Berg 2009). They are notably involved in key processes such as improving plant nutrient acquisition, and they also play a major role in stimulating plant growth and in protecting plants against pathogens by producing bioactive substances. Conversely, agricultural practices influence the physical and chemical properties of the soil and hence affect the abundance and diversity of soil microorganisms (Kladivko 2001; Helgason et al. 2009; Lienhard et al. 2013). This generates interest in studying the responses of microbial communities to agricultural practices.

Powerful new tools are now available for assessing at very high resolution the huge diversity of microbial communities and the composition thereof. One is massive DNA (pyro)sequencing, which generates thousands of DNA sequences (Cardenas and Tiedje 2008) in record time. In addition, the recent introduction of multivariate analysis in microbial ecology has made it possible to summarize and explore such data, to detect microbial patterns and relate them to the environment (Ramette 2007). A central question in such studies remains: how to choose the taxonomic level used to detect microbial patterns?

The most recent surveys based on massive DNA sequencing and multivariate analysis, aiming to detect microbial patterns in an agricultural context, have focused on a high taxonomic level, i.e., class or phylum (Lienhard et al. 2013; Ceja-Navarro et al. 2010; Navarro-Noya et al. 2013). Such studies allow a coarse assessment of the variability of large microbial groups in relation to agricultural practices. This approach, however, ignores a large part of the accessible information concerning lower taxonomic levels, which could be more relevant to agriculture. For example, *Ascomycota* is a vast group of fungi containing both beneficial and harmful organisms, the latter being illustrated by the genus *Gibberella*, which includes the causative agent of *Fusarium* head blight of wheat (Bottalico and Perrone 2002). In addition, a phylum or class can contain subgroups of organisms responding differently to environmental factors. For example, subgroups of *Acidobacteria*, one of the most abundant bacterial phyla in many soils, are reported to respond differently to tillage practice (Yin et al. 2010).

On the other hand, detecting microbial patterns at a finer taxonomic level such as genus or species remains difficult or even unfeasible, because a great many soil microbes remain unknown at these levels, and because pyrosequenced DNA fragments are still too short to allow accurately assigning the sequence at these levels.

Consequently, we propose an original method to increase the resolution of the analysis by exploiting a maximum of information in the dataset, a method that could provide better discrimination between microbial communities according to the agricultural soil practice. The method is to exploit the available sequence information at the lowest taxonomic level attainable for each operational taxonomic unit.

To test the usefulness of this method, we have used it to examine the effects of tillage and crop residue management practice (Fig. 1) on microbial community composition and have compared the results obtained with those of a phylum-level analysis of the same soil samples. For this, we have used 16S and 28S pyrosequencing followed by redundancy ordination analysis.

2 Materials and methods

2.1 Site description

The studied site is located on the experimental farm of Gembloux Agro-Bio Tech (University of Liège, Gembloux, Belgium, at 50° 33′ 45.92″ N and 4° 42′ 48.97″ E). According to the WRB soil system, the soil type of the studied site is classified as Cutanic Luvisol. The soil texture is silt loam (FAO) 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. The Belgian climate is maritime temperate, with cool, humid summers and mild, rainy winters. The monthly average temperature is highest in July, at 18.4 °C, and lowest in January, at 3.3 °C. The monthly average rainfall is highest in December, at 81 mm, and lowest in April, at

Fig. 1 Different soil treatments applied to the experimental field: **a** reduced tillage, crop residues being left at the soil surface; **b** conventional tillage, the crop residues having been mixed into the soil by plowing. Both pictures show the appearance of the soil before and after passage of the machine which prepares the soil and sows simultaneously

51.3 mm (data from the Belgian Royal Meteorological Institute).

2.2 Soil treatments and experimental design

The experimental design consisted of a Latin square arrangement with four replicates of four soil treatments. Each soil treatment consisted of a combination of different soil practices: a tillage practice (conventional or reduced tillage) with a crop residue management practice (residue retention or removal). The combinations were as follows: conventional tillage with residue removal (CT/−R, the agricultural practice most commonly used in Belgium), conventional tillage with residue retention (CT/+R), reduced tillage with residue retention (RT/+R), and reduced tillage with residue removal (RT/−R). Conventional tilled plots were ploughed to a depth of 25 cm, while in reduced-tillage plots, only the top 10 cm of soil was mixed. The estimated quantity of crop residues from the 2012 season was 8.3 t/ha for the plots with residue retention (+R) and 4.5 t/ha for the plots with residue removal (−R). Crops are rotated on the studied field, and the experimental design and different soil treatments have been applied since autumn, 2008. Crop history is as follows: *Brassica napus* (2009), *Triticum aestivum* (2010, 2011, and 2012), and *Vicia faba* (2013).

2.3 Soil sampling and physicochemical analyses

We took 16 soil samples from the faba bean field in April 2013, 10 days after sowing and 1 month after glyphosate application. Each sample was a composite of five 25-g subsamples. Each subsample consisted of a 5-cm core collected corresponding to a depth of 15 to 20 cm. This depth was chosen to allow comparisons with other studies conducted by our laboratory and because we wanted to focus on the soil layer located between the depth reached by reduced tillage (7 cm) and that reached by conventional tillage (25 cm). One should note that the response of microbial communities is related to crop residue location, which is different for conventional and reduced tillage (Helgason et al. 2014). Under conventional tillage, residues are mixed within the soil profile, while under reduced tillage, there is a stratification of residues. For each sample, we performed physical and chemical soil analyses. Volumetric water content and porosity were measured by the normalized cylinder method (AFNOR NF X31-501). Clay content was measured by the normalized pipette method (AFNOR NF X31-107). Soil nitrates were determined by the QuickChem®: method 12-107-04-1-B. Soil pH was measured in 1 N KCl (2:5 *w*/*v*). 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 602 H 1/2. Carbon was quantified as described by Ghani et al. (2003).

The average soil physicochemical parameters characterizing each treatment (CT/R+, CT/R−, RT/R+, and RT/R−) are presented in Table 1. We performed a statistical test (ANOVA, $n=4$) to assess the impacts of tillage practice and crop residue management on the log-transformed soil parameters. The results (Table 1) show a variation in potassium, phosphorus, sodium, porosity, pH, and nitrates between conventional and reduced tillage, all these values being higher under conventional tillage. There was no impact of residue management (residue retention or removal) on soil parameters.

2.4 DNA extraction and pyrosequencing of 16S and 28S rRNA gene sequences

We used the PowerMax® soil DNA isolation kit (MO BIO Laboratories, Solana Beach, CA) to extract metagenomic DNA from 8 g (wet weight) of each composite sample, according to the manufacturer's recommendations. We checked the quality of the DNA by gel electrophoresis, and we quantified it with the Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) prior to storage at −20 °C.

We used Roche 454 pyrosequencing technology to sequence fragments of the 16S and 28S rRNA genes. For bacterial DNA, the procedure was briefly as follows: We carried out a PCR to amplify a 500-bp fragment of the 16S rRNA gene from the total bacterial DNA. We used primers designed by fusion of (1) primers targeting the 16S rRNA gene fragments E9-29, 5′-GAGAGTTTGATCATGGCTCAG-3′, and E530-541, 5′-ACCGCGGCTGCTGGCAC-3′ (Baker et al. 2003), (2) multiplex identifiers (MIDs), and (3) the Roche 454 pyrosequencing adaptors (Roche Diagnostics, Vilvoorde, Belgium). Our PCR method could be biased, as we directly amplified our target using a fusion primer (Berry et al. 2011). However, the bias is the same for each sample we studied. We performed the PCR under the following conditions: the amplification mix contained 5 U FastStartHigh Fidelity DNA polymerase (Roche Diagnostics, Vilvoorde, Belgium), 1× 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. 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. We carried out amplification on a Mastercycler ep Gradient thermocycler (Eppendorf, Hamburg, Germany). PCR products were electrophoresed through a 1 % agarose gel and the DNA fragments were plugged out and purified with the SV PCR Purification Kit (Promega Benelux, Leiden, the Netherlands). We assessed the quality and quantity of the products with a Picogreen dsDNA quantitation assay (Isogen, St-Pieters-Leeuw, Belgium). We sequenced all amplicons with the Roche GS-Junior Genome Sequencer

Table 1 Average physicochemical soil parameters according to the soil treatment (tillage practice: conventional or reduced tillage) and type of crop residue management (residue retention or residue removal)

Physical and chemical soil parameters	Unit	Conventional tillage–residue retention	Conventional tillage–residue removal	Reduced tillage–residue retention	Reduced tillage–residue removal
Texture					
Sand	%	5.7±0.8	5.9±0.9	5.9±0.5	5.5±0.6
Silt	%	78.2±2.3	78.0±1.6	78.1±1.4	78.9±1.6
Clay	%	16.2±2.1	16.2±2.3	16.1±1.3	15.6±1.7
Water-extractable elements					
Carbon	mg/kg 105 °C	368.3±53	350.2±52	373.1±57	385.0±60
Calcium		45.4±10.2	38.8±3.4	44.8±13.5	42.8±11.4
Potassium Tillage**		17.1±3.8**(a)**	13.7±4.2**(a)**	11.3±4.3**(b)**	8.6±3.6**(b)**
Phosphorus Tillage*		3.9±1.0**(a)**	3.9±1.1**(a)**	3.4±1.4**(b)**	2.7±0.9**(b)**
Sodium Tillage*		21.2±0.9**(a)**	20.6±0.4**(a)**	20.3±1.1**(b)**	19.6±0.8**(b)**
Magnesium		2.7±0.5	2.6±0.3	2.9±0.7	2.7±0.6
Porosity Tillage**	%	46.9±1.7**(a)**	45.8±2.0**(a)**	43.3±1.4**(b)**	43.0±2.9**(b)**
Water content	%	33.4±0.3	32.4±1.0	32.2±1.0	32.3±0.5
pH Tillage*	–	6.6±0.1**(a)**	6.6±0.2**(a)**	6.4±0.2**(b)**	6.3±0.1**(b)**
Nitrates Tillage**	kg/ha	13.3±2.4**(a)**	15.5±5.0**(a)**	7.3±2.2**(b)**	6.9±4.8**(b)**
Total organic carbon	%	1.1±0.1	1.1±0.1	1.2±0.1	1.1±0.1

A statistical test (ANOVA, $n=4$) was performed to assess the impact of soil treatment on log-transformed soil parameters. Lines in bold with letters mean that there is an effect of soil treatment. Different letters correspond to significantly different values. The significance level is as follows: * significant at the 0.05 probability level and ** significant at the 0.01 probability level

(Roche, Vilvoorde, Belgium). For fungi, the procedure was the same, except that we amplified and sequenced a 500-pb fragment of the 28S rRNA gene with the following primers: NL-1, 5′-GCATATCAATAAGCGGAGGAAAAG-3′, and NL-4, 5′-GGTCCGTGTTTCAAGACGG-3′ (Kurtzman and Robnett 1997).

2.5 Bioinformatic analysis of the pyrosequencing data

A total of 85,935 raw reads were obtained for bacteria and 82,119 for fungi. The obtained partial 16S and 28S rRNA gene sequences were processed with the MOTHUR package (Schloss et al. 2009). We denoised all sequence reads with the Pyronoise algorithm implemented in MOTHUR and filtered them according to the following criteria: minimal length of 425 bp; an exact match to the barcode, and one mismatch allowed for the proximal primer. We used ChimeraSlayer to check the sequences for the presence of chimeric amplifications (Haas et al. 2011). The numbers of high-quality reads obtained after read processing were 68,230 for bacteria and 66,337 for fungi. We compared the resulting high-quality read sets with a reference dataset of aligned sequences of the corresponding region derived from the SILVA 111 database of full-length rDNA sequences implemented in MOTHUR. To cluster the final reads into operational taxonomic units, we used in MOTHUR the nearest neighbor algorithm with a 0.03 distance unit cutoff. A taxonomic identity was attributed to each operational taxonomic unit by comparison with the SILVA database (80 % homogeneity cutoff). The raw data sets are available in the SRA database (Sequence Read Archive) under project accession number SRP043491 for bacteria and under project accession number SRP044036 for fungi.

2.6 Statistical analyses

We performed all statistical analyses with R statistical software (Team 2013). We analyzed the impact of tillage practice and crop residue management on microbial alpha diversity and microbial community composition. This analysis was done at two taxonomic levels: phylum level and the most precise taxonomic level that could be reached for each operational taxonomic unit.

2.6.1 Microbial alpha diversity analysis

As using samples with different sequencing depths can bias alpha diversity indexes, a random sequence subsampling step was carried out so as to compare samples containing the same number of sequences: that of the sample having the lowest sampling depth (2693 sequences for bacteria and 2453 for fungi). To measure the alpha diversity of bacteria and fungi in the different subsamples, we used MOTHUR to evaluate the richness and Shannon indexes. We performed an ANOVA ($n=4$) to determine if the alpha diversity changed with the soil

treatment applied. We used the operational taxonomic unit level to measure alpha diversity.

2.6.2 Microbial community composition analysis

We used multivariate analysis to relate microbial community composition to soil treatment, i.e., tillage practice (conventional or reduced tillage) and type of crop residue management (residue retention or removal). We determined the impact of soil treatment on bacterial and fungal community composition at two taxonomic levels: phylum level and the most precise taxonomic level possible for each operational taxonomic unit, called the "precise level" in the following text. For the analysis, microbial abundance data was first log2 transformed with the decostand() function implemented in the vegan package (Oksanen et al. 2007). The log2 transformation was chosen to weight the variation of dominant taxa abundance in a reasonable way. We used redundancy ordination analysis to analyze and compare relationships between microbial community composition at each taxonomic level and soil practice. We constrained the redundancy ordination analysis by three explanatory variables: tillage practice, crop residue management practice, and the interaction of both. We used the ordistep() function of vegan to select the most significant explanatory variable. In addition, we used the envfit() function of vegan to fit soil physical and chemical parameters to the ordination graph, as these parameters, related to soil practices, might explain microbial composition variability. Finally, we revealed the bacteria and fungi most strongly affected by the best explanatory variable with the goodness() function of vegan.

3 Results and discussion

With a view to achieving better discrimination power than is usual in such studies, we compared two methods of microbial community composition analysis applied to soils subjected to different tillage practices—conventional and reduced tillage—and different residue management practices—crop residue retention and removal. One approach was to limit our analysis to the phylum level (the level most studied in soil microbial surveys), the second being to use the most precise taxonomic level reachable for each operational taxonomic unit. We then assessed the information gain provided by the more precise analysis.

3.1 Phylum composition of microbial communities

Our analyses showed that, for each soil treatment, *Proteobacteria* (25–29 %), *Acidobacteria* (18–24 %), and *Bacteroidetes* (9–14 %) were the most abundant bacterial phyla (Fig. 2a). These phyla are often dominant in very diverse agricultural soils (Janssen 2006; Lienhard et al. 2013; Navarro-Noya et al. 2013). We also showed that the most abundant fungal phyla for each combination were *Ascomycota* (74–86 %) and *Basidiomycota* (12–25 %), which are saprotrophic soil fungi (de Boer et al. 2005) frequently dominant in soil ecosystems (Lienhard et al. 2013; Buée et al. 2009) (Fig. 2b).

3.2 Analysis of alpha diversity in relation to soil treatment

The average soil alpha diversity characterizing each soil treatment and the numbers of sequences before and after the subsampling step are summarized in Table 2. We performed a statistical test (ANOVA, $n=4$) to assess the effect of soil practice (conventional or reduced tillage) and crop residue management practice (residue retention or removal) on the alpha diversity indexes.

Fungal richness appeared lower than bacterial richness (Table 2), with fewer than 300 operational taxonomic units for fungi and more than 1000 for bacteria. Strangely, this very low fungal richness is comparable to that observed in agricultural soils with high aluminum toxicity (Lienhard et al. 2013). In our soil, the low fungal richness might be due to cultivation history, as for a long time before 2008, the experimental field was subjected to conventional tillage with tilling tools liable to disturb fungal hyphae.

After only 4 years of experiment, we can already observe differences in bacterial and fungal alpha diversity indexes between conventional and reduced tillage.

The diversity of bacteria appeared higher under conventional tillage (Table 2). This could be due to the physical disturbance caused by tillage. Aggregates are broken and the organic matter is released and available for bacterial activity (Cheeke et al. 2012). Our results are consistent with those of Lienhard et al. (2013) and Navarro-Noya et al. (2013), showing an increase in bacterial diversity with increased soil disturbance and cropping intensity. In addition, Siciliano et al. (2014) have shown soil fertility, including nitrates and organic matter, to be the most important factor influencing bacterial and fungal richness and diversity indexes. In our soil, the nitrate content was consistently higher under conventional tillage (Table 1), which could also explain the observed higher bacterial diversity under conventional tillage.

Fungal richness appeared higher under conventional tillage than under reduced tillage, and higher with residue removal than with residue retention. Our results differ from those of Lienhard et al. (2013), who observed a negative effect of tillage on fungal richness and suggested that this effect could be due to a negative effect of tilling tools on the growth of fungal hyphae. Yet, as for bacterial diversity, the higher fungal richness observed under conventional tillage could be caused by the higher nitrate content observed under conventional

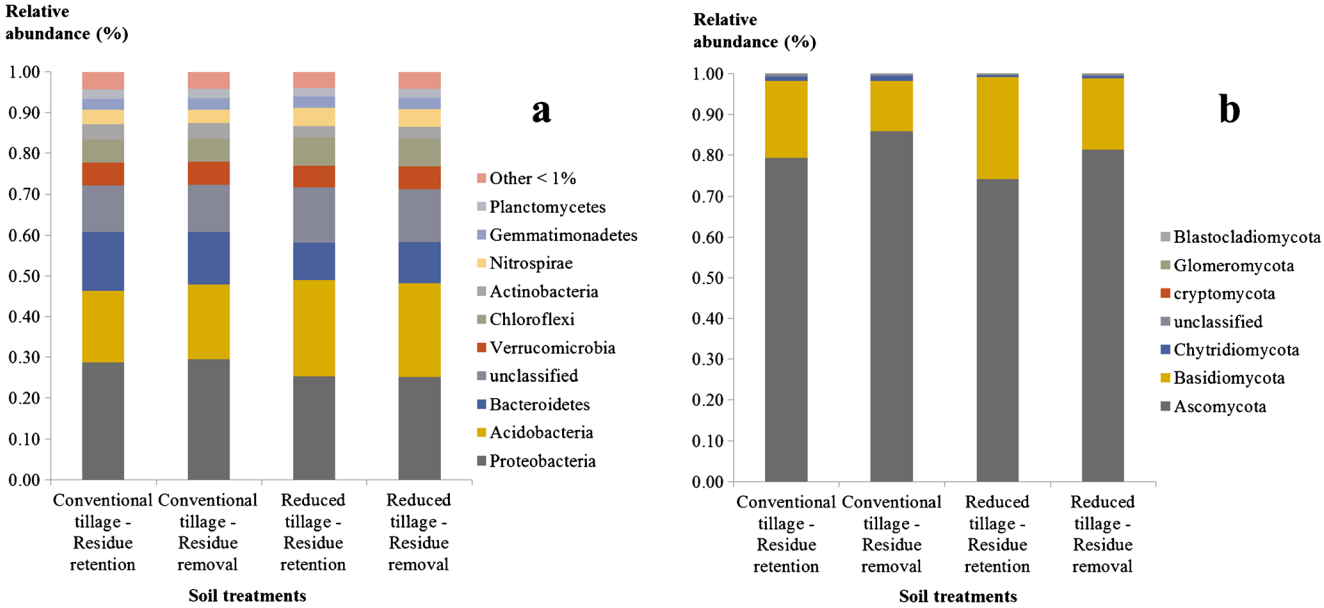

Fig. 2 Barplot representation of the relative abundances for each treatment (based on the sums of the four replicates) of **a** soil bacterial and **b** soil fungal phyla

tillage. Here, the higher nitrate content under conventional tillage seems to be a factor influencing fungal richness more strongly than the disturbance of fungal hyphae caused by tilling tools.

Although the use of such indexes is an easy way for an ecologist to assess diversity, these indexes ignore taxonomic identity, treating operational taxonomic units as anonymous entities (Hartmann and Widmer 2006). It is therefore interesting to analyze further such complex soil microbial communities with a method such as ordination that takes microbial community composition into account. However, using such a method requires choosing an appropriate taxonomic level.

3.3 Effect of soil practice on microbial community composition evaluated at two taxonomic levels

3.3.1 Bacterial community composition analysis

At phylum level (Fig. 3a), we observed no effect of soil practice on bacterial community composition. We did not focus on cropping intensity, but it is worth noting that Lienhard et al. (2013) report clear phylum-level differences in bacterial community composition along a cropping intensity gradient. This suggests that changing the cropping intensity alters the soil conditions more drastically than do our changes in soil practice, making it possible to detect coarser (phylum-level)

Table 2 Soil alpha diversity indexes for each treatment, on the basis of operational taxonomic units (based on averages of four replicates)

	Conventional tillage – Residue retention	Conventional tillage – Residue removal	Reduced tillage – Residue retention	Reduced tillage – Residue removal
Bacteria				
Number of reads before/after subsampling	4408/2693	4274/2693	3926/2693	4451/2693
Richness index	1776±313	1762±311	1413±357	1571±236
Shannon index	6.62±0.02 **(a)**	6.63±0.03 **(a)**	6.37±0.10 **(b)**	6.38±0.13 **(b)**
Tillage***				
Fungi				
Number of reads before/after subsampling	3993/2453	4048/2453	4418/2453	4186/2453
Richness index	251±24 **(b)**	291±40**(a)**	227±11 **(d)**	235±13 **(c)**
Tillage**				
Residues*				
Shannon index	3.82±0.38	3.88±0.12	3.80±0.17	3.83±0.06

We performed a statistical test (ANOVA, $n=4$) to assess the impact of soil management practice (tillage practice and crop residue management practice) on log-transformed indexes. Lines in bold with letters mean that there is an effect of soil treatment. Different letters correspond to significantly different values. The numbers of sequences before and after the subsampling step are also given. Significance values are as follows: * significant at the 0.05 probability level, ** significant at the 0.01 probability level, and *** significant at the 0.001 probability level

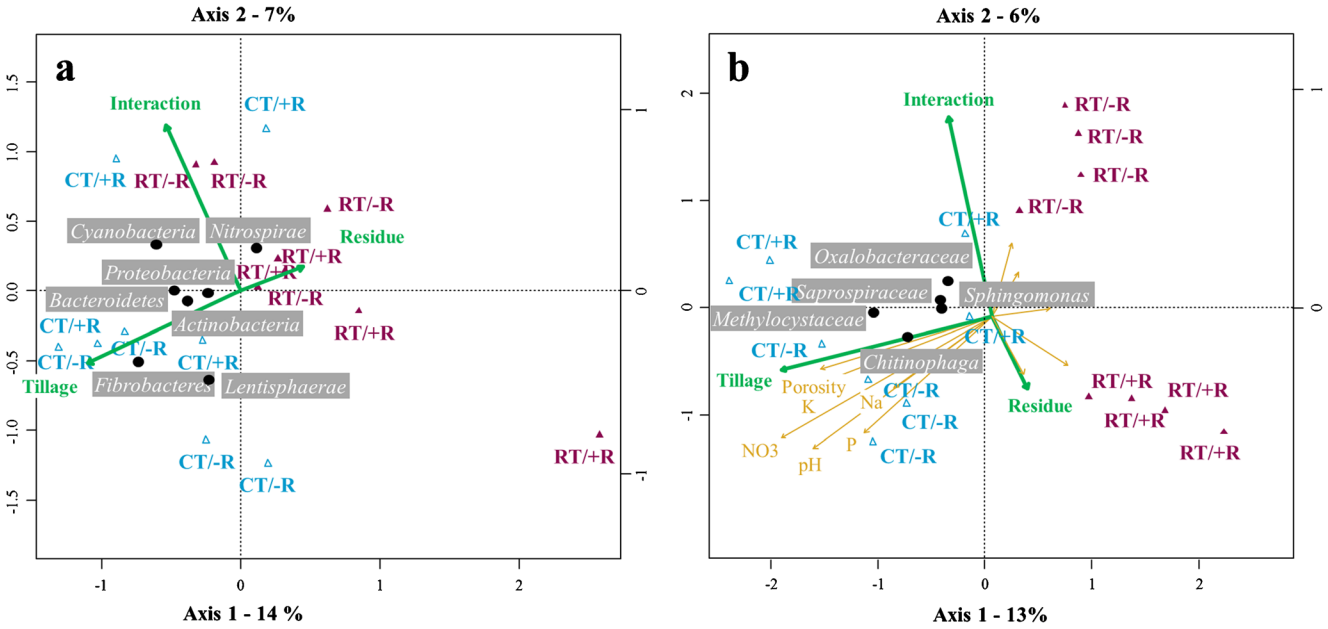

Fig. 3 Factorial map of the redundancy analysis carried out on soil bacterial community composition at **a** phylum level and **b** the most precise taxonomic level attainable. The axes 1 and 2 represent the maximum percentage of variance that can be explained by soil practice: tillage practice (conventional tillage, CT; or reduced tillage, RT) and crop residue management practice (residue retention, R+; or residue removal, R−). For both analyses, a statistical test (ANOVA, n=4) was performed to assess the effect of soil management practice on bacterial community composition. At phylum level, there appeared no difference in bacterial community between soil management practices, while differences due to tillage practice were observed at the more precise level, this factor accounting for 13 % of the bacterial community variation (p<0.01)

changes in bacterial communities. That our changes in soil practice are milder is supported by the low percentage of variance along the first two axes of our ordination plot (Fig. 3a).

At the precise level, however (Fig. 3b), we did observe a significant shift in bacterial composition according to the soil practice used. Tillage practice appeared as the best explanatory variable, explaining 13 % of the variation in bacterial composition (p<0.01). Our results demonstrate that it is useful to exploit the information that can be obtained at sub-phylum level, particularly in a system with lesser contrast between soil treatments, since the effect of tillage practice was not detectable at phylum level. At the more precise level, we were able to obtain sub-phylum-level information on the bacteria impacted by tillage practice. For example, we showed that the relative abundances of bacteria of the groups *Methylocystaceae*, *Sphingomonas*, *Saprospiraceae*, *Oxalobacteraceae*, and *Chitinophaga* were higher under conventional tillage.

Some of the groups just mentioned could play key roles in crop health and growth. For example, *Methylocystaceae* (Fig. 3b) is a group of methanotrophs, i.e., bacteria using methane (CH4) as energy source under aerobic conditions and thus capable of reducing methane emissions (Conrad 1996). Our results suggest that conventional tillage generates favorable conditions for *Methylocystaceae* development. The higher P and K contents observed (Table 1) under conventional tillage might explain our results, as Zheng et al. (2013) have found P and K amendments to increase the methanotroph population significantly. Interestingly, their survey evidenced a negative correlation between methanotrophic activity and methanotroph abundance. Species of the genus *Sphingomonas* (Fig. 3b) are involved in degrading refractory contaminants such as herbicides (Sørensen et al. 2001). Our results suggest that conventional tillage favors such organisms. The application of glyphosate to our field 1 month before soil sampling might have induced microbial glyphosate-degrading activity, which is higher under aerobic conditions (Rueppel et al. 1977). As we observed higher soil porosity under conventional tillage than under reduced tillage but similar water content regardless of the tillage practice (Table 1), we could expect a higher oxygen content under conventional tillage and hence better development of microorganisms capable of degrading glyphosate under aerobic conditions.

3.3.2 Fungal community composition analysis

At phylum level (Fig. 4a), we observed a significant shift in fungal community composition according to the tillage practice (p<0.05). The shift was largely due to *Chytridiomycota (C)*, favored under conventional tillage, and *Basidiomycota*, favored under reduced tillage. Fungi of the phylum *Basidiomycota* are known to degrade lignin and cellulose under anaerobic conditions (de Boer et al. 2005). Given the soil humidity, which was similar for conventional and reduced tillage, and the soil porosity, which was higher under reduced tillage (Table 1), we could expect such anaerobic conditions to be more frequent under reduced tillage. Little information is

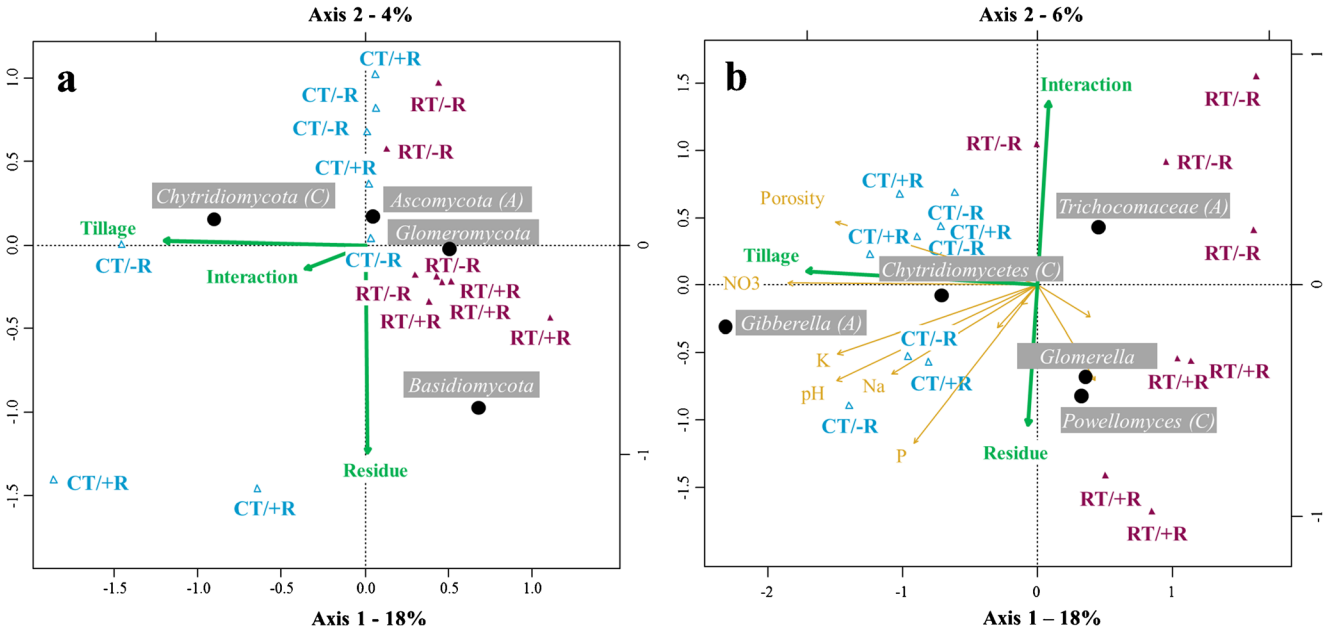

Fig. 4 Factorial map of the redundancy ordination analysis of soil fungal community composition at **a** phylum level and **b** the most precise taxonomic level attainable. Axes 1 and 2 represent the maximum percentages of variance that can be explained by soil practice, i.e., by tillage practice (conventional tillage, CT; reduced tillage, RT) and crop residue management practice (residue retention, R+; residue removal, R−). For both analyses, a statistical test (ANOVA, $n=4$) was performed to assess the effect of soil practice on fungal community composition. At both phylum level and the more precise level, there appear differences in fungal community composition between soil practices, with tillage practice accounting, respectively, for 16 and 18 % of the variation ($p<0.05$ and $p<0.01$)

available on the diverse groups of fungi composing the *Chytridiomycota*, but soil *Chytridiomycota* appear capable of recovering from dryness and high temperature (Gleason et al. 2004), more likely to occur in tilled soil.

These same two phyla were likewise highlighted in the survey of Lienhard et al. (2013), showing a greater relative abundance of *Chytridiomycota* under high cropping intensity (conventional tillage) and a greater relative abundance of *Basidiomycota* under lower cropping intensity (zero tillage). It thus appears that the contrast between our soil treatments, insufficient to induce differences between bacterial communities detectable at phylum level, was sufficient to induce differences between fungal populations detectable at this level. This might be due to the lower diversity of fungi as compared to bacteria (Table 2). It is generally accepted that a population with a low diversity should be less stable under environmental stress, as species affected by the stress will not be replaced by others, as in the case of a more diverse population (Giller et al. 1997).

At the more precise level (Fig. 4b), however, we observed a significant shift in fungal community composition according to the tillage practice, which explained 18 % of the variation in community composition ($p<0.01$). The precise analysis showed that different members of the phylum *Chytridiomycota (C)* responded differently to tillage practice: The relative abundance of *Chytridiomycetes (C)* was higher under conventional tillage, but the relative abundance of *Powellomyces (C)* was higher under reduced tillage.

Some phyla, furthermore, showed no impact of soil management practice (Fig. 4a), while analysis at the precise level (Fig. 4b) revealed an effect of tillage on the relative abundance of certain phylum members. A difference was observed, for example, between two subgroups of the phylum *Ascomycota*: a higher relative abundance was observed for *Gibberella (A)* under conventional tillage and for *Trichocomaceae (A)* under reduced tillage. These results again highlight the importance of comparing communities of soil fungi at the most precise taxonomic level accessible.

Some of these taxa are known to have specific roles in ecosystems. For example, *Gibberellazeae*, also known as *Fusarium graminearum*, is the causative agent of *Fusarium* head blight of wheat (Bottalico 1998). This disease can cause root, stem, and ear decay, resulting in a significant reduction in crop yield. As reported by Booth (1971), *F. graminearum* can survive saprophytically on a wide range of gramineous host debris, such as wheat residues. As our samples were taken at a depth between 15 and 20 cm, the higher relative abundance of *F. graminearum* observed under conventional tillage might be due to the presence of crop residues from previous wheat crops at this depth, while crop residues remain in the topsoil (<10 cm) under reduced tillage.

For both bacteria and fungi, the observed pattern changes can be explained by differences in soil conditions between conventional and reduced tillage. We show here that several soil parameters, including porosity, potassium, nitrates, pH, sodium, and phosphorus, were higher under conventional

tillage and might explain variations in bacterial community composition. Among these factors, the pH has been recognized as the best driver of changes in bacterial community composition and diversity, while fungal community composition appears closely associated with changes in nutrient status, such as phosphorus and the C/N ratio (Lauber et al. 2008). Here we show a variation in nitrates and phosphorus between conventional and reduced tillage, which might explain the observed fungal pattern changes.

By exploiting the data obtainable at a more precise taxonomic level, we are able to go further in our analysis and to identify groups of organisms that are affected by soil management practice. For example, because *Gibberellazeae* has a negative effect on wheat, information on its higher relative abundance under conventional tillage is relevant to farmers, who can expect to see the disease under such soil practice. This information is missed when the data are analyzed at phylum level. However, to exploit the available data on microbial community composition, agronomists need to know more about the roles played by soil microorganisms in their environment, and about their effects on plant health and growth. For many taxa, such information is still hard to obtain.

4 Conclusion

In the present work, we have attempted to improve the discrimination power of microbial community analysis applied to soils subjected to different tillage and residue management practices. For this, we have assessed the importance of exploiting 16S and 28S rRNA gene sequencing data at sub-phylum level to identify effects of soil management practice. Our results highlight tillage practice as an important factor influencing microbial community composition. Plowing notably affects several physico-chemical parameters that contribute greatly to shaping the microbial habitat: soil porosity, pH, and the NO3, P, K, and Na contents. These can be expected to affect microbial community composition. Most importantly, we show that some effects of tillage observed at sub-phylum level escape notice at phylum level and that some effects detectable at this higher taxonomic level mask differences in the responses of different members of a same phylum. Clearly, phylum-level analysis cannot do justice to the diversity of organisms within a phylum. As on the other hand it is currently impossible to assign a genus or species to each operational taxonomic unit, we recommend the compromise described in this paper: using for each operational taxonomic unit the most precise taxonomic level attainable. This method should facilitate a fine-scaled and detailed assessment of microbial communities across different soil practices.

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