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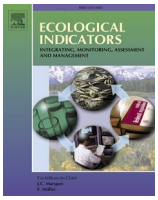

Catching change in microbial diversity indicators under different soil organic matter managements: Higher taxonomic resolution, better discrimination?

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ABSTRACT

Recent advances in molecular ecology have dramatically improved our knowledge of soil microbial diversity and offers new indicators of soil quality. The usefulness of diversity indices has never been greater as the astronomical amounts of data generated in the literature needs to be synthesized. Despite technical guidelines have been proposed to characterize soil microbiomes using high throughput sequencing, the effect of taxonomic resolution on diversity indices is still largely unknown. Here, we explored how downscaling to higher taxonomic resolution levels may affect α- and β-diversity indices of bacterial communities exposed to different soil organic matter management. To this aim, we collected soil samples in a long-term experimental site (Ultuna, Sweden) where different mineral and organic fertilizers have been applied for since 1956. We used both massive amplicon sequencing from phylum to species (OTU) and molecular fingerprints (PLFA, DGGE and T-RFLP). Our results showed that the discrimination potential increased at finer taxonomic resolution for β-diversity but not for α-diversity indices such as richness and evenness. Also, the relative importance of hierarchical drivers of soil microbial communities such as C, N and pH varied depending on the taxonomic resolution. This study also demonstrated that indicators generated by molecular fingerprints such as PLFA, DGGE and T-RFLP are still consistent to monitor the effect of agricultural management on β-diversity but not on α-diversity, which is useful information as it allows for a better use of results in past literature. We encourage performing such comparative studies on wider surveys, including different contexts and other indicators, in order to increase the efficiency and the robustness of the use of sequencing data in soil biodiversity monitoring.

1. Introduction

Soil microbes play a central role in many biogeochemical cycles on earth, driving global carbon and nutrient cycling with direct feedbacks on plant productivity (Wagg et al., 2019). Developing robust, reliable and sensitive biological indicators is crucial for establishing early warning systems of potential soil multi-functionality losses (Schloter et al., 2018). These indicators should not only contribute to the assessment of the current status of agricultural soils and the impact of past management, but they should also help to determine the effectiveness of new practices for improving soil biological quality. The most popular microbial indicators in soil science were first, and are still, based on integrative parameters such as microbial biomass C, potential N mineralization, metabolic quotient or enzymatic activities (Nannipieri et al. 2003, Nannipieri et al., 2012). Although they are key indicators for studying the functioning of soils, they do not inform on microbial diversity.

Until the mid 90′s, little research had been conducted to quantify the relationships between microbial diversity, soil quality, and ecosystem sustainability (Kennedy and Smith, 1995). Since then, the development of molecular fingerprinting partially fulfill this need for knowledge. Among these methods, phospholipid fatty acids (PLFA), Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal Restriction Fragment Length Polymorphism (T-RFLP) have been probably the most commonly used in routine to study soil microbial ecology, with almost 8000 publications (Web of Knowledge, 2020). However, these

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fingerprints are limited by their low resolution, which restrict the investigation to the most dominant groups of microorganisms. Besides, they cannot be used to determine genetic affiliation, unless complemented by a cloning and Sanger sequencing procedure including a time consuming clone library construction. These major methodological constraints have been overcome thanks to the emergence of the so-called next-generation sequencing (NGS) about 15 years ago (Daniel, 2005).

The constant improvement of molecular biology techniques in soil sciences has dramatically improved our knowledge of soil microbial diversity and offers new indicators of soil quality. A global-range survey of the soil biodiversity research community across Europe showed that microbial diversity determined by molecular methods are now the top indicators for monitoring progress towards policy goals for soil quality (Stone et al., 2016). According to the "logical sieve" procedure developed by Ritz et al. (2009), microbial diversity measurements were only in the middle rankings for technical factors [F_T] but scored in the top five for discrimination potential [S_D] and high for relevance to function [F_{SF}]. However, it should be noted that the relationship between the soil microbial diversity and soil functions is still not clear. Given the relevance of these molecular techniques for soil microbial monitoring, it is crucial to establish standard operating procedures (SOPs) such as those recommended by the FAO for soil chemical and physical properties (https://www.fao.org/global-soil-partnership/glosolan).

To ensure the reproducibility of results and respond to the need for harmonizing the huge microbial data provided by molecular tools, one should first resolve practical issues. Before sequencing, each step of the DNA metabarcoding workflow can potentially introduce its own sources of artifacts and biases (Zinger et al., 2019). For instance, DNA extraction methods can alter microbial diversity measurements and even mask long-term fertilization effects (Changey et al., 2021). Also, microbial diversity indexes can be affected by read annotation stringency (Delmont et al., 2012), inappropriate primers (Clarke et al., 2014), reagent contaminations (Salter et al., 2014) or sequencing adapters (Taberlet et al., 2018). In addition, there are other potential biases such as the denoising procedure, sequencing depth, reference sequence database or data normalizations. Even the choice of the diversity indicator used to quantify and compare microbial taxonomic diversity has been questioned (Haegeman et al., 2013). Although guidelines have been proposed to design of metagenomic surveys and characterize community composition and function of soil microbiomes (Vestergaard et al., 2017), there are still no reference on the taxonomic level at which analyses of diversity indicators should be performed.

One might expect that most soil microbial diversity studies performed with High-Throughput Sequencing (HTS) tend to use information at the lowest taxonomic rank (i.e. species or OTU level when possible). Indeed, DNA-based assignment of microorganisms to fine taxonomic resolution is equally expensive as assigning them to coarser levels (assuming the same criteria of quality or sequencing depth). However, a rapid survey of about one hundred articles published in soil microbiology during the last 5 years (Web of Knowledge, 2020) shows that most of these studies (83%) use different, and paradoxically high taxonomical levels to illustrate the composition of microbial communities: more than 95% used the phylum, 43% the genus and 35% the class. This obvious disparity in the literature certainly stems from the difficulty of synthesizing the astronomical amount of data produced at a very fine taxonomic scale. One can therefore wonder about the most appropriate level of aggregation to answer questions of microbial ecology. In this respect, it has been suggested that high bacterial taxonomic ranks can be relevant for bacterial taxonomy, evolution and ecology (Philippot et al., 2010). Practically speaking, this lack of congruity between studies concerning the taxonomic level at which analyses of diversity indicators are calculated may limit our capacity to monitor microbial diversity of soils.

This question has been addressed for decades in the fields of ecology and biogeography of macroorganisms through the concepts of Higher Taxon Approach (HTA) or taxonomic sufficiency (Ellis, 1985). This approach consists in the identification of organisms to the taxonomic level needed to meet the requirements of the study. A recent meta-analysis performed on different ecosystem types (aquatic and terrestrial) and biological groups (e.g., microorganisms, invertebrates, vertebrates and plants) showed that higher taxonomic ranks are sufficient for representing biodiversity patterns (de Oliveira et al., 2020). Strong within-taxon congruence was demonstrated at family, genus and species levels for bacteria living in a large lake system (Vilmi et al., 2016). A study on bacterial and fungal biogeography conducted in in subarctic ponds showed that taxonomic downscaling indicated a notable change in the relative importance of biodiversity determinants with stronger local environmental filtering, but decreased importance of climatic variables (Yeh et al., 2019). To our knowledge, the congruence of diversity indicators obtained at different taxonomic levels has never been examined in soils contrary to aquatic ecosystems, although it may gives valuable information for the applications of sequencing to soil biodiversity monitoring.

This study focuses on the relationship between soil organic matter (SOM) and bacterial diversity. It is known that SOM quantity and quality greatly influence soil microbial diversity (e.g. Fierer and Jackson 2006; Maestre et al., 2015; Wiesmeier et al., 2019). The study had two objectives. First, we hypothesized that the finer the taxonomic resolution, the better the differentiation among bacterial communities of soils subjected to different SOM managements. Second, we compared diversity indicators obtained with molecular fingerprinting techniques that are of lower resolution but still very popular. Therefore, we analyzed the bacterial communities of samples collected in one of the oldest long-term field experiments in Europe, initially designed to study the effects of different organic and mineral fertilizers on crop production and soil properties. We used both molecular fingerprints (PLFA, DGGE and T-RFLP) and massive amplicon sequencing (Illumina® Technologies) at different taxonomic resolutions (from phylum to species or OTU). The ability of the different methods to discriminate the effect of fertilization treatments on soil bacterial communities was evaluated by comparing α- and β-diversity indexes and the relationships with environmental variables.

2. Materials and methods

2.1. Experimental site and soil sampling

Soil samples were taken from the Ultuna Long-Term Soil Organic Matter Experiment (Uppsala, Sweden; 60N, 17E). The experiment was started in 1956 on a post-glacial clay loam soil classified as an Eutric Cambisol (Herrmann and Witter, 2008). The mineral fractions consist of 37% clay, 41% silt and 22% sand. In this experiment, samples were taken from the plots (2 × 2 m) that had been treated with mineral nitrogen (N) fertilizers and/or organic amendments (biennial addition of 8 Mg ash-free organic matter ha^{-1} y^{-1}). These treatments resulted in a wide range of soil soil physico-chemical parameters (Table 1, data from Lerch et al., 2011). The treatments were initially replicated in four blocks but in the present study, only three replicates per treatment were used. Eight sub-samples from 0 to 7 cm depth were taken from each plot in June 2009, sieved < 2 mm, mixed per replicate block and stored at − 20 °C prior DNA or PLFA extraction.

2.2. Phospholipid fatty acids (PLFA)

Lipid fractions were obtained after extraction from 10 g soil (dry weight equivalent) using the method described by Frostegård et al. (1993). Phospholipids were separated using a solid-phase extraction column (Extract-Clean® Silica, Grace). After methylation, fatty acid methyl esters (FAME) were identified using the retention time of chromatograms and mass spectral comparison on a Hewlett-Packard 6890 gas chromatograph equipped with either a Flame Ionisation Detector (GC-FID) or an Agilent 5973 Electronic Impact (70 eV) quadruple Mass

Table 1
Soil physico-chemical characteristics (C, N, C/N, pH) of Ultuna Long-Term experiment field collected in 2009. Mean values ± standard deviation ($n = 3$) are shown. Different lowercase letters indicate significant ($P < 0.05$) differences among treatments.

Soils treatments*	Code	C (mg g^{-1})	N (mg g^{-1})	C/N ratio	pH (H_2O)
Unfertilised	B	11.1 ± 0.2^{f}	1.18 ± 0.02^{g}	9.4 ± 0.2^{e}	6.2 ± 0.1^{c}
$Ca(NO_3)_2$	C	14.0 ± 0.6^{e}	1.45 ± 0.04^{f}	9.6 ± 0.1^{d}	6.7 ± 0.1^{a}
Straw	F	16.1 ± 0.9^{d}	1.56 ± 0.08^{e}	10.4 ± 0.1^{b}	6.4 ± 0.1^{b}
Straw + $Ca(NO_3)_2$	G	19.8 ± 0.8^{c}	1.87 ± 0.06^{c}	10.6 ± 0.1^{a}	6.6 ± 0.1^{a}
Green manure	H	16.9 ± 0.3^{d}	1.73 ± 0.03^{d}	9.8 ± 0.1^{d}	6.1 ± 0.1^{c}
Farmyard manure	J	23.0 ± 0.4^{b}	2.27 ± 0.01^{b}	10.1 ± 0.1^{c}	6.5 ± 0.2^{ab}
Sewage sludge	O	28.6 ± 0.9^{a}	3.08 ± 0.09^{a}	9.3 ± 0.03^{e}	4.9 ± 0.1^{d}

* Soil chemical data are for spring 2009 (Lerch et al., 2013). Initial in 1956: soil C 1.5 mg g^{-1} soil. pH 6.5.

Spectrometer (GC–MS). A standard qualitative bacterial acid methyl esters mix (Sigma-Aldrich) that ranged from C11:0 to C20:0 was used for the identification of sample FAME based on retention time. The relative abundance of each fatty acid was used to compare the community structure between the samples (Lerch et al., 2009). All fatty acids contributing <0.5% to the fatty acids pool were removed before analysis. In order to compare to the other methods focusing on bacteria, the fungal biomarker C18:2w(9,12) was removed from the analyses.

2.3. DNA extraction and quantification

The DNA extraction was performed on 250 mg (dry weight equivalent) of soil using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) following manufacturer instruction and using SL2 and SX buffers. The total DNA concentration in each sample was quantified with a Qubit™ fluorometer (Invitrogen, NZ). In addition, the purity of the DNA was estimated by measuring the 260/280 nm and 260/230 nm ratios using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.4. Denaturing Gel gradient Electrophoresis (DGGE)

The DGGE analysis of the bacterial community was performed according to Muyzer et al. (1993). Briefly, the V3 region of the 16S rDNA was amplified using primers 338F and 518R (See Table A.1). Each PCR tube (25 µL) contained 12.5 µL of Taq PCR Master Mix Kit (Qiagen, USA), 1.25 µL of each primer, 2 µL of DNA template and 8 µL of nuclease free water. PCR reaction was performed in a thermocycler (Biorad T100, France) with the following program: 95 °C for 2 min, followed by 15 cycles at 95 °C for 1 min, 60 °C for 1 min and final extension step 72 °C for 2 min followed by 15 cycles at 95 °C for 1 min, 65 °C for 1 min and 72 °C for 2 min and 10 cycles at 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and an additional extension time at 72 °C (10 min). DGGE was performed on 6% polyacrylamide gels with a gradient of 45% to 65% denaturants (urea/formamide). Denaturation gradient was 30–60% (urea/formamide) and migration of 25 µL of PCR products was performed for 6 h at 150 V. Gels were stained with ethidium bromide and were imaged Gel-Doc (BioRad, USA) exploitation system. Data analysis was realized using the software Quantity One (BioRad, USA) according to (Ranjard et al., 2000). Bands with a relative abundance below 0.5% were removed from the matrices.

2.5. Terminal-restriction fragment length polymorphism(T-RFLP)

For T-RFLP, bacterial 16S rRNA genes were amplified using primers 63F and 1389R (Marchesi et al., 1998; Osborn et al., 2000) (See Table A.1). A Biorad T100 thermal cycler was used for the amplification with the following program: initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 57 °C for 45 s, and 72 °C for 90 s, followed by a final extension time at 72 °C for 10 min. Restriction fragments of amplified 16S rRNA genes were obtained using enzyme *AluI* (Thermo Fisher) following the protocol described by Blaud et al. (2015). Samples were electrophoresed on an ABI 3730 PRISM® capillary DNA sequencer (Applied Biosystems) using LIZ500 internal size standard (Applied Biosystems). Genetic profiles obtained were analyzed using GeneMapper® v. 3.7 software (Applied Biosystems). The terminal restriction fragments (T-RFs) were binned with a 0.5 bp interval. T-RFs with a relative abundance below 0.5% were removed from the matrices.

2.6. Amplicon sequencing and bioinformatics analysis

The 16S rRNA fragment was amplified from each DNA sample using the surrounding conserved regions' V3–V4 primers (Klindworth et al., 2013) with overhang adapters: FwOvAd_341F and ReOvAd_785 (Table A.1). For each sample, 5 ng of DNA template were used for a 25 µL PCR mix with 5 µL of each primers (1 µM), 12.5 µL, of HiFi Hot Start Ready Mix (Kapa Biosystems) and ultra-pure water. PCR were performed on a T100 thermal cycler (Biorad, USA) as follows: 95 °C for 3 min, followed by 27 cycles of 98 °C for 20 s, 61 °C for 10 s, and 72 °C for 15 s. A final extension step was performed at 72 °C for 5 min. All amplicon products were purified using AMPure magnetic beads (Agencourt Bioscience, USA), quantified with Qubit™ fluorometer (Thermo Fisher Scientific) and mixed in equimolar concentrations (5 ng µL). The library construction (Nextera XT DNA Library Preparation Kit) and the sequencing (Illumina MiSeq 300 bp paired-end with V2 chemistry with a sequencing depth of 1 million reads) were performed at the Curie Institute (Paris, France). After a quality control, 16S paired-end sequences were merged into contigs with PEAR (v0.9.10) (Zhang et al., 2014). Data were subsequently imported into the FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented on a galaxy instance (v.2.3.0) (http:// sigenae-workbench.toulouse.inra.fr/galaxy/) (Escudié et al., 2018). Sequences were dereplicated before being clustered using SWARM algorithm (v.2.1.5) (Mahé et al., 2014) with a first denoising step using an aggregation distance equal to 1 and a second one equal to 3. Chimera were removed using VSEARCH (Rognes et al., 2016). Filters were applied to remove clusters which are not present in at least 3 samples or with an abundance below a 0.005% threshold (Bokulich et al., 2013). The taxonomic assignation of each OTU was performed using the BLAST tools against (Camacho et al., 2009) the database SILVA 132 16S (Pruesse et al., 2007). Phyloseq (1.26.1) R package was used to identify community composition analysis, to normalize and to generate α-diversity indexes (richness and evenness) after a rarefaction curve using the transform counts method (McMurdie et al., 2013).

2.7. Statistical analysis

All statistical analyses were carried out using R software version 3.3.1 (R Development Core Team, http://www.R-project.org). To compare each method of soil bacterial community analysis, we first compared α-diversity indexes. Here, we used the number of OTU as community richness and Simpson-Yule index as evenness. The two latter parameters were also used to characterize PLFA, DGGE and T-RFLP profiles although we are aware that richness and evenness values obtained from molecular fingerprints are not reliable descriptors of the bacterial community (Blackwood et al., 2007). The evenness was

estimated using the Simpson-Yule index: $E = 1/\sum pi^2$ (Magurran, 1988), where pi is the proportion of a given peak, band, T-RF or amplicon. After normality and homoscedasticity verifications (Shapiro and Bartlett test), ANOVA (significance was declared at $P < 0.05$) were performed on richness and evenness indexes and Tukey HSD test was used for pairwise comparisons using "agricolae" package. The β-diversity was estimated by using the relative abundance matrices and multivariate analyses. The dissimilarities (Bray-Curtis distances) among soil treatments were assessed using Principal Coordinate Analysis (PCoA) were performed followed by Between-Class Analysis (BCA) using the "ade4TkGUI" package. In addition, significant difference among treatments was determined using a Mantel test performed on 10^5 permutations (vegan 2.2.1 R package) (Oksanen et al., 2011). After testing the multivariate homogeneity of group dispersions (Anderson, 2006), PERMANOVA were performed using the "adonis" function (vegan 2.2.1 R package) to test the link between bacterial community composition obtained with the different method and the environmental variables (9999 permutations). All graphic representations were performed using SigmaPlot 14.0 software.

3. Results

3.1. Soil bacterial α-diversity

The bacterial richness (number of OTUs) obtained by Illumina Miseq sequencing doubled with the taxonomic resolution (Fig. 1.A). PLFA and DGGE profiling gave similar number of variables to the OTUs at the phylum level and T-RFLP profiling was similar to the class level. The intra-group variation tended to decrease with the taxonomic resolution, representing half of the total variability at the species (OTU) level (Fig. S1). With molecular fingerprints, significant ($P < 0.05$) differences among soil treatments were only found for DGGE and T-RFLP but the hierarchy among groups was not the same as that obtained with amplicon sequencing (Table 2). With NGS techniques, significant ($P < 0.05$) differences in the number of OTU among soil treatments were observed at all taxonomic level, except at the genus level. The same hierarchy was observed from the order to the species (OTU) level, with the lowest richness in the green manure treatment (H) and the highest in the straw + $Ca(NO_3)_2$ (G) and farmyard manure (J). The richness z-scores decreased with taxonomic resolution for the unfertilized (B) or green manure amended (H) soils but increased for $Ca(NO_3)_2$ fertilized (C) and straw (F) amended soils (Fig. S2).

The average evenness values obtained by sequencing tended to decrease linearly with the taxonomic resolution (Fig. 1B). Profiles obtained by molecular fingerprints were much more even than OTU distributions. Similar values were calculated for PLFA and T-RFLP while the highest evenness was found for DGGE profiles. The variation coefficient of the evenness index was the lowest for PLFA method and the highest for NGS at the species (OTU) level (Fig S1.B). DNA based fingerprints showed similar values as amplicon sequencing at the phylum to the genus level (Fig. A.1). The intra-group variation was the lowest for T-RFLP and NGS at the species (OTU) level. Significant ($P < 0.05$) differences among soil treatments were observed both with molecular fingerprints and amplicon sequencing at all taxonomic level, except for the order (Table 3). However, the hierarchy among treatments were not the same according to the method. The highest value in the PLFA data was observed for the straw treatment (F) and the lowest for sewage sludge (O). The DGGE profiling showed the highest value for the unfertilized plot (B) and the lowest for farmyard manure treatment (J). The highest value in the T-RFLP data was found for the green manure treatment (H) and the lowest for sewage sludge (O). The patterns were very similar between phyla and classes in the NGS data, with the highest evenness values for the $Ca(NO_3)_2$ and sewage sludge treatments (C and O). At the family, genus and species (OTU) level, the highest evenness value was observed for the farmyard manure (J) treatment. The evenness z-scores decreased with taxonomic resolution for the unfertilized soil (B) and soils amended with farmyard manure (J), sewage sludge (O) or green manure (H). Inversely, evenness z-scores increased for the $Ca(NO_3)_2$ fertilized (C), the straw (F) and the green manure (H) amended soils (Fig. A.2).

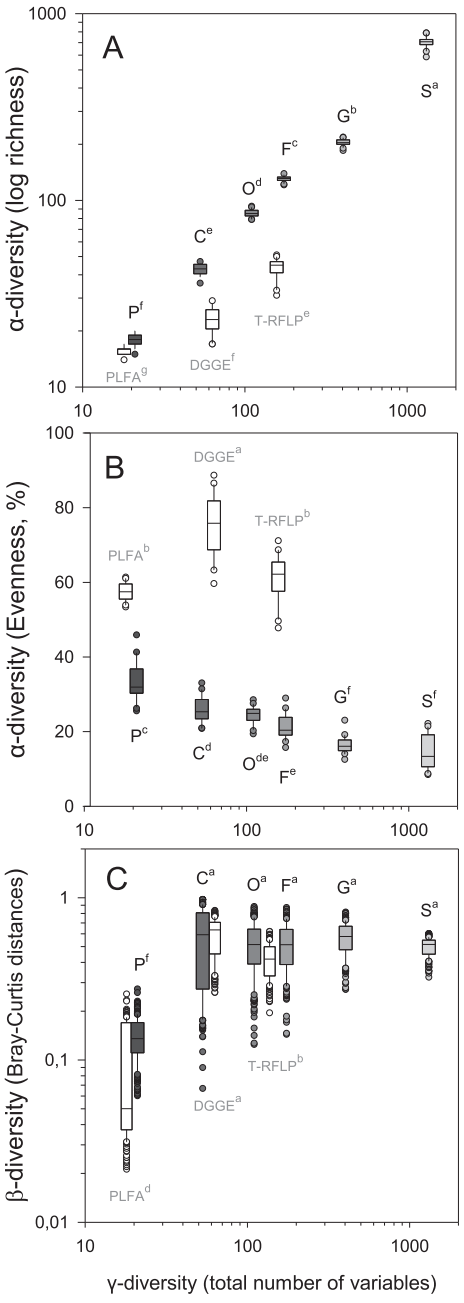

Fig. 1. Richness (A) and Evenness (B) of all samples (n = 21) and Bray-Curtis inter-group distances (C; n = 128) as function of the number of variables obtained with molecular fingerprints (white boxes) or amplicon sequencing (grey boxes) at different taxonomy levels (P: Phylum; C: Class; O: Order; F: Family; G: Genus; S: Species). Different lowercase letters indicate significant ($P < 0.05$) differences among methods.

3.2. Soil bacterial β-diversity

The analyses of Bray-Curtis inter-group distances (Fig. 1.C) obtained with amplicon sequencing showed that dissimilarities among samples was the highest at the class and genus level and the lowest at the phylum level. They were the lowest for PLFA and the highest for DGGE. For all methods, inter-group dissimilarities were higher than intra-group ones

Table 2
Number of FAME, DGGE bands, T-RFs and OTU obtained by amplicon sequencing (from Phylum to Species) Means values ± standard deviation ($n = 3$) are shown. Different lowercase letters indicate significant ($P < 0.05$) differences among treatments.

	Fingerprinting			Amplicon Sequençing					
Soils treatments	PLFA	DGGE	T-RFLP	Phylum	Class	Order	Family	Genus	Species
Unfertilised	16 ± 1	21 ± 2^{b}	45 ± 2 ^{a}	19 ± 1 ^{a}	43 ± 3 ^{ab}	85 ± 4 ^{abc}	131 ± 3 ^{bc}	201 ± 3	676 ± 28^{b}
$Ca(NO3)_2$	16 ± 1	23 ± 1^{b}	46 ± 3 ^{a}	17 ± 1 ^{bc}	40 ± 1 ^{bc}	83 ± 1^{c}	127 ± 1 ^{cd}	206 ± 3	709 ± 10 ^{ab}
Straw	17 ± 2	26 ± 2 ^{a}	44 ± 3 ^{a}	16 ± 1^{c}	38 ± 2^{c}	84 ± 1 ^{bc}	131 ± 2 ^{bc}	207 ± 7	713 ± 27 ^{ab}
Straw + $Ca(NO3)_2$	16 ± 1	27 ± 2 ^{a}	46 ± 4 ^{a}	19 ± 1 ^{a}	44 ± 1 ^{a}	89 ± 3 ^{a}	134 ± 3 ^{ab}	209 ± 6	755 ± 56 ^{a}
Green manure	16 ± 1	19 ± 1^{c}	48 ± 2 ^{a}	18 ± 1 ^{ab}	43 ± 2 ^{ab}	81 ± 3^{c}	124 ± 4 ^{d}	194 ± 13	619 ± 29^{c}
Farmyard manure	15 ± 1	26 ± 2 ^{a}	38 ± 5^{b}	18 ± 2 ^{ab}	44 ± 3 ^{a}	89 ± 3 ^{a}	137 ± 2 ^{a}	214 ± 6	738 ± 20 ^{a}
Sewage sludge	16 ± 1	21 ± 3^{b}	35 ± 4^{b}	17 ± 2 ^{abc}	44 ± 3 ^{a}	85 ± 4 ^{abc}	129 ± 2^{c}	202 ± 3	705 ± 11 ^{ab}
P value	0.66	**0.021**	**0.011**	**0.029**	**0.015**	**0.025**	**0.006**	0.062	**0.002**

Table 3
Evenness (in %) of PLFA, DGGE or T-RFLP profiles and OTU obtained by amplicon sequencing (from Phylum to Species). Mean values ± standard deviation ($n = 3$) are shown. Different lowercase letters indicate significant ($P < 0.05$) differences among treatments.

	Fingerprinting			Amplicon Sequençing					
Soils treatments	PLFA	DGGE	T-RFLP	Phylum	Class	Order	Family	Genus	Species
Unfertilised	55 ± 1 ^{de}	77 ± 4 ^{a}	60 ± 2 ^{bc}	33 ± 3^{b}	26 ± 3 ^{cd}	23 ± 2	18 ± 2^{c}	14 ± 2^{b}	10 ± 1^{c}
$Ca(NO3)_2$	58 ± 2 ^{bcd}	72 ± 3 ^{ab}	59 ± 5^{c}	38 ± 3 ^{a}	31 ± 1 ^{a}	26 ± 1	22 ± 2^{b}	16 ± 1^{b}	17 ± 4^{b}
Straw	60 ± 2 ^{a}	71 ± 5 ^{ab}	63 ± 1 ^{abc}	32 ± 1^{b}	24 ± 1 ^{cd}	26 ± 2	21 ± 2 ^{bc}	16 ± 1^{b}	13 ± 3^{c}
Straw + $Ca(NO3)_2$	58 ± 1 ^{abc}	68 ± 2 ^{ab}	63 ± 2 ^{abc}	28 ± 3^{b}	22 ± 2 ^{d}	24 ± 2	22 ± 2^{b}	16 ± 2^{b}	18 ± 3 ^{ab}
Green manure	57 ± 1 ^{cde}	66 ± 3^{b}	68 ± 3 ^{a}	30 ± 2^{b}	22 ± 2 ^{d}	23 ± 3	21 ± 2^{b}	16 ± 2^{b}	10 ± 1^{c}
Farmyard manure	60 ± 2 ^{ab}	57 ± 2^{c}	65 ± 6 ^{ab}	32 ± 4^{b}	27 ± 1 ^{bc}	27 ± 1	27 ± 2 ^{a}	20 ± 2 ^{a}	21 ± 1 ^{a}
Sewage sludge	54 ± 1 ^{e}	67 ± 2^{b}	55 ± 2 ^{d}	41 ± 4 ^{a}	30 ± 3 ^{ab}	23 ± 2	19 ± 1 ^{bc}	16 ± 1^{b}	13 ± 1^{c}
P value	**0.002**	**0.009**	**<0.001**	**<0.001**	**<0.001**	0.209	**0.004**	**0.022**	**<0.001**

(data not shown). The coefficient of variation of the total and intra-group distances tend to decrease with the taxonomic resolution, with a minimum reached at the species (OTU) (Fig. A.1.). The analyses of z-scores of the inter-group distances shows that, whatever the method or resolution, the sewage sludge treatment (O) had very different bacterial communities (highest distances) compared to the other treatments (Fig. A.2.). For few treatments like the farmyard amendment (J) or non-fertilized soil (B), z-scores are highly influenced by the method or the resolution.

The first two axes of the between class analysis (Fig. 2) explained 76%, 54%, 64% for PLFA, DGGE and T-RFLP, respectively and between 80 and 82% for amplicon sequencing (depending on the taxonomic level). For all methods used, the analysis of multivariate homogeneity of group dispersions showed no differences and the Monte Carlo test revealed significant ($P < 0.001$) shifts in bacterial community structure associated with long-term fertilization. Amplicon sequencing gave the highest differentiation among treatments. On the first (horizontal) axis, the unfertilized soil (B) and the sewage sludge (O) treatment were separated from straw + $Ca(NO_3)_2$ and the farmyard manure (G and J) while on the second (vertical) axis, the soil (C) fertilized with $Ca(NO_3)_2$ was separated from the straw and green manure amended soils (F and H). Correlations of Hellinger distances between closest taxonomic levels were all significant except between the Phylum and Order levels (Fig. 3). It should be noted that molecular fingerprints profiles were significantly correlated with amplicon sequencing data at all taxonomic level except the class and the phylum for PLFA (Table A.2.).

The proportions of OTU present in all samples (core microbiota), shared with few groups (2 to 6) and specific to a particular soil treatment varied with the taxonomic level considered (Fig. 4). The results showed a decrease of the apparent core microbiota from 62% to 16% from the phylum to the species (OTU) level. Inversely, the proportion of OTUs partially shared among treatments increased from 33 to 75% between the higher and the lower taxonomic ranks. Specific OTUs were not detected at the phylum and class levels but their proportion increased from 4% to 9% between the order and the species (OTU) levels. Most of the specific OTUs were found in the sewage sludge (O) and farmyard manure (J) treatments (Fig. A.2).

3.3. Relationship between bacterial diversity and soil parameters

A positive correlation ($P < 0.01$) was found between C/N ratio of the soil and the richness at the order ($r^2 = 0.65$), family ($r^2 = 0.56$) and species (OTU) levels ($r^2 = 0.55$). The number of T-RFs was also positively correlated to the pH ($P < 0.01$; $r^2 = 0.63$). The C/N ratio was positively correlated ($P < 0.01$, $r^2 = 0.59$) to the evenness at the species (OTU) level but negatively correlated ($P < 0.05$, r^2 = -0.53) at the phylum level. With PLFA fingerprinting, a positive correlation ($P < 0.05$, $r^2 = 0.49$) was found between soil pH and the evenness of the profile. With T-RFLP fingerprinting, a positive correlation ($P < 0.001$, $r^2 = 0.82$) was found between soil pH and the evenness and a negative one ($P < 0.001$, r^2 = -0.54) with the soil N content. For all methods, the PERMANOVA based on Bray-Curtis dissimilarities gave significant ($P < 0.001$) relationships between the microbial community structure and the type of fertilization (Table 4). The strongest relationships were found at the species (OTU) ($r^2 = 0.77$) and phylum ($r^2 = 0.74$) levels and the lowest relationships at intermediate taxonomic resolutions (r^2 between 0.61 and 0.65). In comparison, molecular methods had an r^2 between 0.69 and 0.70.

When testing a PERMANOVA model using soil physico-chemical variables, the higher taxonomic resolution increased the number of significant relationships. At the phylum level, the microbial community composition was correlated with N, pH and C/N ratio while at the genus and species (OTU) level, it was correlated to C, N, pH, C/N ratio and also the interaction of C and N and C and pH. The PLFA matrix was significantly correlated to N, pH, the interaction of C and N contents. DGGE and T-RFLP matrices were additionally correlated with C and C/N ratio and the interaction of C and pH. DGGE matrix also revealed significant effect of the interactions between C, N, pH and C/N ratio.

4. Discussion

4.1. Changes in diversity indexes across taxonomic resolutions

Globally, the results obtained from amplicon sequencing showed a doubling of richness at each taxonomic level. This trend matches that of

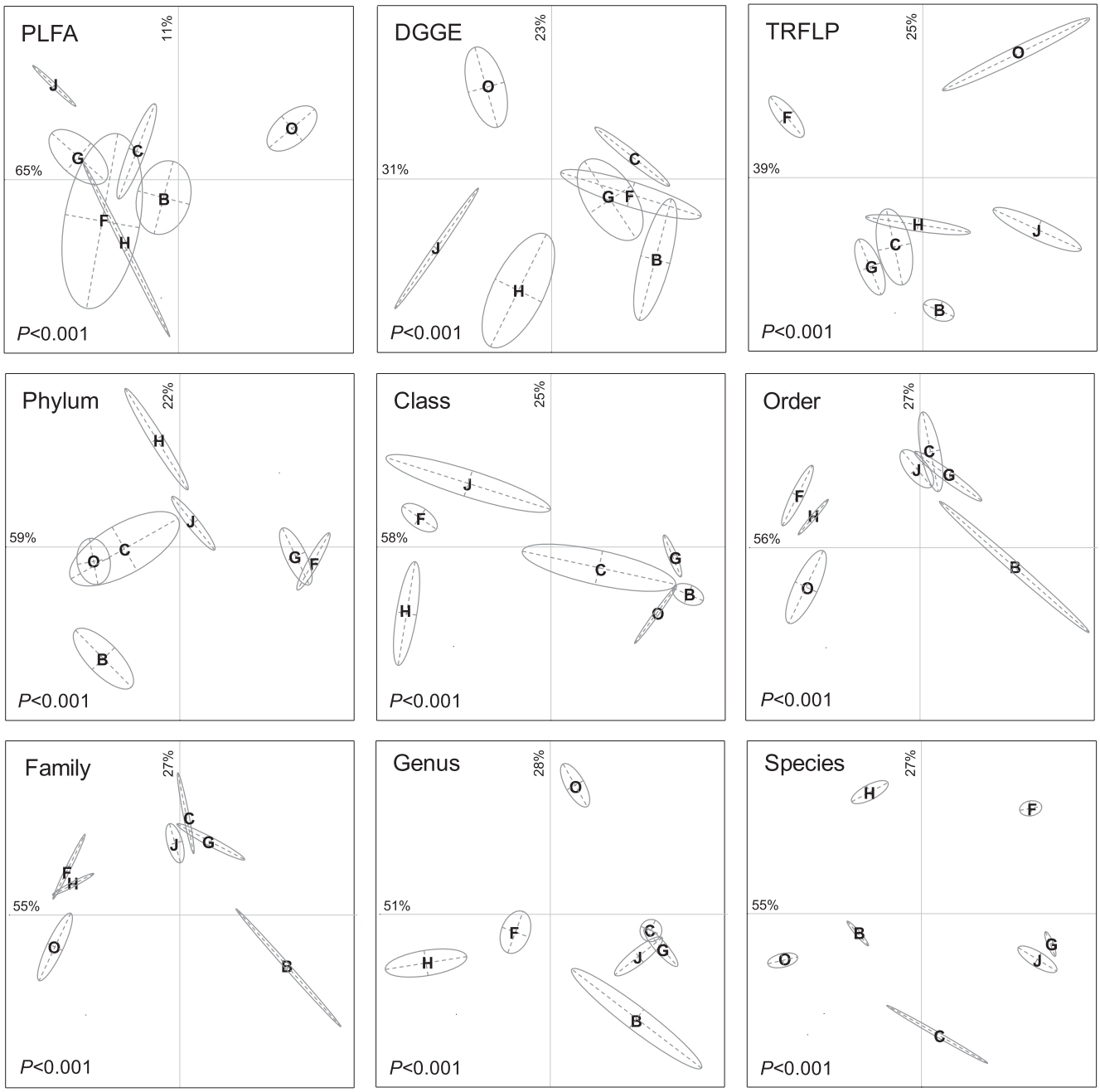

Fig. 2. Differences in bacterial community structure represented by Between Class Analysis (BCA) based on PLFA, TRFLP, DGGE or amplicon sequencing (Phylum to Species level) matrices. Grey lined ellipses represent the variability of each treatment (B: unfertilized; C: Ca(NO3)2; F: straw; G: straw + Ca(NO3)2; H: Green manure; J: Farmyard manure; O: Sewage sludge). Letter represent the barycenter of the replicates (n = 3) for each treatment. Monte Carlo test revealed significant differences among treatments (simulated P < 0.001) for all methods. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the List of Prokaryotic names with Standing in Nomenclature (LPSN; https://www.bacterio.net/), where 34 phyla, 116 classes, 196 orders, 415 families, 2930 genus and 15,448 species are listed (Parte, 2018). From the phylum to the family rank, a linear relationship was found between the number of OTU in the present study and that of the LPSN ($y = 0.47x$; $r^2 = 0.96$; $P < 0.001$). This linearity was subject to a break in slope at lower levels, as the numbers of genus and species found here represent only 0.11 and 0.07 of those classified in the LPSN, respectively. It could be the consequence of stronger selection in soils at fine taxonomic levels, or lower levels of contribution to the database by the soils community, as most of the species and genus belonging to soil environments are not recorded in worldwide databases yet (Nayfach et al., 2021). Concomitantly, we observed a decrease in the evenness of nearly 15% between two different subsequent taxonomic levels as we go down in the taxonomic hierarchy, which is evidence of increasing numbers of minor bacterial groups.

We hypothesized that the increase of information provided at low taxonomic ranks would accentuate the differences in bacterial diversity among soil treatments and thus, improve the discrimination potential [SD] of sequencing techniques (Ritz et al., 2009). Our results do not confirm this trend for α-diversity indices. Here, the best discrimination were found at the family and at the class level, respectively for bacterial richness and evenness. Inversely, no differences among treatments were found at the genus and order level for bacterial richness and evenness,

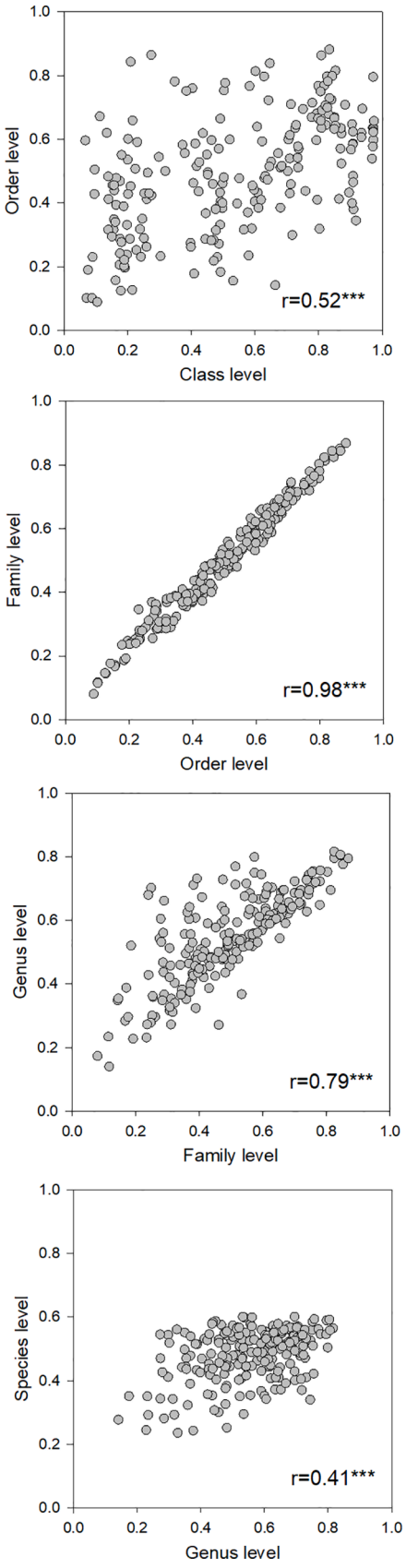

Fig. 3. Comparisons of different taxonomic resolutions of bacterial communities. The pairwise congruencies between closest taxonomic levels from Order to Species are illustrated using Hellinger distances. The Mantel test statistics are presented inside the scatterplots ("***": $P < 0.001$). Note that the relationship between the Phylum and Order levels is not significant (data not shown).

respectively. The high intra-treatment variability (approximately 80% of the total variability) found for both bacterial richness and evenness at these taxonomic ranks may be responsible for the lack of differences. Therefore, attention should be paid to the taxonomic level when using α-diversity indexes to compare the effect of agricultural practices. For example, one can conclude that there is no effect of fertilization on bacterial richness at the genus level, or that the lowest bacterial richness is found in the soil amended with straw at the phylum level, while it harbors one of the highest species level richnesses. These results suggest taking into account the taxonomic resolution when studying more drastic effects on soil microbial communities, such as those induced by toxic pollutants. For instance, a theoretical study by Bozzuto and Blanckenhorn (2017) conducted on fictitious community of organisms showed that a dose-dependent mortality effect on biodiversity can be masked depending on the the taxonomic resolution used.

The trends observed with β-diversity are different and the interpretation less ambiguous. Multivariate analyses performed at the phylum levels allow to separate most treatments, which support the idea of the ecological coherence of high bacterial taxa (Philippot et al., 2010). However, taxonomic downscaling offers a better discrimination among treatments. Here, we conclude that analyzing bacterial communities at the genus or species level provide not only a clear separation among the different fertilizations applied to the soil but also the highest number of significant relationships with environmental variables. This could be related to the increase of the proportion of OTU specific to a treatment from the order and the species levels while the relative weight of core microbiota was divided by 4 between the phylum and the species level. Soils select functions, but there are no function specific to a phylum. The polyphyletic distribution of many functional traits results from gene loss, convergent evolution but also lateral gene transfer. It has been demonstrated that the phylogenetical dispersion of functional traits is higher for the ability to use of simple carbon substrates than for more complex traits involving many genes (Martiny et al., 2013). Thus, the taxonomic level at which the discrimination among treatments is the most relevant also depends on the functional traits on which the effects of soil selection will be exerted. As other approaches using trait-based microbial strategies (Malik et al., 2020; Romillac and Santorufo, 2021) or ecological networks (Banerjee et al., 2018; Hemprich-Bennett et al., 2021), we believe that comparing diversity indices across different taxonomic resolution and soil management could be useful both for the development of monitoring tools and knowledge improvement.

4.2. Comparison of low resolution molecular fingerprinting with metabarcoding

Molecular fingerprint methods have often been compared to each other or used simultaneously to investigate soil microbial communities: PLFA and T-RFLP (Widmer et al., 2006; Turpeinen et al., 2004; Männistö et al., 2007; Wang et al., 2011; Ying et al., 2013), PLFA and DGGE (Ritz et al., 2004; Bossio et al., 2005; Dong et al., 2008; Ben-David et al., 2011; Stagnari et al. 2014) or DGGE and T-RFLP (Nunan et al., 2005; Smalla et al., 2007; Matsuyama et al., 2007; Gao et al., 2012). In general, these studies indicate a similar discrimination potential for all methods, or a slightly better one with T-RFLP, given its high reproducibility. Comparisons of molecular fingerprinting and NGS are less documented. Here, the number of descriptive variables with PLFA and DGGE profiling were equivalent to the number of phyla measured by sequencing, while the number of T-RFs was similar to the number of classes. However, diversity patterns and discriminant potential obtained at these taxonomic levels are not always consistent with those obtained with molecular fingerprints because there is no direct correspondence between a given fatty acid or DNA fragment and a phylum or a class of bacteria.

The use of either lipid- or nucleic-based fingerprinting methods to estimate α-diversity metrics has been often criticized. However, a few studies have found similar diversity results with both metabarcoding and fingerprinting techniques. For example, Elsayed et al. (2014) found

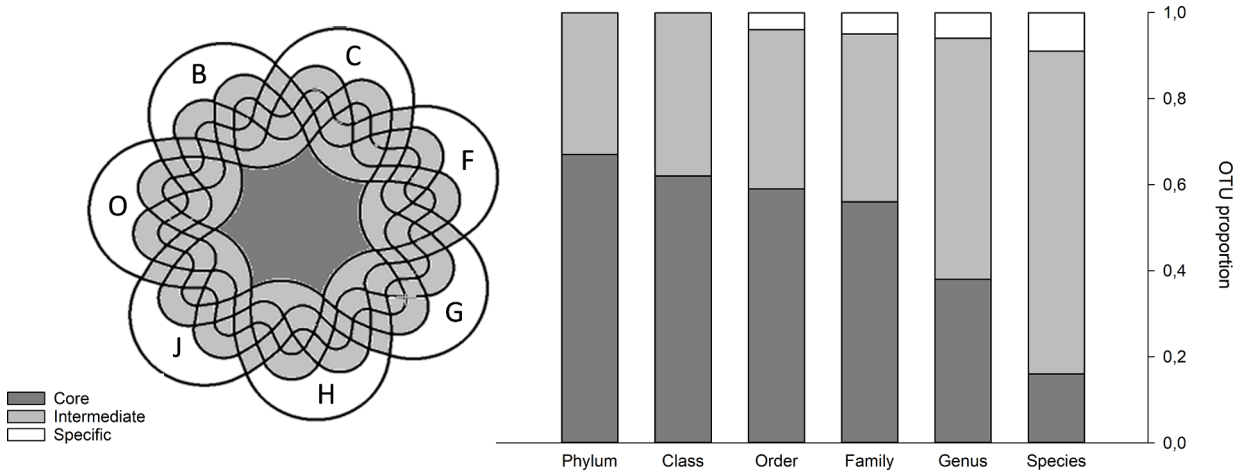

Fig. 4. Symmetric 7-way "Adelaide" Venn diagram (Grünbaum, 1992) depicting the core microbiota (dark grey), OTUs partially shared among 2 to 6 treatments (grey) and OTU specific to a treatment treatment (B: unfertilized; C: $Ca(NO_3)_2$; F: straw; G: straw + $Ca(NO_3)_2$; H: Green manure; J: Farmyard manure; O: Sewage sludge). Stack bars representing the proportion of OTU distributed in the core, intermediate from the phylum to species level. Only OTUs found at least in 67% of the replicates (2/3) were considered. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Relationships obtained by PERMANOVA (r^2) among bacterial community composition characterized by PLFA, DGGE or TRFLP fingerprints or by amplicon sequencing (from Phylum to Species) and soil properties (C, N, C/N ratio and pH) and fertilization treatment (***: $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

	Fingerprinting			Amplicon Sequençing					
	PLFA	DGGE	T-RFLP	Phylum	Class	Order	Family	Genus	Species
C	0.04	**0.05***	**0.06***	0.05	**0.14***	**0.09***	**0.09***	**0.06***	**0.08****
N	**0.43****	**0.19*****	**0.25*****	**0.17****	0.02	**0.18*****	**0.17*****	**0.18*****	**0.19*****
pH	**0.14***	**0.11****	**0.09****	**0.19*****	**0.12***	0.06	**0.08***	**0.08***	**0.09****
C/N	0.02	**0.37*****	**0.08****	0.06	**0.09***	0.05	**0.07***	**0.09****	**0.07****
C*N	**0.10***	**0.04***	**0.18*****	**0.18****	**0.11***	**0.13****	**0.12****	**0.10****	**0.13*****
C*pH	0.02	**0.08****	**0.05***	0.03	0.08	0.05	0.06	**0.11****	**0.09****
N*pH	0.01	0.01	0.02	0.02	0.02	0.06	0.06	0.04	0.03
C*C/N	0.01	0.01	0.03	0.01	0.02	0.04	0.04	0.04	0.03
pH*C/N	0.01	0.01	0.01	0.01	0.03	0.04	0.04	0.03	0.03
C*N*pH	0.01	**0.02***	0.03	0.01	**0.11***	0.04	0.04	0.03	0.02
C*N*C/N	0.01	0.01	0.02	0.02	0.05	0.02	0.02	0.02	0.03
C*pH*C/N	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.02	0.02
C*N*pH*C/N	0.01	**0.02***	0.01	0.01	0.01	0.01	0.02	0.02	0.02
Treatments	**0.69*****	**0.71*****	**0.70*****	**0.73*****	**0.63*****	**0.61*****	**0.62*****	**0.65*****	**0.77*****

that Shannon (H′) indexes of wetland bacterial communities estimated by T-RFLP and pyrosequencing approaches were significantly correlated. More recently, it has been shown that H' and the evenness of PLFA profiles of soil microbial communities were correlated to those obtained by metabarcoding at the phylum-level (Orwin et al., 2018). In this study, the evenness of molecular profiles were 3 to 4 times higher than those calculated with metabarcoding, indicating that all molecular fingerprints clearly overestimate the equitability of the microbial communities by considering only the most dominant groups. Although significant differences among soil treatments were observed with all fingerprint techniques, we did not find any relationship between techniques for either richness or evenness, whatever the taxonomic rank. It is known that lipid based fingerprinting cannot be used to estimate the richness of a microbial community since it relies on the detection of only few biochemical markers which usually indicate the relative abundance of gram-positive, gram-negative, fungi and actinobacteria (Frostegård et al., 2011). Furthermore, the fact that these biomarkers are also sensitive to changes in membrane composition for maintaining its integrity may lead to confounding changes in community structure and phenotypic plasticity (e.g. Wixon and Balser, 2013).

T-RFLP and DGGE are known to be inherently limited by their detection thresholds (Dunbar et al., 2001). Should they allow a perfect determination of a band (DGGE) or a peak (T-RFLP), the total number of OTU would be limited by the length of the amplicon produced and the "cutting" techniques. For instance, the maximum number of DGGE bands that could be obtained with 338F and 518R primers would be 541 corresponding to (180 bp × 4 bases) - (180 bp-1). For T-RFLP, the maximum number of peaks using 63F and 1389R primers and *AluI* as restriction enzyme would be 1322 (1326 bp-4 bp). In both cases, it is dramatically lower than the number of OTU obtained using Illumina MiSeq (2x300), for which the number of species is only limited by the length and region of barcode and the definition of bacterial species itself. We could argue that theoretically, a difference in the number of DGGE bands or of T-RFs may be due to a real modification of microbial richness. This case is probably true when microbial diversity changes dramatically under toxic pollutant exposure (e.g. Ge et al., 2011) or when comparing samples from very distinct habitats (e.g. Fierer and Jackson, 2006), but not for monitoring changes due to soil management. Variation in DGGE and T-RFLP evenness profiles may reveal changes in bacterial structure, but the changes are likely shared by different taxonomic groups. Overall, we conclude that molecular fingerprinting cannot provide reliable α-diversity indices in the context of a change in agricultural practices.

With the exception of the PLFA data, the fingerprinting methods were able to detect significant differences in the structure of the bacterial communities due to fertilization practices. The results obtained by multivariate analyses were quite similar to the metabarcoding results, although the discrimination potential was higher with the latter.

Significant relationships were found between data matrices obtained with all molecular fingerprinting and sequencing data at all taxonomic levels using Mantel's test (Table A.2.). This conclusion is in line with previous comparison studies. T-RFLP and pyrosequencing approaches were shown to result in similar abilities to separate bacterial community composition across sample locations and relate them to environmental variables in wetlands (Elsayed et al., 2014) and polar soils (van Dorst et al., 2014). Similar β-diversity patterns were found for bacterial and fungal communities in nest mounds of ants (Lindström et al., 2018) and for anaerobic bacterial and archaeal communities (De Vrieze et al., 2018), using T-RFLP and Illumina MiSeq. PLFA and 16S rRNA gene metabarcoding showed broadly similar patterns of bacterial community composition change with land use and a similar ability to predict a wide range of ecosystem functions (Orwin et al., 2018). The present one suggest that fingerprinting methods other than PLFA remain a good option for investigating microbial dissimilarities among samples if resources for metabarcoding are lacking.

4.3. Taxonomic resolution and environmental determinants of diversity

Previous studies conducted with molecular tools on the Ultuna long term experiment have shown that the C, N and pH have a strong influence on microbial biomass measured either by total PLFA (Börjesson et al., 2014) or qPCR (Changey et al., 2021). It has also been demonstrated that these variables are also correlated with microbial metabolic quotient (Enwall et al., 2007), catabolic profiles (Lerch et al., 2013) and community structure (Hallin et al., 2009; Blaud et al.2015). In this study, the taxonomic downscaling revealed that the correlation between bacterial richness and C/N was not observed at higher taxonomic ranks than order level and then to the species (OTU) level. This result suggest a higher influence of other environmental variables at a fine taxonomic level. Here, soils amended with straw + $Ca(NO_3)_2$, or farmyard manure had the highest C/N ratio and harbored the highest diversity, both in term of richness and evenness. These two treatments, that are among the richest in C content in the trial, were found to have the highest bacterial biomass (Changey et al., 2021), which suggest that richness and abundance are closely related. This is in line with a recent survey across global biomes which shows that soil C content is associated with the microbial diversity–biomass relationship (Bastida et al., 2021). The soil pH has been shown to be one of the main variables driving soil microbial diversity in long term treatments (Zhalnina et al., 2015). Although not a true indicator of bacterial diversity, the number and the evenness of T-RFs was negatively correlated with pH. The phylotype richness and diversity obtained by T-RFLP have been shown to decrease with soil acidity (Fierer and Jackson, 2006). However, in the present study, T-RFLP was not congruent with amplicon sequencing as the number of OTU was not the lowest in the sewage sludge treatment..

Whatever the method used or the taxonomic resolution, the most important differences among soil treatments were found when comparing bacterial β-diversity. The resolution of the method used PERMANOVA results showed no relationship between the number of variables obtained and the degree of intensity between the bacterial community structure and the type of fertilization. All methods revealed that the composition of the bacterial communities were mainly influenced by C, N and pH. Again, the highest differences were found for the sewage sludge, the farmyard manure, and to lesser extend to the green manure. In this study, only the major soil physico-chemical characteristics (C, N, C/N and pH) were compared to the biological dataset. Other physical or chemical parameters shaping the microbial habitats may also influence the diversity of the microorganisms. For example, the soil organic matter may indirectly affect the microbial community by modifying the porosity of the soil, as demonstrated on the same experimental plot (Kirchmann and Gerzabek, 1999). The presence of trace metals in the sewage sludge treatments (Witter and Dahlin, 1995; Witter, 1996) may also affect the bacterial communities. More generally, the chemical composition of the soil, in the labile and non-labile pool could explain a significant part of the variability observed in microbial diversity among the different treatments. This aspect could be further investigated focusing on a wide range of trace elemental analysis (ICP-MS or XRF techniques) and/or a more refined analysis using spectroscopy or spectrometry (FTIR, NMR or GC–MS). By comparing different spatial scales of drivers, Yeh et al. (2018) showed that taxonomic downscaling increased the relative weight of local environmental variables for aquatic microbial diversity but decreased the weight of climatic variables. It can be hypothesized that in a soil subjected to different organic fertilizers, taxonomic downscaling increases the influence of local physico-chemical variables and decreases that of major variables such as C, N and pH.

5. Conclusion

The ongoing development of high-throughput sequencing techniques allows us to better understand the role of microbial diversity in soils, something that was impossible a few decades ago. However, the astronomical amounts of data generated in the literature needs to be synthesized and the usefulness of diversity indices has never been greater for monitoring the effects of agricultural practices and climate change on microbial diversity. Based on the observation that studies conducted with metabarcoding techniques often present different taxonomic levels, we explored how downscaling to higher taxonomic resolution levels (from phylum to species) may affect the diversity indicators of soil bacterial communities subjected to long term fertilization. Our results show that in this case, the discrimination potential increased at finer taxonomic resolution for β-diversity but not for α-diversity indices such as richness and evenness. Also, the relative importance of hierarchical drivers of soil microbial communities such as C, N and pH varied depending on the taxonomic resolution. This study also demonstrated that indicators generated by molecular fingerprints such as DGGE and T-RFLP are still consistent to monitor the effect of agricultural management on β-diversity but not on α-diversity, which is useful information as it allows for a better use of results in past literature. We encourage performing such comparative studies on wider surveys (regional to national level) and different contexts (e.g. land use change, pollution exposure, urbanization) in order to increase the efficiency and the robustness of the use of sequencing data in soil biodiversity monitoring. From a fundamental point of view, studying biodiversity patterns across taxonomic scales may also be of fundamental interest when combined with high resolution physical and chemical analyses as well as functional traits or ecological networks in order to better understand at which taxonomic level environmental drivers matter and microbial functions operate.

CRediT authorship contribution statement

Frédérique Changey: Writing – original draft, Writing – review & editing. **Naoise Nunan:** Writing – review & editing. **Anke M. Herrmann:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Thomas Z. Lerch:** Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62, 245–253. https://doi.org/10.1111/j.1541-0420.2005.00440.x.

Bastida, F., Eldridge, D.J., García, C., Kenny Png, G., Bardgett, R.D., Delgado-Baquerizo, M., 2021. Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. ISME J. 15, 2081–2091. https://doi.org/10.1038/s41396-021-00906-0.

Ben-David, E.A., Zaady, E., Sher, Y., Nejidat, A., 2011. Assessment of the spatial distribution of soil microbial communities in patchy arid and semi-arid landscapes of the Negev Desert using combined PLFA and DGGE analyses. FEMS Microbiol. Ecol. 76, 492–503. https://doi.org/10.1111/j.1574-6941.2011.01075.x.

Blackwood, C.B., Hudleston, D., Zak, D.R., Buyer, J.S., 2007. Interpreting ecological diversity indices applied to terminal restriction fragment length polymorphism data: insights from simulated microbial communities. Appl. Environ. Microbiol. 73 (16), 5276–5283.

Blaud, A., Diouf, F., Herrmann, A.M., Lerch, T.Z., 2015. Analysing the effect of soil organic matter on bacterial communities using T-RFLP fingerprinting: different methods, different stories? Biol. Fertil. Soils 51, 959–971. https://doi.org/10.1007/s00374-015-1041-0.

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D. A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59. https://doi.org/10.1038/nmeth.2276.

Börjesson, G., Kirchmann, H., Kätterer, T., 2014. Four Swedish long-term field experiments with sewage sludge reveal a limited effect on soil microbes and on metal uptake by crops. J. Soils Sediments 14, 164–177. https://doi.org/10.1007/s11368-013-0800-5.

Bossio, D.A., Girvan, M.S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., Scow, K.M., Ball, A.S., Pretty, J.N., Osborn, A.M., 2005. Soil microbial community response to land use change in an agricultural landscape of Western Kenya. Microb. Ecol. 49, 50–62. https://doi.org/10.1007/s00248-003-0209-6.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421. https://doi.org/10.1186/1471-2105-10-421.

Changey, F., Blaud, A., Pando, A., Herrmann, A.M., Lerch, T.Z., 2021. Monitoring soil microbial communities using molecular tools: DNA extraction methods may offset long-term management effects. Eur. J. Soil Sci. 72, 1026–1041. https://doi.org/10.1111/ejss.13026.

de Oliveira, S.S., Ortega, J.C.G., Ribas, L.G.D.S., Lopes, V.G., Bini, L.M., 2020. Higher taxa are sufficient to represent biodiversity patterns. Ecol. Indic. 111, 105994.

De Vrieze, J., Pinto, A.J., Sloan, W.T., Ijaz, U.Z., 2018. The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. Microbiome 6, 63. https://doi.org/10.1186/s40168-018-0449-9.

Delmont, T.O., Prestat, E., Keegan, K.P., Faubladier, M., Robe, P., Clark, I.M., Pelletier, E., Hirsch, P.R., Meyer, F., Gilbert, J.A., Le Paslier, D., Simonet, P., Vogel, T.M., 2012. Structure, fluctuation and magnitude of a natural grassland soil metagenome. ISME J. 6, 1677–1687. https://doi.org/10.1038/ismej.2011.197.

Elsayed, O.F., Maillard, E., Vuilleumier, S., Imfeld, G., 2014. Bacterial communities in batch and continuous-flow wetlands treating the herbicide S-metolachlor. Sci. Total Environ. 499, 327–335. https://doi.org/10.1016/j.scitotenv.2014.08.048.

Enwall, K., Nyberg, K., Bertilsson, S., Cederlund, H., Stenström, J., Hallin, S., 2007. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. Soil Biol. Biochem. 39, 106–115. https://doi.org/10.1016/j.soilbio.2006.06.015.

Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., Maman, S., Hernandez-Raquet, G., Combes, S., Pascal, G., Berger, B., 2018. FROGS: Find, Rapidly, OTUs with Galaxy Solution. Bioinforma. Oxf. Engl. 34 (8), 1287–1294.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 103 (3), 626–631.

Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. Environ. Microbiol. 59, 3605–3617. https://doi.org/10.1128/aem.59.11.3605-3617.1993.

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621–1625. https://doi.org/10.1016/j.soilbio.2010.11.021.

Gao, G., Yin, D., Chen, S., Xia, F., Yang, J., Li, Q., Wang, W., Gilbert, J.A., 2012. Effect of biocontrol agent pseudomonas fluorescens 2P24 on soil fungal community in cucumber rhizosphere using T-RFLP and DGGE. PLOS ONE 7 (2), e31806.

Haegeman, B., Hamelin, J., Moriarty, J., Neal, P., Dushoff, J., Weitz, J.S., 2013. Robust estimation of microbial diversity in theory and in practice. ISME J. 7, 1092–1101. https://doi.org/10.1038/ismej.2013.10.

Hallin, S., Jones, C.M., Schloter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J. 3, 597–605. https://doi.org/10.1038/ismej.2008.128.

Herrmann, A.M., Witter, E., 2008. Predictors of gross N mineralization and immobilization during decomposition of stabilized organic matter in agricultural soil. Eur. J. Soil Sci. 59, 653–664. https://doi.org/10.1111/j.1365-2389.2008.01023.x.

Kennedy, A.C., Smith, K.L., 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170, 75–86. https://doi.org/10.1007/BF02183056.

Kirchmann, H., Gerzabek, M.H., 1999. Relationship between soil organic matter and micropores in a long-term experiment at Ultuna, Sweden. J. Plant Nutr. Soil Sci. 162, 493–498. https://doi.org/10.1002/(SICI)1522-2624(199910)162:5<493::AID-JPLN493>3.0.CO;2-S.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1. https://doi.org/10.1093/nar/gks808.

Lerch, T.Z., Dignac, M.-F., Nunan, N., Barriuso, E., Mariotti, A., 2009. Ageing processes and soil microbial community effects on the biodegradation of soil 13C–2,4-D nonextractable residues. Environ. Pollut. 157, 2985–2993. https://doi.org/10.1016/j.envpol.2009.05.058.

Lerch, T.Z., Coucheney, E., Herrmann, A.M., 2013. Sensitivity of soil microbial catabolic profiles to a gradient of carbon inputs: does the soil organic matter matter? Soil. Biol. Biochem. 57, 911–915. https://doi.org/10.1016/j.soilbio.2012.11.014.

Lindström, S., Rowe, O., Timonen, S., Sundström, L., Johansson, H., 2018. Trends in bacterial and fungal communities in ant nests observed with terminal-restriction fragment length polymorphism (T-RFLP) and next generation sequencing (NGS) techniques—validity and compatibility in ecological studies. PeerJ 6, e5289.

Magurran, A.E., 1988. Ecological Diversity and Its Measurement. Princeton University Press.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., Dunthorn, M., 2014. Swarm: robust and fast clustering method for amplicon-based studies. PeerJ 2, e593.

Malik, A.A., Martiny, J.B.H., Brodie, E.L., Martiny, A.C., Treseder, K.K., Allison, S.D., 2020. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. ISME J. 14, 1–9. https://doi.org/10.1038/s41396-019-0510-0.

Männistö, M.K., Tiirola, M., Häggblom, M.M., 2007. Bacterial communities in Arctic fjelds of Finnish Lapland are stable but highly pH-dependent. FEMS Microbiol. Ecol. 59, 452–465. https://doi.org/10.1111/j.1574-6941.2006.00232.x.

Marchesi, J.R., Sato, T., Weightman, A.J., Martin, T.A., Fry, J.C., Hiom, S.J., Wade, W.G., 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64 (2), 795–799.

Martiny, A.C., Treseder, K., Pusch, G., 2013. Phylogenetic conservatism of functional traits in microorganisms. ISME J. 7, 830–838. https://doi.org/10.1038/ismej.2012.160.

McMurdie, P.J., Holmes, S., Watson, M., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8 (4), e61217.

Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700. https://doi.org/10.1128/aem.59.3.695-700.1993.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670. https://doi.org/10.1046/j.1351-0754.2003.0556.x.

Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C., Marinari, S., 2012. Soil enzymology: classical and molecular approaches. Biol. Fertil. Soils 48, 743–762. https://doi.org/10.1007/s00374-012-0723-0.

Nayfach, S., Roux, S., Seshadri, R., Udwary, D., Varghese, N., Schulz, F., Wu, D., Paez-Espino, D., Chen, I.-M., Huntemann, M., Palaniappan, K., Ladau, J., Mukherjee, S., Reddy, T.B.K., Nielsen, T., Kirton, E., Faria, J.P., Edirisinghe, J.N., Henry, C.S., Jungbluth, S.P., Chivian, D., Dehal, P., Wood-Charlson, E.M., Arkin, A.P., Tringe, S. G., Visel, A., Abreu, H., Acinas, S.G., Allen, E., Allen, M.A., Alteio, L.V., Andersen, G., Anesio, A.M., Attwood, G., Avila-Magaña, V., Badis, Y., Bailey, J., Baker, B., Baldrian, P., Barton, H.A., Beck, D.A.C., Becraft, E.D., Beller, H.R., Beman, J.M., Bernier-Latmani, R., Berry, T.D., Bertagnolli, A., Bertilsson, S., Bhatnagar, J.M., Bird, J.T., Blanchard, J.L., Blumer-Schuette, S.E., Bohannan, B., Borton, M.A., Brady, A., Brawley, S.H., Brodie, J., Brown, S., Brum, J.R., Brune, A., Bryant, D.A., Buchan, A., Buckley, D.H., Buongiorno, J., Cadillo-Quiroz, H., Caffrey, S.M., Campbell, A.N., Campbell, B., Carr, S., Carroll, JoLynn, Cary, S.C., Cates, A.M., Cattolico, R.A., Cavicchioli, R., Chistoserdova, L., Coleman, M.L., Constant, P., Conway, J.M., Mac Cormack, W.P., Crowe, S., Crump, B., Currie, C., Daly, R., DeAngelis, K.M., Denef, V., Denman, S.E., Desta, A., Dionisi, H., Dodsworth, J., Dombrowski, N., Donohue, T., Dopson, M., Driscoll, T., Dunfield, P., Dupont, C.L., Dynarski, K.A., Edgcomb, V., Edwards, E.A., Elshahed, M.S., Figueroa, I., Flood, B., Fortney, N., Fortunato, C.S., Francis, C., Gachon, C.M.M., Garcia, S.L., Gazitua, M.C.,

Gentry, T., Gerwick, L., Gharechahi, J., Girguis, P., Gladden, J., Gradoville, M., Grasby, S.E., Gravuer, K., Grettenberger, C.L., Gruninger, R.J., Guo, J., Habteselassie, M.Y., Hallam, S.J., Hatzenpichler, R., Hausmann, B., Hazen, T.C., Hedlund, B., Henny, C., Herfort, L., Hernandez, M., Hershey, O.S., Hess, M., Hollister, E.B., Hug, L.A., Hunt, D., Jansson, J., Jarett, J., Kadnikov, V.V., Kelly, C., Kelly, R., Kelly, W., Kerfeld, C.A., Kimbrel, J., Klassen, J.L., Konstantinidis, K.T., Lee, L.L., Li, W.-J., Loder, A.J., Loy, A., Lozada, M., MacGregor, B., Magnabosco, C., Maria da Silva, A., McKay, R.M., McMahon, K., McSweeney, C.S., Medina, M., Meredith, L., Mizzi, J., Mock, T., Momper, L., Moran, M.A., Morgan-Lang, C., Moser, D., Muyzer, G., Myrold, D., Nash, M., Nesbø, C.L., Neumann, A.P., Neumann, R.B., Noguera, D., Northen, T., Norton, J., Nowinski, B., Nüsslein, K., O'Malley, M.A., Oliveira, R.S., Maia de Oliveira, V., Onstott, T., Osvatic, J., Ouyang, Y., Pachiadaki, M., Parnell, J., Partida-Martinez, L.P., Peay, K.G., Pelletier, D., Peng, X., Pester, M., Pett-Ridge, J., Peura, S., Pjevac, P., Plominsky, A. M., Poehlein, A., Pope, P.B., Ravin, N., Redmond, M.C., Reiss, R., Rich, V., Rinke, C., Rodrigues, J.L.M., Rodriguez-Reillo, W., Rossmassler, K., Sackett, J., Salekdeh, G.H., Saleska, S., Scarborough, M., Schachtman, D., Schadt, C.W., Schrenk, M., Sczyrba, A., Sengupta, A., Setubal, J.C., Shade, A., Sharp, C., Sherman, D.H., Shubenkova, O.V., Sierra-Garcia, I.N., Simister, R., Simon, H., Sjöling, S., Slonczewski, J., Correa de Souza, R.S., Spear, J.R., Stegen, J.C., Stepanauskas, R., Stewart, F., Suen, G., Sullivan, M., Sumner, D., Swan, B.K., Swingley, W., Tarn, J., Taylor, G.T., Teeling, H., Tekere, M., Teske, A., Thomas, T., Thrash, C., Tiedje, J., Ting, C.S., Tully, B., Tyson, G., Ulloa, O., Valentine, D.L., Van Goethem, M.W., VanderGheynst, J., Verbeke, T.J., Vollmers, J., Vuillemin, A., Waldo, N.B., Walsh, D. A., Weimer, B.C., Whitman, T., van der Wielen, P., Wilkins, M., Williams, T.J., Woodcroft, B., Woolet, J., Wrighton, K., Ye, J., Young, E.B., Youssef, N.H., Yu, F.B., Zemskaya, T.I., Ziels, R., Woyke, T., Mouncey, N.J., Ivanova, N.N., Kyrpides, N.C., Eloe-Fadrosh, E.A., 2021. A genomic catalog of Earth's microbiomes. Nat. Biotechnol. 39 (4), 499–509.

Nunan, N., Daniell, T.J., Singh, B.K., Papert, A., McNicol, J.W., Prosser, J.I., 2005. Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. Appl. Environ. Microbiol. 71, 6784–6792. https://doi.org/10.1128/AEM.71.11.6784-6792.2005.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2011. Community ecology package. R package version 2.0-2.

Orwin, K.H., Dickie, I.A., Holdaway, R., Wood, J.R., 2018. A comparison of the ability of PLFA and 16S rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions. Soil Biol. Biochem. 117, 27–35. https://doi.org/10.1016/j.soilbio.2017.10.036.

Osborn, A.M., Moore, E.R.B., Timmis, K.N., 2000. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ. Microbiol. 2, 39–50. https://doi.org/10.1046/j.1462-2920.2000.00081.x.

Parte, A.C., 2018. LPSN – List of Prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. Int. J. Syst. Evol. Microbiol. 68 (6), 1825–1829.

Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nat. Rev. Microbiol. 8, 523–529. https://doi.org/10.1038/nrmicro2367.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. https://doi.org/10.1093/nar/gkm864.

Ranjard, L., Poly, F., Nazaret, S., 2000. Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. Res. Microbiol. 151, 167–177. https://doi.org/10.1016/S0923-2508(00)00136-4.

Ritz, K., McNicol, J.W., Nunan, N., Grayston, S., Millard, P., Atkinson, D., Gollotte, A., Habeshaw, D., Boag, B., Clegg, C.D., Griffiths, B.S., Wheatley, R.E., Glover, L.A., McCaig, A.E., Prosser, J.I., 2004. Spatial structure in soil chemical and microbiological properties in an upland grassland. FEMS Microbiol. Ecol. 49, 191–205. https://doi.org/10.1016/j.femsec.2004.03.005.

Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A., Wood, C., 2009. Selecting biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development. Ecol. Indic. 9, 1212–1221. https://doi.org/10.1016/j.ecolind.2009.02.009.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.

Romillac, N., Santorufo, L., 2021. Transferring concepts from plant to microbial ecology: A framework proposal to identify relevant bacterial functional traits. Soil Biol. Biochem. 162, 108415 https://doi.org/10.1016/j.soilbio.2021.108415.

Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P., Parkhill, J., Loman, N.J., Walker, A.W., 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 12, 87. https://doi.org/10.1186/s12915-014-0087-z.

Schloter, M., Nannipieri, P., Sørensen, S.J., van Elsas, J.D., 2018. Microbial indicators for soil quality. Biol. Fertil. Soils 54, 1–10. https://doi.org/10.1007/s00374-017-1248-3.

Smalla, K., Oros-Sichler, M., Milling, A., Heuer, H., Baumgarte, S., Becker, R., Neuber, G., Kropf, S., Ulrich, A., Tebbe, C.C., 2007. Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: do the different methods provide similar results? J. Microbiol. Methods 69, 470–479. https://doi.org/10.1016/j.mimet.2007.02.014.

Stagnari, F., Perpetuini, G., Tofalo, R., Campanelli, G., Leteo, F., Della Vella, U., Schirone, M., Suzzi, G., Pisante, M., 2014. Long-term impact of farm management and crops on soil microorganisms assessed by combined DGGE and PLFA analyses. Front. Microbiol. 5, 644. https://doi.org/10.3389/fmicb.2014.00644.

Stone, D., Ritz, K., Griffiths, B.G., Orgiazzi, A., Creamer, R.E., 2016. Selection of biological indicators appropriate for European soil monitoring. Soil Biodivers. Ecosyst. Funct. Eur. Transect Cover. Var. Bio-Geogr. Zones Land Use Soil Prop. 97, 12–22. https://doi.org/10.1016/j.apsoil.2015.08.005.

Turpeinen, R., Kairesalo, T., Häggblom, M.M., 2004. Microbial community structure and activity in arsenic-, chromium- and copper-contaminated soils. FEMS Microbiol. Ecol. 47, 39–50. https://doi.org/10.1016/S0168-6496(03)00232-0.

van Dorst, J., Siciliano, S.D., Winsley, T., Snape, I., Ferrari, B.C., Kostka, J.E., 2014. Bacterial targets as potential indicators of diesel fuel toxicity in subantarctic soils. Appl. Environ. Microbiol. 80 (13), 4021–4033.

Vestergaard, G., Schulz, S., Schöler, A., Schloter, M., 2017. Making big data smart—how to use metagenomics to understand soil quality. Biol. Fertil. Soils 53, 479–484. https://doi.org/10.1007/s00374-017-1191-3.

Vilmi, A., Karjalainen, S.M., Nokela, T., Tolonen, K., Heino, J., 2016. Unravelling the drivers of aquatic communities using disparate organismal groups and different taxonomic levels. Ecol. Indic. 60, 108–118. https://doi.org/10.1016/j.ecolind.2015.06.023.

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G.A., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nat. Commun. 10, 4841. https://doi.org/10.1038/s41467-019-12798-y.

Wang, W., Niu, J., Zhou, X., Wang, Y., 2011. Long-term change in land management from subtropical wetland to paddy field shifts soil microbial community structure as determined by PLFA and T-RFLP. Pol. J. Ecol. 59.

Widmer, F., Rasche, F., Hartmann, M., Fliessbach, A., 2006. Community structures and substrate utilization of bacteria in soils from organic and conventional farming systems of the DOK long-term field experiment. Appl. Soil Ecol. 33, 294–307. https://doi.org/10.1016/j.apsoil.2005.09.007.

Wiesmeier, M., Urbanski, L., Hobley, E., Lang, B., von Lützow, M., Marin-Spiotta, E., van Wesemael, B., Rabot, E., Ließ, M., Garcia-Franco, N., Wollschläger, U., Vogel, H.-J., Kögel-Knabner, I., 2019. Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. Geoderma 333, 149–162. https://doi.org/10.1016/j.geoderma.2018.07.026.

Witter, E., 1996. Soil C balance in a long-term field experiment in relation to the size of the microbial biomass. Biol. Fertil. Soils 23, 33–37. https://doi.org/10.1007/BF00335815.

Witter, E., Dahlin, S., 1995. Microbial utilization of [U 14C]-labelled straw and [U 13C]-labelled glucose in soils of contrasting pH and metal status. Soil Biol. Biochem. 27, 1507–1516. https://doi.org/10.1016/0038-0717(95)00107-P.

Wixon, D.L., Balser, T.C., 2013. Toward conceptual clarity: PLFA in warmed soils. Soil Biol. Biochem. 57, 769–774. https://doi.org/10.1016/j.soilbio.2012.08.016.

Yeh, C.-F., Soininen, J., Teittinen, A., Wang, J., 2019. Elevational patterns and hierarchical determinants of biodiversity across microbial taxonomic scales. Mol. Ecol. 28, 86–99. https://doi.org/10.1111/mec.14935.

Ying, J.-Y., Zhang, L.-M., Wei, W.-X., He, J.-Z., 2013. Effects of land utilization patterns on soil microbial communities in an acid red soil based on DNA and PLFA analyses. J. Soils Sediments 13, 1223–1231. https://doi.org/10.1007/s11368-013-0704-4.

Zhalnina, K., Dias, R., de Quadros, P.D., Davis-Richardson, A., Camargo, F.A.O., Clark, I. M., McGrath, S.P., Hirsch, P.R., Triplett, E.W., 2015. Soil pH determines microbial diversity and composition in the park grass experiment. Microb. Ecol. 69, 395–406. https://doi.org/10.1007/s00248-014-0530-2.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinforma. Oxf. Engl. 30, 614–620. https://doi.org/10.1093/bioinformatics/btt593.

Zinger, L., Bonin, A., Alsos, I.G., Bálint, M., Bik, H., Boyer, F., Chariton, A.A., Creer, S., Coissac, E., Deagle, B.E., De Barba, M., Dickie, I.A., Dumbrell, A.J., Ficetola, G.F., Fierer, N., Fumagalli, L., Gilbert, M.T.P., Jarman, S., Jumpponen, A., Kauserud, H., Orlando, L., Pansu, J., Pawlowski, J., Tedersoo, L., Thomsen, P.F., Willerslev, E., Taberlet, P., 2019. DNA metabarcoding—need for robust experimental designs to draw sound ecological conclusions. Mol. Ecol. 28, 1857–1862. https://doi.org/10.1111/mec.15060.