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Research Article

RESEARCH ARTICLE

Distinct rhizomicrobiota assemblages and plant performance in lettuce grown in soils with different agricultural management histories

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One sentence summary: Long-term tillage, fertilization intensity and cropping history shaped the soil and rhizosphere microbiota assemblage of the model plant lettuce with implications for plant growth and health.

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ABSTRACT

A better understanding of factors shaping the rhizosphere microbiota is important for sustainable crop production. We hypothesized that the effect of agricultural management on the soil microbiota is reflected in the assemblage of the rhizosphere microbiota with implications for plant performance. We designed a growth chamber experiment growing the

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model plant lettuce under controlled conditions in soils of a long-term field experiment with contrasting histories of tillage (mouldboard plough *vs* cultivator tillage), fertilization intensity (intensive standard nitrogen (N) + pesticides/growth regulators *vs* extensive reduced N without fungicides/growth regulators), and last standing field crop (rapeseed *vs* winter wheat). High-throughput sequencing of bacterial and archaeal 16S rRNA genes and fungal ITS2 regions amplified from total community DNA showed that these factors shaped the soil and rhizosphere microbiota of lettuce, however, to different extents among the microbial domains. *Pseudomonas* and *Olpidium* were identified as major indicators for agricultural management in the rhizosphere of lettuce. Long-term extensive fertilization history of soils resulted in higher lettuce growth and increased expression of genes involved in plant stress responses compared to intensive fertilization. Our work adds to the increasing knowledge on how soil microbiota can be manipulated by agricultural management practices which could be harnessed for sustainable crop production.

Keywords: tillage; fertilization; crop rotation; 16S rRNA gene; ITS region; plant gene expression

INTRODUCTION

Soil is one of the most important natural resources and provider of ecosystem functions. Around 95% of food is directly or indirectly produced on soils, highlighting the importance of soil for food production (FAO 2015). Different anthropogenic activities such as improper land use, pollution or global climate change are causing extensive soil degradation worldwide (Smith *et al.* 2016). Currently, 23% of earth's terrestrial area faces land degradation, increasing at an annual rate of 5–10 million ha and jeopardizing food security for ca. 1.5 billion people (Stavi and Lal 2015). Intensive high-input agriculture increased productivity, but has resulted in a number of negative impacts on soil quality, such as loss of soil organic carbon (Steinmann *et al.* 2016; Sanderman, Hengl and Fiske 2017), loss of soil biodiversity (Wagg *et al.* 2014; Tsiafouli *et al.* 2015; Rutgers *et al.* 2019), loss of fertility, erosion (Smith *et al.* 2016) as well as accumulation of pathogens and pesticides (Foley *et al.* 2005; Brevik and Burgess 2014). Low-input agricultural practices like organic farming (Birkhofer *et al.* 2008), reduced tillage (Hobbs, Sayre and Gupta 2008), wide crop rotation (Tilman *et al.* 2002) and reduced mineral fertilization (Williams, Börjesson and Hedlund 2013) can mitigate the negative effects of high-input farming and contribute to more sustainable plant production. However, low-input agricultural systems are still not competitive when it comes to yield or yield stability compared to conventional intensive farming (Pittelkow *et al.* 2015; Kravchenko, Snapp and Robertson 2017; Knapp and van der Heijden 2018). Therefore, proper stewardship is required to ensure food security while maintaining or restoring natural soil resources.

Soil microbes play a pivotal role for maintaining soil functions (Brussaard *et al.* 1997; Berendsen, Pieterse and Bakker 2012). Agricultural practices affect the structural and functional composition of the soil microbiota directly or indirectly via altering physico-chemical soil properties (Figuerola *et al.* 2012; de Vries *et al.* 2015; Figuerola *et al.* 2015; Francioli *et al.* 2016; Hartman *et al.* 2018; Sommermann *et al.* 2018; Babin *et al.* 2019; Banerjee *et al.* 2019). Plants recruit their rhizosphere microbiota from adjacent soil via root exudates (rhizodeposits) (Berg and Smalla 2009; Philippot *et al.* 2013). It was estimated that plants invest ca. 11% of their photosynthetically fixed carbon and 10%–16% of total plant nitrogen in the rhizosphere to attract or repel soil microorganisms (Jones, Nguyen and Finlay 2009). Many processes with direct effects on plant performance are governed in the rhizosphere, demonstrating its utmost importance for agricultural plant production. The rhizosphere microbiota is more and more regarded as an additional plant genetic resource stimulating or activating plant traits with functions in plant nutrition, growth and pathogen defense (Lau and Lennon 2011; Berendsen, Pieterse and Bakker 2012; Mendes, Garbeva and Raaijmakers 2013; Berg *et al.* 2014; Panke-Buisse *et al.* 2015; Vandenkoornhuyse *et al.* 2015).

It has recently been postulated that the agricultural soil legacy or soil memory is conveyed to the next plant generation along with other factors via soil microbial communities (Lapsansky *et al.* 2016; Bakker *et al.* 2018). In line with this, Chowdhury *et al.* (2019) showed that long-term fertilization practices (organic *vs* mineral) affected not only the soil microbiota but also influenced the associated rhizosphere microbiota assemblage. Gene expression data obtained from lettuce indicated that such fertilization-dependent differences in the rhizomicrobiota might have implications for plant-microbe-interactions and thus for plant growth and health (Chowdhury *et al.* 2019). According to the plant-soil-feedback concept, the plant affects the soil and *vice versa* which might be altered by agricultural management practices (van der Putten *et al.* 2016).

Over the last years there has been a rising awareness of the untapped potential of the indigenous soil microbiota, and their exploitation for sustainable agriculture has been suggested (Bender, Wagg and van der Heijden 2016; Berg *et al.* 2017; Das, Ho and Kim 2019; Wall *et al.* 2019). This necessitates an improved understanding on how agricultural practices affect the soil, rhizosphere microbiota and plant health in order to benefit from the potential of the microbiota. This can ideally be studied with soils from long-term field experiments (LTEs). In the present study we used soils from a LTE established in 1992 in Bernburg, Germany (Deubel, Hofmann and Orzessek 2011). This LTE facilitates the study of the effects of tillage practice, fertilization intensity and cropping history. The effect of tillage can be assessed by comparing conventional mouldboard plough (MP) *vs* cultivator tillage (CT). Two contrasting fertilization intensities are implemented, i.e. intensive nitrogen (N)-fertilization including required pesticide/growth regulator application (Int) *vs* extensive N-fertilization without fungicide/growth regulator usage (Ext). The effect of the standing field crop can be addressed due to a crop rotation within five adjacent fields (maize-wheat-barley-rapeseed-wheat). Previous studies of this LTE showed that the soil microbiota composition was significantly affected by the preceding crop and tillage practice, while the fertilization intensity had only minor effects (Sommermann *et al.* 2018; Babin *et al.* 2019). In the current study, we established a growth chamber experiment with soils collected from this LTE and investigated to which extent these factors affect the rhizosphere microbiota assemblage and performance of lettuce grown under growth chamber conditions in these soils. We hypothesized that (i) tillage practice, fertilization intensity and the last standing field crop affect the soil microbiota and this would be reflected in the assemblage of the rhizosphere microbiota; (ii) plant growth

and performance depend on the long-term agricultural management. We conducted the experiment with the model plant lettuce (*Lactuca sativa* L., cv. Tizian), which was never planted at the LTE site and shows high sensitivity towards soil biotic and abiotic stresses. We present a multidisciplinary approach consisting of microbiota analysis via high-throughput amplicon sequencing, RNA-based lettuce gene expression studies, analyses of macro- and micro-nutrient content in soils and plants as well as plant biomass assessments.

MATERIALS AND METHODS

Site conditions and soil sampling

To assess the impact of agricultural practices on the soil and rhizosphere microbiota as well as on lettuce performance, a growth chamber experiment was established with soils originating from the LTE in Bernburg (Germany; 51.82°N, 11.70°E). The field soil was classified as loess chernozem over limestone (8% sand, 70% silt, 22% clay) with a neutral to slightly alkaline pH. Further characteristics of the Bernburg LTE site were previously published (Deubel, Hofmann and Orzessek 2011; Sommermann *et al*. 2018; Babin *et al*. 2019). Briefly, the LTE is composed of five 1.2 ha-sized plots which are divided into four replicate subplots. The plots are managed under a yearly crop rotation of grain maize (*Zea mays*), winter wheat 1 (*Triticum aestivum*), winter barley (*Hordeum vulgare*), winter rapeseed (*Brassica napus* ssp. *napus*) and winter wheat 2 (*Triticum aestivum*). Each plot allows the comparison of conventional MP (20-30 cm ploughing depth, soil inversion) with conservation CT (12-15 cm depth, flat soil loosening). Within the MP and CT stripes, the soils are managed by two contrasting fertilization intensities which is either intensive N-fertilization and pesticide/growth regulator application according to usual farming practice (Int; 220 kg N ha^{-1} or 260 kg N ha^{-1} for wheat or rapeseed, respectively) or reduced, extensive N-fertilization without addition of fungicides and growth regulators (Ext; 90 kg N ha^{-1} or 100 kg N ha^{-1} for wheat or rapeseed, respectively).

Soils from each management practice were sampled after harvest from the plots with the last standing crops winter wheat (W; cv. Dichter) or rapeseed (R; cv. SY Saveo) in September 2016. This resulted in the following eight management treatments used for lettuce cultivation in the growth chamber (York, Mannheim, Germany): MP-Int-W, MP-Ext-W, CT-Int-W, CT-Ext-W, MP-Int-R, MP-Ext-R, CT-Int-R, CT-Ext-R. For each treatment, 15 soil cores (0-30 cm depth), sampled randomly across the respective total field plot area, were combined. Subsequently, the soils were air-dried and sieved (4 mm mesh-size).

Design of the growth chamber experiment

Prior to the start of the growth chamber experiment, the collected soils were incubated at the intended cultivation conditions for lettuce for two weeks in the dark (20°C/15°C, 60%/80% relative humidity day/night). The water potential of each soil was adjusted to 100 hPa (T5 tensiometer, UMS AG, Munich, Germany). Lettuce seeds (*Lactuca sativa* L., cv. Tizian; Syngenta, Bad Salzuflen, Germany) were sown in each soil and incubated at 18°C and 80% relative humidity for two days. Afterwards the seedlings were grown in a growth chamber (20°C/15°C, 420 μmol m^{-2} s^{-1} photosynthetic active radiation, 60%/80% relative humidity, 100 hPa water potential, 16 h/8 h day/night). At BBCH13 to BBCH14 plantlets were transferred into single pots (10 × 10 × 11 cm) containing the respective soils.

The amount of available N was adjusted to the recommendations for lettuce cultivation (0.32 g N/pot) by adding calcium nitrate in two portions during planting of seedlings (50%) and two weeks later (remaining 50%). Each treatment was composed of four replicates with four plants per replicate arranged in a randomized block design. Each treatment included four additional pots without plants that were incubated under the same conditions for bulk soil (BS) analysis.

Sampling of the growth chamber experiment

Sampling of plants and soils was conducted after 10 weeks of lettuce cultivation (BBCH19). Shoot and root fresh masses were recorded. The macro- and micro-nutrient content in BS and in the plant shoots were analyzed after harvest according to the certified protocols of Agricultural Analytic and Research Institutions Association (VDLUFA, Germany).

Root-associated soil (RA), defined as soil loosely adhering to roots, was sampled from two planted pots per replicate by vigorous shaking of roots. From the same two plants per replicate, complete root systems were pooled for rhizosphere (RH) microbial community analysis. After briefly washing roots in sterile tap water (Schreiter *et al*. 2014b), microbial cells were obtained from up to 5 g of roots by three times 1 min Stomacher 400 Circulator (Seward Ltd, Worthing, UK) treatment at high speed followed by centrifugation according to Schreiter *et al*. (2014a). Samples from BS, RA and RH were kept at −20°C until total microbial community (TC)-DNA extraction. For qPCR-based plant gene expression studies, four leaves (from outer to inner whorls) of the same two plants used for RH analysis were pooled and snap frozen in liquid nitrogen.

Plant gene expression analysis

A total of 20 target genes was selected from the lettuce (cv. Tizian) draft genome at NCBI (Verwaaijen *et al*. 2018) based on the comparison with functional genes involved in oxidative and biotic stress regulation pathways from *Arabidopsis thaliana* using 'The Arabidopsis Information Resource' (www.arabidopsis.org, Berardini *et al*. 2015). All genes and their primer pairs used in this study have been described in Chowdhury *et al*. (2019). The reference gene glyceraldehyde-3-dehydrogenase (*gadph*) was used as an endogenous control in qPCR analyses. The target and endogenous control genes were validated and only primers with 100% (±10%) amplification efficiencies were used for further analyses following the MIQE guidelines (Bustin *et al*. 2009). The genes, their putative functions and primer pairs used in this study are described in Table S1 (Supporting Information).

The RNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) was used to extract RNA from 100 mg pulverized lettuce leaves. After quantification of RNA by NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), cDNA was synthesized from 2 μg total RNA with the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Foster City, CA, USA). The qPCR was performed with Power SYBR Green Supermix (Applied Biosystems, Foster City, CA, USA) using a peqSTAR 96Q thermal cycler (PEQLAB Biotechnologie, Erlangen, Germany). cDNA dilutions (1 μL, 1:4) were used as PCR templates. Each PCR reaction contained 12.5 μL of 2 × Power SYBR Green Supermix, 0.4 μM primers (Eurofins MVG Operon, Ebersberg, Germany), and 1 μL of template in a 25 μL reaction volume. PCR mixtures were heated to 95°C for 3 min and then for 40 cycles with steps of 95°C for 30 s and 60°C for 60s. Specific PCR products were confirmed by melting curve analysis and

gel electrophoresis prior to relative quantification by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Data were first normalized to the endogenous control and logarithmically transformed to fold change differences. The standard error of the mean was calculated from the average of four biological replicates.

TC-DNA extraction and amplicon sequencing

TC-DNA was extracted from 0.5 g (fresh mass) of BS or RA, respectively, and from total RH pellets using the FastPrep-24 bead-beating system and FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following manufacturer's recommendations. TC-DNAs were further purified with the GeneClean Spin Kit (MP Biomedicals, Santa Ana, CA, USA). The analysis of bacterial and archaeal communities was reduced to RH and BS since previous data showed no differences between RA and BS (data not shown). The V3-V4 regions of bacterial and archaeal 16S rRNA genes were amplified from BS and RH using the primer pair 341F/806R (Caporaso *et al.* 2011; Sundberg *et al.* 2013; modified after Yu *et al.* 2005). PCRs were performed in 25 μL volumes containing 0.625 U Hot Start Taq Polymerase (New England Biolabs GmbH, Frankfurt am Main, Germany), 1 × Standard Taq Reaction buffer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 0.4 μM of each primer and 1 μL of target DNA. Bovine serum albumin (final concentration 0.1 mg mL^{-1}) was added optionally. PCR conditions were used as previously described (Chowdhury *et al.* 2019). In a second PCR step the primers additionally included Illumina specific sequencing adapters and a unique combination of indexes for each sample. Resulting amplicons were purified using HighPrep™ PCR Clean Up System (AC-60500, MagBio Genomics Inc., Gaithersburg, MD, USA) using a 0.65:1 (beads: PCR volume) ratio to remove DNA fragments below 100 bp in size. Samples were normalized equimolar using SequalPrep Normalization Plate (96) Kit (Invitrogen, Carlsbad, CA, USA) and pooled using 5 μL of each sample. The final pool volume was concentrated using DNA Clean and Concentrator™-5 kit (Zymo Research, Irvine, CA, USA) and adjusted to 4 nM. High-throughput amplicon sequencing of the pooled library was carried out on an Illumina® MiSeq® platform (Illumina, San Diego, CA, USA) using MiSeq v2 sequencing kit (2 × 250 bp, paired-end) following manufacturer's instructions.

Fungal communities were characterized by high-throughput sequencing of the internal transcribed spacer (ITS2) region amplified from BS, RA and RH TC-DNAs. The PCR conditions were as previously described (Sommermann *et al.* 2018). In brief, three PCRs per sample were conducted at different annealing temperatures (54°C, 56°C, 58°C). The number of cycles was set to 24 (midpoint of exponential phase) and 10 ng template DNA was used. Especially for some rhizosphere samples (MP-Ext-W, CT-Int-R and MP-Int-R), cycles and annealing time were increased to 28 and 35 s (instead of 25 s), respectively, to obtain sufficient amplicon amounts. Due to extremely low DNA concentrations, three of four replicates of RH samples of MP-Int-W and CT-Int-W had to be pooled to obtain at least two samples for each treatment. The sample CT-Ext-W-RH3 could not be analyzed because of low DNA amounts. Bovine serum albumin (final concentration 0.5 mg mL^{-1}) was added optionally. Subsequently, amplicons of the same treatment were pooled and purified using the MinElute PCR Purification Kit (QIAGEN, Hilden, Germany) and eluted in 12 μL 10 mM Tris-HCl, pH 8.5. The concentration of each sample was determined, and amplicons were pooled to equimolar amounts.

The ITS2 amplicon pool was further processed as recently described with a few modifications (Sommermann *et al.* 2018). In brief, after quality control and library preparation, sequencing was carried out on the Illumina® MiSeq® platform (ca. 25% of an Illumina flow cell) in paired-end mode (2 × 300 bp).

Raw sequence data were submitted to NCBI Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA659405 (16S rRNA gene) and PRJEB40328 (ITS2).

Processing and clustering of DNA sequences

For 16S rRNA genes, primer sequences used in first PCR were trimmed using cutadapt (Martin 2011) and only read pairs containing both primers were retained for subsequent analysis. Primer trimmed sequences were then merged, clustered in OTUs using UPARSE-OTU algorithm (Edgar 2013) with a 97% sequence similarity threshold. Representative OTUs were taxonomically classified using the RDP14 trainset (http://rdp.cme.msu.edu/) and mothur commands (Schloss *et al.* 2009; confidence threshold 80%). After removing Cyanobacteria/Chloroplasts, mitochondrial or unclassified sequences (kingdom level), a total of 7316 bacterial and archaeal OTUs with an average of 25 471 quality 16S rRNA gene sequences per sample were obtained. One replicate of the rhizosphere of CT-Ext-W was not considered for further analysis due to low sequence amount.

For fungal ITS sequences, barcode, primer and adapter trimming were performed based on a customized in-house perl script including the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). This was followed by raw sequence merging using FLASH (Magoč and Salzberg 2011). Furthermore, the resulting sequences were analyzed with a local version of the GALAXY Bioinformatics Platform (https://galaxyproject.org) based on a database-dependent strategy (Antweiler *et al.* 2017) using UNITE database v8.0 (UNITE Community 2019; Kõljalg *et al.* 2005) by applying the closed reference approach (Carter *et al.* 2017). All sequences were aligned with the database (e-value $\leq$ 0.001) and only results with an alignment length $>$ 200 bp were kept. Sequences with $<$ 97% similarity to the reference database were removed. Finally, BLAST-PARSER (Antweiler *et al.* 2017) was used for taxonomic assignment based on the lowest e-value. The sequences per assignment were counted and the OTU abundance table was generated by using the SH-number from the database as identifier. An average of 64 155 ITS sequences per sample and a total of 1296 fungal OTUs were obtained.

Statistical analyses

Multivariate analyses were carried out in R (R Core Team 2019) using the following packages *agricolae* (de Mendiburu 2017), *vegan* (Oksanen *et al.* 2019), *phyloseq* (McMurdie and Holmes 2013), *RColorBrewer* (Neuwirth 2014), *indicspecies* (de Cáceres and Legendre 2009), *data.table* (Dowle 2020), *ggplot2* (Wickham 2016), *pheatmap* (Kolde 2019), *dplyr* (Wickham *et al.* 2019), *MASS* (Venables and Ripley 2002), *rcompanion* (Mangiafico 2020), *car* (Fox *et al.* 2020), *emmeans* (Lenth 2020), *stats* (R Core Team 2019). The data set used for alpha-diversity analysis (species richness, Shannon, Chao-1, Pielou's evenness) was randomly subsampled to the lowest amount of reads, i.e. 6489 for 16S rRNA gene and 31 830 for ITS datasets. This was repeated 100 times and diversity indices were averaged. Effects of microhabitat (BS, RA and RH), tillage practice, last standing field crop and fertilization intensity on the microbial community composition were tested by PERMANOVA analysis (Bray-Curtis dissimilarity, 10 000 permutations) based on count data. Ordination of microbial communities was carried out by non-metric multidimensional scaling (NMDS;

Bray-Curtis dissimilarity) based on count data. Heatmaps of the 30 most abundant microbial genera were created based on relative abundances (Euclidean distance clustering). In order to identify treatment-specific microbial taxa in the different microhabitats, OTU indicator analyses were conducted with the multipatt function (option = 'r.g', 10 000 permutations) from the package 'indicspecies' followed by Benjamini-Hochberg correction for multiple testing ($P \leq 0.05$). Shoot and root dry masses, physicochemical properties of BS samples, nutritional status of lettuce shoots, microbial alpha-diversity indices, and relative abundances at phylum or class level were tested separately for each microhabitat for the effect of tillage practice, fertilization intensity and last standing field crop by applying three-way analysis of variance (ANOVA) followed by post-hoc Tukey's HSD ($P \leq 0.05$). If ANOVA assumptions failed, data was transformed by Tukey's Ladder of Powers approach. Plant gene expression data was analyzed by PERMANOVA (10 000 permutations) based on Bray-Curtis dissimilarity calculated from ΔCt values. To compare which genes were differentially expressed in Ext compared to Int soils in both W and R, one-way ANOVA with pairwise post-hoc Tukey's HSD tests ($P \leq 0.05$) were performed. To connect the observed plant gene expression pattern to the observed relative abundance of microorganisms in the RH, a distance-based redundancy analysis (dbRDA, Bray-Curtis distance) was carried out with the expression of 20 selected lettuce genes (ΔCt values). The relative abundances of the 50 most abundant bacterial and archaeal OTUs in RH were controlled for linear dependencies. The contribution of OTU_11818, OTU_54, OTU_35, OTU_10235, OTU_105, OTU_11657, OTU_8493, OTU_51, OTU_4945, OTU_2742, OTU_8426, OTU_122 and OTU_329 to the plant gene expression was tested and fitted on the dbRDA ordination by the 'Envfit' function at 999 permutations.

RESULTS

Fertilization intensity affected fungal but not bacterial and archaeal diversity

In order to investigate whether tillage practice, fertilization intensity and last standing field crop affected the microbial diversity in our experiment, different alpha-diversity estimates (Pielou, Shannon, species richness, Chao-1) were calculated for BS, RH (16S rRNA gene) and for BS, RA and RH (ITS; Tables S2, S3, Supporting Information). A significant influence of the tillage practice and the last standing crop was found on the bacterial and archaeal diversity in both BS and RH. Treatment-dependent differences were more pronounced in RH revealing also significant interaction effects (Table S2A, Supporting Information). Regarding the RH of individual treatments, a significantly higher bacterial and archaeal alpha-diversity was observed for lettuce grown in MP compared to respective CT soils except for Ext-W (Fig. 1A; Table S2B, Supporting Information). BS of MP-Int-R had the lowest and BS of CT-Ext-W the highest richness and Chao-1 indices (Table S2B, Supporting Information).

In contrast to bacteria and archaea, the fungal diversity was tignificantly affected by fertilization intensity across the different microhabitats (Table S3, Supporting Information). The effects of the last standing field crop and tillage practice were mainly detected in RH. Significant interaction effects between factors were only observed in BS for richness and Chao-1 as well as in the RH for Pielou and Shannon (Table S3A, Supporting Information). When looking at the individual treatments, alpha-diversity indices of fungal communities in terms of Pielou, Shannon and Chao-1 were similar in BS and RA (Table S3B, Supporting Information). The Chao-1 index was significantly higher in CT-Ext-R compared to CT-Int-R in BS. Regarding Pielou's and Shannon indices, fungal alpha-diversity in RH was significantly reduced when lettuce was grown in extensively fertilized soils especially with rapeseed as last standing field crop (Table S3B, Supporting Information). Fungal richness differed significantly between treatments in each microhabitat (Fig. 1B, Table S3B, Supporting Information). In both BS and RA, the treatments CT-Ext-R and CT-Ext-W exhibited the highest fungal richness. Richness was low in MP-Int-W when considering BS and RA but showed the highest value in RH. Fungal communities in RH of lettuce grown in MP-Ext-R soils exhibited a significantly reduced richness compared to other treatments.

In summary, alpha-diversity of the bacterial and archaeal community was mainly affected in RH by the factors last standing field crop and tillage practice, while fungal alpha-diversity was influenced by fertilization intensity irrespective of the microhabitat.

Differential effects of tillage practice, fertilization intensity and last standing field crop on soil and rhizosphere microbial community composition of lettuce

Due to the observed effects of tillage practice, fertilization intensity and last standing field crop on alpha-diversity, we elucidated also the changes between microbial community compositions in the different microhabitats and treatments (beta-diversity). PERMANOVA analysis revealed that microbial communities differed significantly between microhabitats ($R^2 = 45\%$ for bacteria and archaea, $R^2 = 39\%$ for fungi; both $P < 0.001$). Therefore, microbial community compositions were analyzed separately per microhabitat (BS and RH for bacterial and archaeal, RA and RH for fungal communities). According to PERMANOVA analysis (Table 1A), the last standing field crop and to lesser extents tillage practice and interaction between both factors had a significant effect on the bacterial and archaeal community composition in BS. The RH bacterial and archaeal community composition was shaped by all the factors studied as well as by the interactions between them. In contrast to BS, tillage practice explained most of the observed variance among RH bacterial and archaeal communities and also the fertilization intensity significantly shaped the communities (Table 1A). NMDS ordination of the bacterial and archaeal communities in BS showed that MP samples of the last standing field crop rapeseed (MP-Int-R, MP-Ext-R) had a high variability among replicates and differed clearly from CT-R as well as from all samples with wheat as the last standing field crop (Fig. 2A). Among the different soil treatments obtained from the wheat field, a slight separation of bacterial and archaeal community compositions according to tillage practice (MP-W, CT-W) was observed. In the RH, bacterial and archaeal communities of lettuce grown in MP soils (Int, Ext) had a high similarity among each other and clustered tightly in the NMDS analysis irrespective of the last standing field crop (Fig. 3A). Rhizosphere bacterial and archaeal communities from CT soils were affected by the last standing field crop and exhibited a higher variability between the replicates compared to MP. A clear fertilization intensity effect was observed for CT-W RH samples (Fig. 3A).

Regarding fungal community composition, the tillage practice was the strongest driver in BS and RA followed by the last standing field crop and fertilization intensity and their interaction effects (Table 1B). Only in the RH, fertilization intensity was the strongest driver (Table 1B). Ordination by NMDS in BS (Fig. S1,

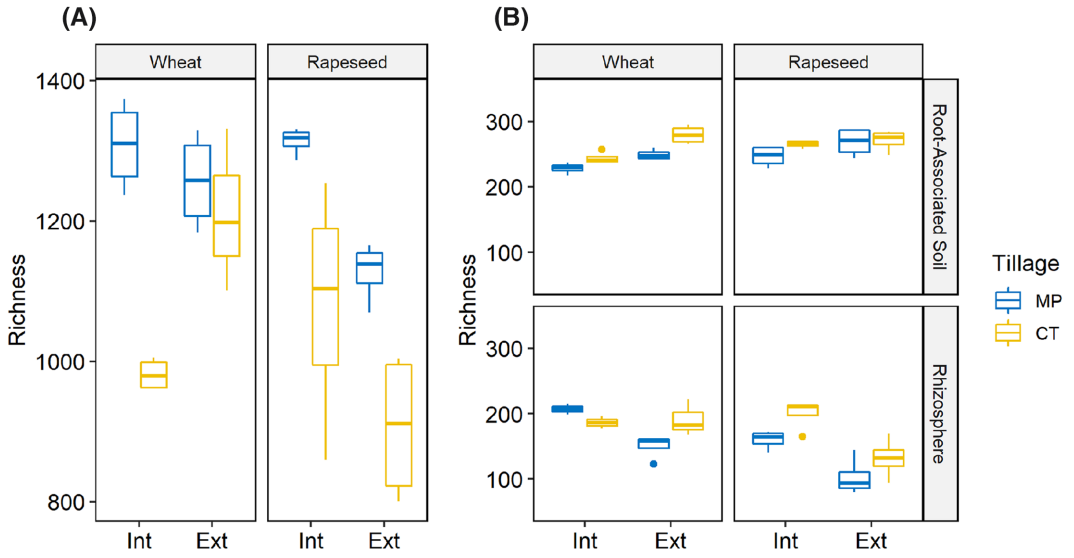

Figure 1. Effect of tillage practice, fertilization intensity and last standing field crop on richness of **(A)** bacterial and archaeal communities in rhizosphere and **(B)** fungal communities in the root-associated soil and rhizosphere of lettuce (cv. Tizian) grown in different soils from LTE Bernburg. Three-way ANOVA-Tukey results can be found in Tables S2 and S3 (Supporting Information). MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators.

Table 1. PERMANOVA analysis based on Bray–Curtis distances (10 000 permutations) of the A) bacterial and archaeal or B) fungal community compositions in bulk soil (BS), root-associated soil (RA) and rhizosphere (RH) of lettuce (cv. Tizian) grown in different long-term soil treatments from LTE Bernburg to test the effect of factors tillage practice, last standing field crop and fertilization intensity.

A)	BS		RH	
Factor	Explained variance [%]	P-value	Explained variance [%]	P-value
Tillage practice (TP)	11.9	**<0.01**	25.0	**<0.001**
Last standing field crop (LstC)	16.8	**<0.001**	11.8	**<0.001**
Fertilization intensity (Fertl-int)	3.9	0.11	7.1	**<0.01**
TP × LstC	8.5	**<0.01**	7.2	**<0.001**
TP × Fertl-int	2.4	0.28	3.8	**<0.05**
LstC × Fertl-int	1.8	0.50	4.0	**<0.05**
TP × LstC × Fertl-int	2.2	0.35	4.4	**<0.01**
Residuals	52.6		36.8	

B)	BS		RA		RH	
Factor	Explained variance [%]	P-value	Explained variance [%]	P-value	Explained variance [%]	P-value
Tillage practice (TP)	30.6	**<0.001**	25.7	**<0.001**	5.5	**<0.05**
Last standing field crop (LstC)	12.9	**<0.001**	15.8	**<0.001**	3.3	**<0.05**
Fertilization intensity (Fertl-int)	6.2	**<0.001**	11.4	**<0.001**	66.1	**<0.001**
TP × LstC	5.7	**<0.01**	5.7	**<0.001**	1.2	0.23
TP × Fertl-int	3.8	**<0.05**	3.6	**<0.01**	3.1	**<0.05**
LstC × Fertl-int	5.2	**<0.01**	6.8	**<0.001**	2.1	0.11
TP × LstC × Fertl-int	2.6	0.07	2.6	**<0.05**	1.8	0.14
Residuals	33.1		28.4		16.9	

Supporting Information) and RA (Fig. 2B) showed a separation of fungal communities into MP and CT with further differentiation according to the last standing field crop. In RA, also an effect of fertilization intensity was visible by the formation of subclusters (except for MP-W). Fertilization intensity resulted in a distinct separation of RH fungal communities from lettuce grown in Int and Ext soils (Fig. 3B).

In summary, the microbial community composition responded to the different management factors studied, however, microhabitat-dependent differences between microbial domains were observed.

Management-dependent taxonomic changes in soil and rhizosphere microbial communities

In the following analyses, we explored whether the observed effects on microbial alpha-diversity and changes in community composition among treatments can be linked to shifts in the relative abundance of certain taxonomic groups at different taxonomic levels. The analyses of the bacterial and archaeal community composition at higher taxonomic ranks (phylum and proteobacterial classes) revealed Acidobacteria (13–16% relative abundance), Actinobacteria (13–16%), Alphaproteobacteria

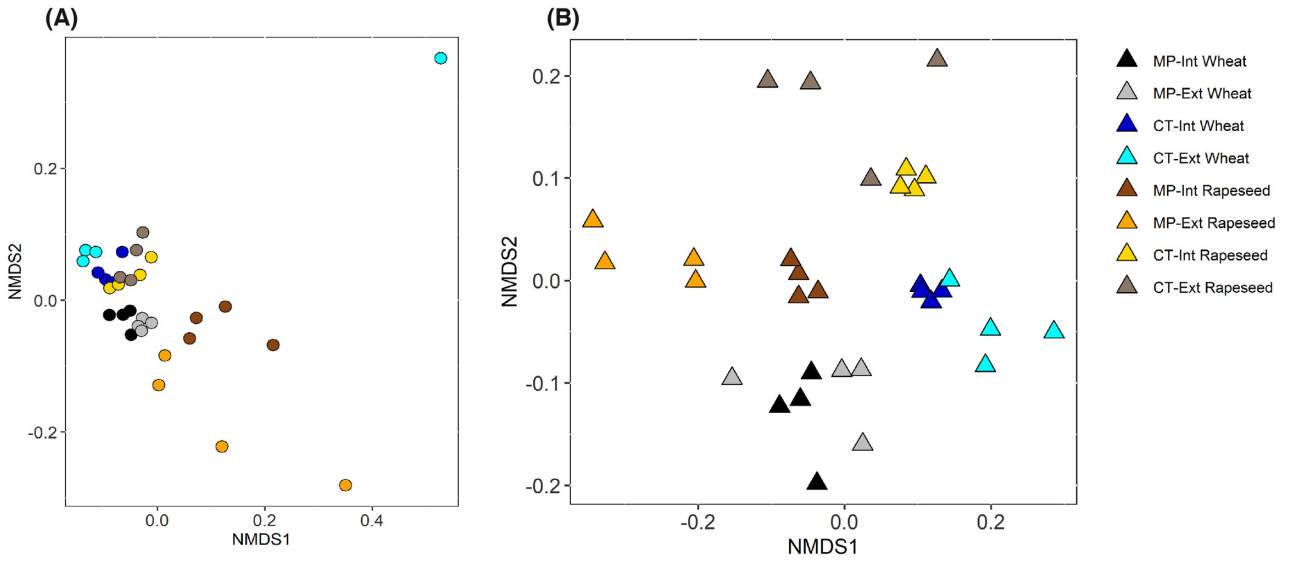

Figure 2. Non-metric multidimensional scaling (NMDS) plots of **A)** bacterial and archaeal communities in bulk soils (stress = 0.09) and **B)** fungal communities in root-associated soils (stress = 0.14) obtained from different long-term soil treatments in LTE Bernburg. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators.

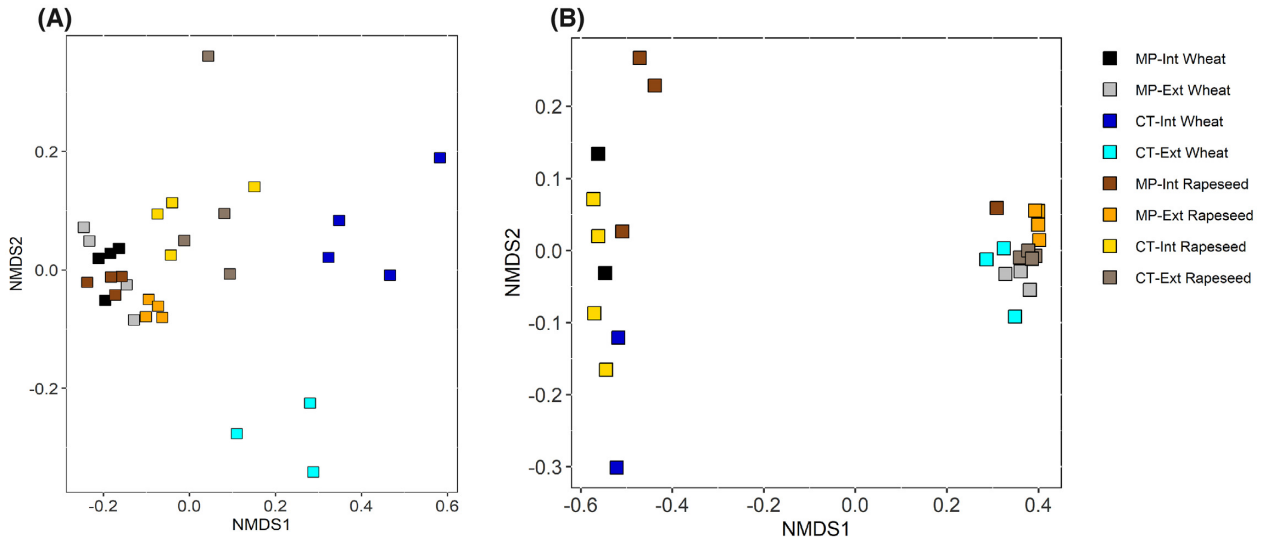

Figure 3. Non-metric multidimensional scaling (NMDS) plots of **(A)** bacterial and archaeal communities in the rhizosphere (stress = 0.13) and **(B)** fungal communities in the rhizosphere (stress = 0.05) of lettuce (cv. Tizian) grown in different long-term soil treatments from LTE Bernburg. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators.

(7–10%), Bacteroidetes (7–8%), Firmicutes (8–14%) and Thaumarchaeota (15–20%) as dominant taxa in BS (Fig. S2, Supporting Information). Most of the OTUs in BS, irrespective of agricultural history, were affiliated to the genera *Nitrososphaera*, *Bacillus* or belonged to unclassified genera of *Chitinophagaceae* and acidobacterial subdivisions Gp4 and Gp6 (Fig. 4). Although a few differences in relative abundances were found among treatments in BS, no general effects of tillage practice, last standing field crop or fertilization intensity could be identified (Fig. S2, Supporting Information; Fig. 4).

Highly prevalent genera detected in the lettuce RH were *Pseudomonas*, *Cellvibrio*, Candidatus Saccharibacteria, *Methylophilus*, *Dyadobacter* and *Rhizobium*. In the RH, significant differences in relative abundances of bacterial and archaeal phyla (or proteobacterial classes) and genera were found among treatments as well (Fig. S3, Supporting Information; Fig. 4). Strikingly, CT-treatments (except CT-Ext-W) exhibited a significantly higher relative abundance of Gammaproteobacteria specifically of *Pseudomonas* compared to the respective MP treatments (Fig. S3, Supporting Information; Fig. 4). The enrichment of *Pseudomonas* in CT-Ext-R, CT-Int-R, and CT-Int-W was attributed to only a few OTUs (Table S4, Supporting Information), mainly OTU_10699 and OTU_11269, which had the highest similarity to *Pseudomonas brassicacearum* and *P. corrugata*, respectively. When lettuce was grown in MP soils, more OTUs were affiliated to *Devosia*, *Sphingomonas* and *Paenibacillus* as compared to RH of CT soils (except CT-Ext-W).

The fungal community composition at phylum level (BS, RA) was dominated by Ascomycota (33%–45%), Basidiomycota (10%–26%), Mortierellomycota (25%–39%) and Mucoromycota (2%–16%) irrespective of the agricultural practice (Table S5, Supporting Information). Ext soils (especially in RA) exhibited a significantly higher relative abundance of Glomeromycota (0.1%–1%) and Olpidiomycota (3%–18%) compared to Int treatments. OTUs

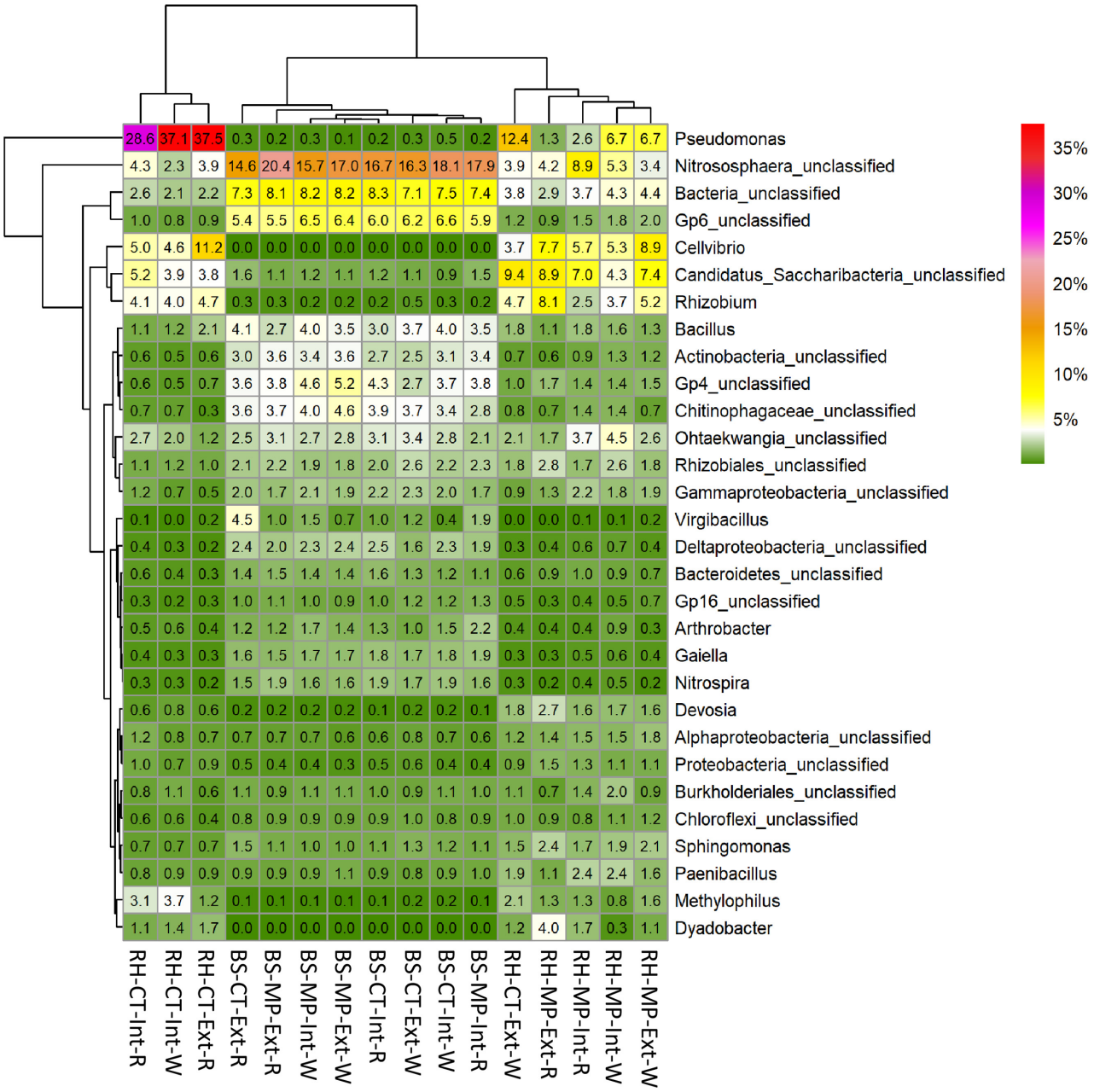

Figure 4. Heatmap showing the relative abundance distribution of the 30 most abundant bacterial and archaeal genera in bulk soil (BS) and rhizosphere (RH) of lettuce (cv. Tizian) grown in different long-term soil treatments from LTE Bernburg. Numbers represent relative abundances. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators, W- last standing field crop winter wheat, R- last standing field crop rapeseed.

unidentified at genus level and *Mortierella* were predominant in all RA samples (Fig. 5). Thus, a high similarity in terms of the relative abundance and distribution of major genera was observed in these samples. Further predominant genera in RA were *Solicoccozyma, Minimedusa, Didymella* and *Exophiala* irrespective of farming practices (Fig. 5). *Rhizopus* was less abundant in RA of both MP-R treatments compared to CT-R and all treatments with wheat as last standing field crop. Among the abundant genera in RA, treatment-dependent differences in the relative abundance were observed, e.g. for *Gibberella, Fusarium, Pseudogymnoascus* and *Actinomucor*. *Gibberella* and *Fusarium* were prevalent in all CT treatments, especially in CT-R. More sequences were classified as *Pseudogymnoascus* or *Actinomucor* in MP-W or in soils with W being the last standing field crop compared to other treatments.

The predominance of Olpidiomycota represented by the genus *Olpidium* in RH of lettuce grown in Ext treatments (82%–97%) separated them from Int and from all RA samples (Table S5, Supporting Information; Fig. 5). The enrichment was attributed to only two OTUs with the closest relationship to *Olpidium brassicae* (see fungal indicator section). Besides RH-Ext, *Olpidium* was also found among the predominant genera in RH of MP-Int-R (25%; Table S5, Supporting Information, Fig. 5). The RH of lettuce

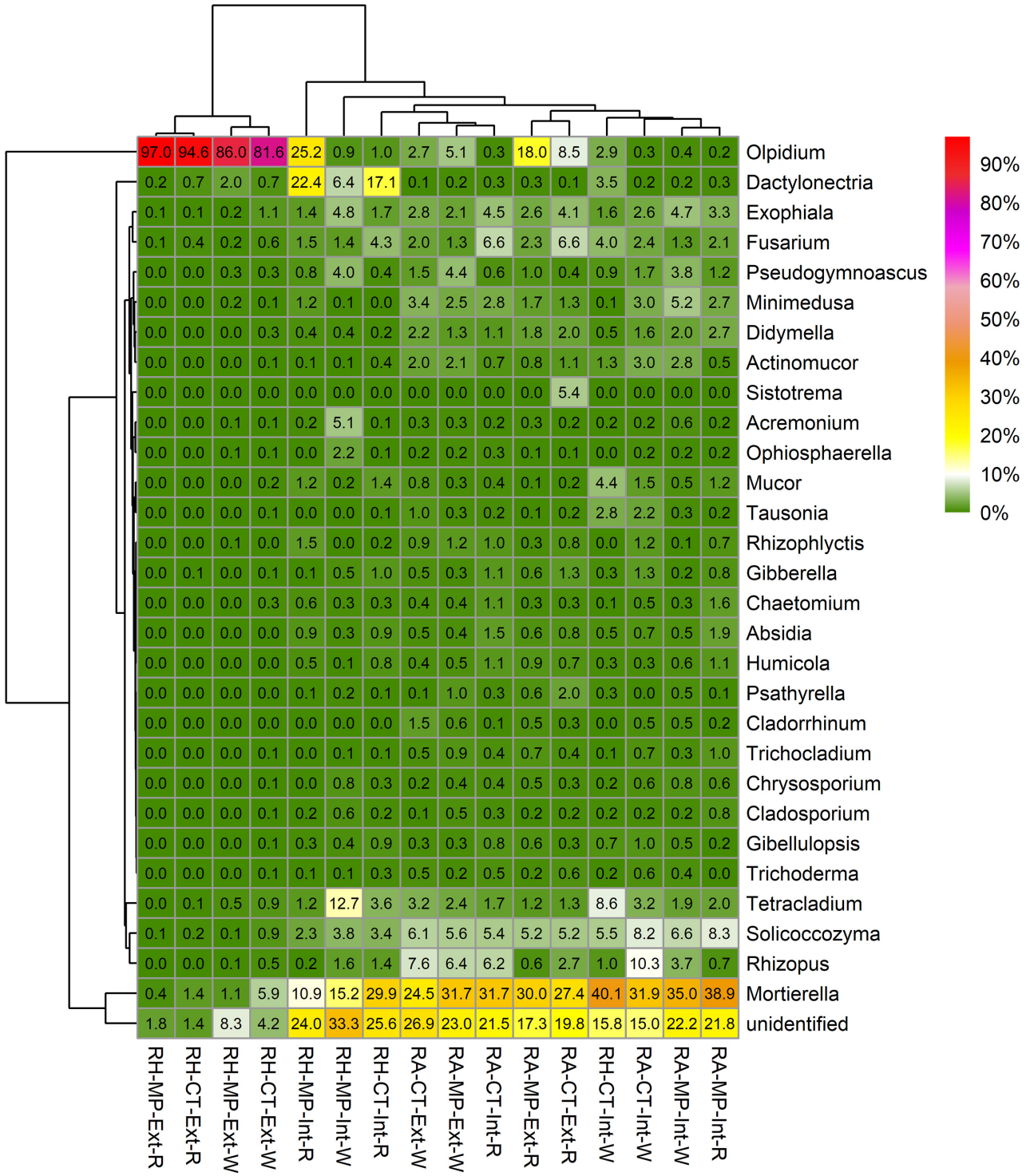

Figure 5. Heatmap showing the relative abundance distribution of the 30 most abundant fungal genera in root-associated soil (RA) and rhizosphere (RH) of lettuce (cv. Tizian) grown in different long-term soil treatments from LTE Bernburg. Numbers represent relative abundances. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators, W- last standing field crop winter wheat, R- last standing field crop rapeseed.

exhibited a significantly higher relative abundance of Ascomycota (40%–76%), Mortierellomycota (11%–40%), Basidiomycota (4–9%) and Mucoromycota (2%–7%) in Int as compared to Ext treatments (Table S5, Supporting Information). Due to this and comparable with the RA samples, OTUs unidentified at genus level and *Mortierella* dominated the Int soils in lettuce RH.

Pseudomonas acts as major bacterial indicator in the lettuce rhizosphere

The factors tillage practice, last standing field crop and fertilization intensity caused shifts in the microbial communities when considering the relative abundances of lower taxonomic ranks.

Statistical tests were carried out to obtain more insights into taxa significantly associated (indicators) to these factors, their interactions as well as to distinct treatments. Among all bacterial and archaeal OTUs ($n = 7316$), 168 OTUs were identified as indicators for agricultural practices in BS (Table S6, Supporting Information). More indicators were found for CT (10 OTUs) than MP (2 OTUs) and W (4 OTUs) yielded more significantly enriched OTUs than R (0 OTUs). No bacterial and archaeal indicators were found in BS for long-term fertilization intensities (Int, Ext). However, when considering distinct treatments, indicators were detected for all except MP-Ext-R (Table S6, Supporting Information). Many of them exhibited only a low relative abundance and belonged to different phyla (Table S7, Supporting Information).

In the RH, considerably more indicator OTUs were found (561 OTUs) compared to BS (Table S6, Supporting Information). Table 2 gives an overview about all RH treatment indicators with relative mean abundances >0.5%. Among the indicators for MP in RH were mainly OTUs affiliated to Acidobacteria (10% of all MP indicators) and Proteobacteria (68%), especially belonging to the alphaproteobacterial orders Rhizobiales and Sphingomonadales, but also to Betaproteobacteria (12%) and Gammaproteobacteria (18%). For the other factors (CT, Int, Ext, R, W) no or only RH indicators with low relative abundance were found (data not shown). When lettuce was grown in MP-Int soils, two OTUs identified as *Ohtaekwangia* and *Paenibacillus* showed strong enrichment (Table 2). In lettuce RH from CT-R soils 80% of the indicator OTUs belonged to Proteobacteria, especially to *Pseudomonadaceae* with OTU_8093 (*Pseudomonas*) being most abundant (Table 2). When lettuce was cultivated in MP-R soils, OTUs affiliated to acidobacterial Gp4, *Dyadobacter*, Candidatus Saccharibacteria and *Sphingomonadaceae* were enriched (Table 2). Considering distinct treatments, OTU_10699 (*Pseudomonas*) was revealed as indicator for RH of lettuce from CT-Ext-R due to its high relative abundance (17%) whereas OTU_104 (*Methylophilaceae*) was indicative for RH of CT-Int-R (Table 2). The RH indicator of MP-Ext-R with highest relative abundance was affiliated to *Acidovorax* (Table 2). A total of 190 OTUs was found to be significantly enriched in RH of lettuce from MP-Int-R soils as compared to other soil treatments (Table S6, Supporting Information). They were mainly classified as Proteobacteria (38% of all indicators), Bacteroidetes (19%), and Thaumarchaeota (9%). Among the predominant MP-Int-R indicators in RH, OTUs identified as *Nitrososphaera* and *Rhizobacter* were detected (Table 2). OTU_131 (*Ohtaekwangia*) was indicative for lettuce RH from MP-Int-W (Table 2). Indicators found for RH of CT-Int-W, CT-Ext-W, and MP-Ext-W exhibited low relative abundances (<0.5%; data not shown). To summarize, RH bacteria and archaea were affected by the interaction between factors resulting in more treatment-specific than general indicators.

Olpidium indicative for soils with extensive fertilization history

For fungi, most of the indicator OTUs were found in RA (105 OTUs) followed by BS (79) and RH (14) (Table S8, Supporting Information). Most indicators were detected for MP-Ext-R (13 OTUs) and MP-Ext-W (12 OTUs) in RA though numerous had low relative abundances (e.g. Glomeromycota, data not shown). No indicators could be detected for the treatment group MP-Ext in all three microhabitats (Table S8, Supporting Information). Indicator OTUs for MP-Int-W, MP-Ext-W, and CT-Int-R in RA were only assigned at higher taxonomic levels with a relative mean abundance >0.5% (Table 3). In RA, the genus *Mortierella* was often detected as an indicator. For instance, two OTUs with closest sequence identity to *Mortierella alpina* were significantly enriched in MP treatments. Furthermore, *Mortierella* sp. OTUs were indicative for MP-R or CT-R, respectively. In CT-R, in addition one OTU most closely affiliated to the genus *Fusarium* was identified as indicator. Furthermore, OTU *M. elongata* significantly increased in CT. OTUs most closely affiliated to *Pseudogymnoascus appendiculatus* and *P. pannorum* were significantly enriched in MP-W treatments. OTUs *Actinomucor elegans* or *M. exigua* were indicative for soils with the last standing field crop W or R, respectively. In each, CT-Int-W and CT-Ext-W, a yeast (*Tausonia pullulans* and *Cystofilobasidium macerans*, respectively) was found as indicator. The genus *Sistotrema* was enriched in CT-Ext-R. In MP-Ext-R, *Funneliformis* as representative of the phylum Glomeromycota and one OTU classified as *Olpidium brassicae* displayed high relative abundances (18%, Table 3) in RA.

Several indicators reported for RA were also identified in BS (Table S9, Supporting Information). Among them were for instance *M. alpina* (two OTUs), *M. elongata, M. exigua, P. appendiculatus* and *Fusarium* sp. Additionally in BS of CT-R, *Gibberella tricincta,* the teleomorph of *F. tricinctum* (Table S9, Supporting Information), was as well enriched besides *Fusarium* sp.

In RH of all Ext soils, OTU *O. brassicae* was found as indicator exhibiting very high relative abundances while in Int treatments OTU *F. concentricum* was indicative (Table 4). In CT-Int, two OTUs belonging to *Mortierella* were significantly enriched compared to other treatments. MP-Int-W was the only treatment with indicative OTUs in RH, however, similar to RA, these were classifiable only at higher taxonomic levels.

One OTU classified as *O. brassicae* was highly abundant in RA of MP-Ext-R and was generally enriched in all Ext soils from BS and RH (Tables 3 and 4; Table S9, Supporting Information). One OTU identified as *M. elongata* was not only suggested as indicator in RH for CT-Int but also for CT in BS and RA. Additionally, an OTU classified as *Bionectriacea* sp. was indicative for CT-Int (in BS and RH) and for CT in RA (Tables 3 and 4; Table S9, Supporting Information).

In summary, in RA and BS, fungal indicators could be identified for most of the single factors studied as well as their interactions. Due to the strong impact of fertilization intensity, mainly fungal indicators for Int and Ext were found in RH.

Tillage practice and fertilization intensity significantly affected lettuce growth

Shoot (SDM) and root dry masses (RDM) of lettuce were measured after 10 weeks of cultivation in soils from different long-term management practices. Results of ANOVA analysis showed significant effects of tillage practice (MP *vs* CT; $P < 0.01$) and fertilization intensity (Int *vs* Ext, $P < 0.0001$) on lettuce growth (Table S10, Supporting Information). No significant impact was found for the last standing field crop (W *vs* R; $P > 0.5$). The highest SDM and RDM were measured when lettuce was cultivated in CT-Ext-W soil (Table 5). In contrast, with R as last standing field crop, the highest SDM and RDM of lettuce were found in the treatment MP-Ext-R. Int fertilization practice significantly reduced lettuce growth compared to Ext, independent of tillage practice and last standing field crop (Table 5). The lowest lettuce SDM and RDM were recorded in MP-Int soils.

Fertilization intensity exerted major influence on lettuce gene expression patterns

To emphasize the influence of the different agricultural management practices on lettuce gene expression levels, a multivariate analysis of qPCR data from 20 lettuce genes with known

Table 2. Bacterial and archaeal indicator OTUs ($P \leq 0.05$, Benjamini–Hochberg corrected) for the different treatments in the rhizosphere of lettuce (only indicators >0.5% relative abundance [mean] are shown). Means ± standard deviation are displayed. Bold marked numbers indicate increased relative abundances. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators, W- last standing field crop winter wheat, R- last standing field crop rapeseed.

Treatment	OTU	Family	Genus	MP-Int-W	MP-Ext-W	CT-Int-W	CT-Ext-W	MP-Int-R	MP-Ext-R	CT-Int-R	CT-Ext-R
CT-Ext-R	OTU_10699	*Pseudomonadaceae*	*Pseudomonas*	1.6 ± 0.8	2.2 ± 3.1	8.8 ± 6.9	2.1 ± 2.4	0.1 ± 0.1	0.3 ± 0.2	5.8 ± 1.3	17.2 ± 10.5
CT-Int-R	OTU_104	*Methylophilaceae*	*Methylophilaceae_unclassified*	0.1 ± 0.1	0.1 ± 0	0.2 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0.8 ± 0.6	0 ± 0
MP-Ext-R	OTU_11657	*Comamonadaceae*	*Acidovorax*	0.2 ± 0.3	0.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	**2.1 ± 1.5**	0.4 ± 0.4	0.6 ± 0.6
	OTU_142	*Candidatus_Saccharibacteria_unclassified*	*Candidatus_Saccharibacteria_unclassified*	0.3 ± 0	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.3	0.2 ± 0.1	**1.2 ± 0.3**	0.4 ± 0.1	0.3 ± 0.1
	OTU_2192	*Rhodobacteraceae*	*Gemmobacter*	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0	0.1 ± 0.1	0.2 ± 0	**1.2 ± 0.3**	0.1 ± 0	0.3 ± 0.2
	OTU_67	*Rhizobiaceae*	*Rhizobium*	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0.6 ± 0.5	0 ± 0	**1 ± 0.6**	0.1 ± 0	0.1 ± 0
MP-Int-R	OTU_1463	*Oxalobacteraceae*	*Herminiimonas*	0.4 ± 0.3	0.4 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	**1.1 ± 0.4**	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
	OTU_21	*Nitrososphaera*	*Nitrososphaera_unclassified*	2.1 ± 0.8	1.7 ± 0.8	0.8 ± 0.3	1.4 ± 0.4	**4.1 ± 1.6**	2 ± 0.8	1.4 ± 0.7	1.5 ± 0.9
	OTU_2742	*Nitrososphaera*	*Nitrososphaera_unclassified*	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0	0.3 ± 0	**0.7 ± 0.2**	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.2
	OTU_277	*Nitrososphaera*	*Nitrososphaera_unclassified*	0.3 ± 0.1	0.2 ± 0	0.1 ± 0	0.2 ± 0	**0.5 ± 0.2**	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2
	OTU_329	*Ohtaekwangia_unclassified*	*Ohtaekwangia_unclassified*	0.4 ± 0.2	0.4 ± 0.3	0.3 ± 0.1	0.1 ± 0	**0.6 ± 0.2**	0.1 ± 0	0.3 ± 0.1	0.2 ± 0
	OTU_35	*Pseudomonadaceae*	*Rhizobacter*	0.6 ± 0.3	0.3 ± 0.1	0.6 ± 0.4	0.3 ± 0.2	**2.2 ± 0.9**	0.2 ± 0.2	0.8 ± 0.5	0.2 ± 0.1
	OTU_5034	*Nitrososphaera*	*Nitrososphaera_unclassified*	0.4 ± 0.2	0.3 ± 0.2	0.1 ± 0	0.2 ± 0.1	**0.6 ± 0.3**	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
	OTU_5114	*Burkholderiales_incertae_sedis*	*Burkholderiales_incertae_sedis_unclassified*	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0	**0.6 ± 0.2**	0.1 ± 0	0.2 ± 0.2	0.1 ± 0.1
	OTU_6227	*Candidatus_Saccharibacteria_unclassified*	*Candidatus_Saccharibacteria_unclassified*	0.1 ± 0.1	0.3 ± 0.1	0 ± 0	0.1 ± 0	**0.5 ± 0.3**	0.2 ± 0	0.1 ± 0	0 ± 0
	OTU_63	*Nitrososphaera*	*Nitrososphaera_unclassified*	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0	0.5 ± 0.1	**0.6 ± 0.1**	0.3 ± 0.1	0.5 ± 0.3	0.4 ± 0.2
	OTU_8325	*Pseudomonadaceae*	*Rhizobacter*	0.3 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.2 ± 0.2	**0.6 ± 0.4**	0.1 ± 0	0.2 ± 0.2	0.1 ± 0.1
MP-Int-W	OTU_131	*Ohtaekwangia_unclassified*	*Ohtaekwangia_unclassified*	**1.1 ± 1.1**	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.1
MP-Int	OTU_10394	*Ohtaekwangia_unclassified*	*Ohtaekwangia_unclassified*	**0.7 ± 0.1**	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	**0.6 ± 0.1**	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
	OTU_184	*Paenibacillaceae_1*	*Paenibacillus*	**1.2 ± 0.4**	0.8 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	**1.2 ± 0.4**	0.6 ± 0.2	0.3 ± 0.2	0.4 ± 0.2
CT-R	OTU_8093	*Pseudomonadaceae*	*Pseudomonas*	0.4 ± 0.1	0.3 ± 0.1	1 ± 0.8	0.5 ± 0.4	0.2 ± 0.1	0.1 ± 0.1	**0.9 ± 0.1**	**0.8 ± 0.6**
MP-R	OTU_2664	*Gp4_unclassified*	*Gp4_unclassified*	0.4 ± 0.2	0.5 ± 0.3	0.2 ± 0.1	0.5 ± 0.2	**0.4 ± 0.1**	**0.8 ± 0.2**	0.2 ± 0.2	0.2 ± 0.1
	OTU_54	*Cytophagaceae*	*Dyadobacter*	0.3 ± 0.1	1 ± 0.5	1.2 ± 0.5	1.1 ± 0.1	**1.6 ± 0.6**	**3.6 ± 0.3**	1.1 ± 0.3	1.6 ± 1.4
	OTU_7	*Candidatus_Saccharibacteria_unclassified*	*Candidatus_Saccharibacteria_unclassified*	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0	0.9 ± 0.6	**0.6 ± 0.2**	**0.6 ± 0.1**	0.2 ± 0	0.1 ± 0.1
	OTU_8426	*Sphingomonadaceae*	*Sphingomonadaceae_unclassified*	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.8 ± 0.2	**0.4 ± 0.1**	**0.7 ± 0.2**	0.1 ± 0	0.1 ± 0.1
MP	OTU_116	*Hyphomicrobiaceae*	*Devosia*	**0.8 ± 0.1**	**0.8 ± 0.3**	0.4 ± 0.1	1 ± 0.3	**0.8 ± 0.2**	**0.8 ± 0.2**	0.4 ± 0.1	0.3 ± 0.1
	OTU_197	*Erythrobacteraceae*	*Erythrobacteraceae_unclassified*	**1.7 ± 0.8**	**1.1 ± 0.8**	0.5 ± 0.1	1.5 ± 0.7	**1.3 ± 0.5**	**1.3 ± 0.4**	0.4 ± 0.2	0.2 ± 0.1
	OTU_349	*Methylophilaceae*	*Methylobacillus*	**0.6 ± 0.2**	**0.8 ± 0.3**	0.4 ± 0.1	0.4 ± 0.1	**0.5 ± 0.2**	**0.7 ± 0.3**	0.2 ± 0	0.1 ± 0.1

Table 3. Fungal indicator OTUs ($P \leq 0.05$, Benjamini–Hochberg corrected) for the different treatments in root-associated soils of lettuce (only indicators >0.5% relative abundance [mean] are shown). Bold marked numbers indicate increased relative abundances. Means ± standard deviation are displayed. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators, W- last standing field crop winter wheat, R- last standing field crop rapeseed.

Treatment	OTU	Family	Species	MP-Int-W	MP-Ext-W	CT-Int-W	CT-Ext-W	MP-Int-R	MP-Ext-R	CT-Int-R	CT-Ext-R
CT-Ext-R	SH1506095.08FU	*Cantharellales fam inc. sed.*	*Sistotrema* sp.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**5.4 ± 8.2**
CT-Ext-W	SH1650089.08FU	*Cystofilobasidiaceae*	*Cystofilobasidium macerans*	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.2	**0.6 ± 0.3**	0.1 ± 0.1	0.2 ± 0	0.2 ± 0	0.2 ± 0.1
CT-Int-R	SH1525459.08FU	*unidentified*	*Pleosporales* sp.	0 ± 0	0 ± 0	0 ± 0	0± 0	0.1 ± 0	0.1 ± 0	**0.8 ± 0.1**	0.1 ± 0
CT-Int-W	SH1650607.08FU	*Mrakiaceae*	*Tausonia pullulans*	0.3 ± 0.1	0.3 ± 0.1	**2.2 ± 0.9**	1.0 ± 0.1	0.2 ± 0.1	0.1 ± 0	0.2 ± 0	0.2 ± 0.1
MP-Ext-R	SH1529297.08FU	*unidentified*	*Chytridiomycota* sp.	0 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	**0.6 ± 0.3**	0 ± 0	0 ± 0
	SH1519091.08FU	*Olpidiaceae*	*Olpidium brassicae*	0.1 ± 0	4.5 ± 7.5	0.1 ± 0	1.3 ± 0.8	0.2 ± 0	**17.9 ± 6.9**	0 ± 0	7.6 ± 7.9
	SH1559922.08FU	*Glomeraceae*	*Funneliformis* sp.	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	**0.9 ± 1.0**	0 ± 0	0.1 ± 0.1
	SH1578228.08FU	*Herpotrichiellaceae*	*Phialophora cyclaminis*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**3.5 ± 4.0**	0 ± 0	0 ± 0
MP-Ext-W	SH1522748.08FU	*unidentified*	*Leucosporidiales* sp.	0.2 ± 0	**0.5 ± 0.2**	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
MP-Int-R	SH2232036.08FU	*Didymellaceae*	*Didymella glomerata*	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	**1.7 ± 0.2**	1.1 ± 0.2	0.6 ± 0.1	1.0 ± 0.2
	SH2180558.08FU	*Pleosporaceae*	*Alternaria betae-kenyensis*	0 ± 0	0.1 ± 0	0 ± 0	0 ± 0.1	**0.6 ± 0.4**	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
MP-Int-W	SH1516799.08FU	*unidentified*	*Ascomycota* sp.	**0.8 ± 0.1**	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0	0.5 ± 0.1	0.4 ± 0.2	0.4 ± 0	0.2 ± 0
CT-Int	SH1517571.08FU	*unidentified*	*Hypocreales* sp.	0.5 ± 0.1	0.3 ± 0.1	**0.7 ± 0.2**	0.4 ± 0.2	0.5 ± 0.4	0.5 ± 0.3	1.0 ± 0.2	0.5 ± 0.2
Int-W	SH1621090.08FU	*Mucoraceae*	*Actinomucor* sp.	**0.8 ± 0.3**	0.4 ± 0.2	**0.8 ± 0.1**	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0	0.3 ± 0.1
Int-R	SH1558059.08FU	*Cunninghamellaceae*	*Absidia glauca*	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	**1.6 ± 0.5**	0.4 ± 0.1	**1.2 ± 0.2**	0.6 ± 0.2
	SH1615796.08FU	*Chaetomiaceae*	*Chaetomium* sp.	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	**1.5 ± 0.4**	0.3 ± 0.1	**1.1 ± 1.0**	0.3 ± 0.2
CT-R	SH1610194.08FU	*Nectriaceae*	*Fusarium* sp.	0.4 ± 0.1	0.5 ± 0.1	1.5 ± 0.2	1.3 ± 0.3	0.9 ± 0.2	1.1 ± 0.1	**5.1 ± 0.5**	**5.6 ± 1.5**
	SH1529200.08FU	*Mortierellaceae*	*Mortierella* sp.	0.2 ± 0.1	0.1 ± 0	0.3 ± 0.2	0.2 ± 0	0.2 ± 0.1	0.3 ± 0.1	**0.9 ± 0.2**	**0.8 ± 0.2**
MP-W	SH1938854.08FU	*Pseudeurotiaceae*	*Pseudogymnoascus appendiculatus*	**2.5 ± 0.3**	**3.2 ± 0.4**	1.6 ± 0.4	1.3 ± 0.2	1.0 ± 0.4	0.6 ± 0.1	0.6 ± 0.1	0.3 ± 0.1
	SH2267912.08FU	*Pseudeurotiaceae*	*Pseudogymnoascus pannorum*	**1.3 ± 0.6**	**1.1 ± 0.6**	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0	0.1 ± 0
MP-R	SH1557076.08FU	*Mortierellaceae*	*Mortierella* sp.	1.5 ± 0.6	1.5 ± 0.6	1.3 ± 0.3	1.1 ± 0.2	**2.4 ± 0.2**	**2.2 ± 0.3**	1.6 ± 0.1	1.6 ± 0.6
CT	SH1557020.08FU	*Mortierellaceae*	*Mortierella elongata*	1.2 ± 0.7	1.1 ± 0.2	**4.7 ± 0.5**	**6.0 ± 0.8**	2.5 ± 0.4	1.8 ± 0.2	**3.8 ± 0.6**	**5.9 ± 1.4**
	SH1648786.08FU	*unidentified*	*Helotiales* sp.	0.8 ± 0.3	0.9 ± 0.4	**2.2 ± 0.1**	**1.7 ± 0.2**	1.0 ± 0.3	0.8 ± 0.1	**1.5 ± 0.5**	**1.4 ± 0.5**
	SH1522839.08FU	*Bionectriaceae*	*Bionectriaceae* sp.	0.3 ± 0.3	0.3 ± 0.1	**1.0 ± 0.2**	**0.9 ± 0.4**	0.4 ± 0.1	0.5 ± 0.3	**1.7 ± 0.1**	**0.9 ± 0.6**
MP	SH1650286.08FU	*Mortierellaceae*	*Mortierella alpina*	**5.7 ± 0.7**	**4.4 ± 1.2**	2.1 ± 0.1	1.0 ± 0.2	**4.5 ± 0.2**	**3.8 ± 0.4**	1.1 ± 0.2	1.0 ± 0.2
	SH1525093.08FU	*unidentified*	*Pleosporales* sp.	**4.2 ± 0.9**	**6.0 ± 0.7**	1.3 ± 0.2	1.6 ± 0.3	**3.9 ± 0.8**	**4.8 ± 0.9**	2.3 ± 0.2	2.3 ± 0.3
	SH1650283.08FU	*Mortierellaceae*	*Mortierella alpina*	**1.7 ± 0.5**	**1.4 ± 0.4**	0.6 ± 0.1	0.4 ± 0.1	**2.1 ± 0.4**	**1.4 ± 0.3**	0.6 ± 0.1	0.5 ± 0.1
	SH1506805.08FU	*Lasiosphaeriaceae*	*Lasiosphaeriaceae* sp.	**1.0 ± 0.8**	**0.8 ± 0.4**	0.1 ± 0	0.1 ± 0	**1.2 ± 0.5**	**0.6 ± 0.5**	0.2 ± 0.2	0.2 ± 0.1
W	SH1621089.08FU	*Mucoraceae*	*Actinomucor elegans*	**2.0 ± 0.7**	**1.6 ± 0.8**	**2.2 ± 0.2**	**1.5 ± 0.4**	0.3 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.8 ± 0.2
R	SH1557028.08FU	*Mortierellaceae*	*Mortierella exigua*	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	**1.8 ± 0.2**	**1.0 ± 0.2**	**1.3 ± 0.3**	**1.0 ± 0.3**

Table 4. Fungal indicator OTUs ($P \leq 0.05$, Benjamini-Hochberg corrected) for the different treatments in the rhizosphere of lettuce (only indicators >0.5% relative abundance [mean] are shown). Bold marked numbers indicate increased relative abundances. Means ± standard deviation are displayed. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators, W- last standing field crop winter wheat, R- last standing field crop rapeseed.

Treatment	OTU	Family	Species	MP-Int-W	MP-Ext-W	CT-Int-W	CT-Ext-W	MP-Int-R	MP-Ext-R	CT-Int-R	CT-Ext-R
MP-Int-W	SH1506805.08FU	*Lasiosphaeriaceae*	*Lasiosphaeriaceae* sp.	**0.5 ± 0.3**	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
	SH1525093.08FU	*unidentified*	*Pleosporales* sp.	**3.2 ± 0.5**	0.3 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.5	0.1 ± 0.1	0.4 ± 0.2	0.1 ± 0
CT-Int	SH1522839.08FU	*Bionectriaceae*	*Bionectriaceae* sp.	0.1 ± 0.1	0 ± 0	**0.4 ± 0**	0.2 ± 0.1	0.2 ± 0.2	0 ± 0	**0.5 ± 0.1**	0 ± 0
	SH1557020.08FU	*Mortierellaceae*	*Mortierella elongata*	0.4 ± 0.2	0 ± 0	**8.5 ± 0.1**	1.3 ± 0.9	0.2 ± 0.4	0 ± 0	**4.0 ± 2.2**	0.3 ± 0.2
	SH1557038.08FU	*Mortierellaceae*	*Mortierella* sp.	2.0 ± 1.1	0.2 ± 0.1	**8.2 ± 2.4**	0.4 ± 0.1	0.4 ± 0.5	0.1 ± 0	**8.2 ± 3.7**	0.2 ± 0.1
Ext	SH1519091.08FU	*Olpidiaceae*	*Olpidium brassicae*	0.9 ± 0.2	**85.9 ± 8.3**	2.9 ± 2.2	**81.5 ± 8.6**	25.2 ± 32.0	**97.0 ± 1.2**	1.0 ± 0.6	**94.5 ± 3.3**
Int	SH2456034.08FU	*Nectriaceae*	*Fusarium concentricum*	**1.0 ± 0.4**	0.1 ± 0.1	**1.1 ± 0.8**	0.3 ± 0.1	**1.2 ± 0.7**	0 ± 0	**1.3 ± 0.6**	0.1 ± 0.1

Table 5. Effect of long-term farming practice on shoot (SDM) and root (RDM) dry masses of lettuce (cv. Tizian) grown in different long-term soil treatments from LTE Bernburg. SDM and RDM data were analyzed by Tukey's HSD after transformation by Tukey's Ladder of Power. Different lowercase letters indicate significant differences ($P \leq 0.05$). Means ± standard deviation are displayed ($N = 4$). MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators, W- last standing field crop winter wheat, R- last standing field crop rapeseed.

Treatment	SDM [g/plant]	RDM [g/plant]
MP-Int-W	0.21 ± 0.15 f	0.10 ± 0.01 f
MP-Ext-W	2.76 ± 1.35 cd	1.13 ± 0.12 cd
CT-Int-W	1.74 ± 0.42 de	0.70 ± 0.07 de
CT-Ext-W	7.31 ± 1.39 a	2.28 ± 0.59 a
MP-Int-R	0.83 ± 0.31 ef	0.35 ± 0.09 ef
MP-Ext-R	5.80 ± 0.67 ab	1.77 ± 0.18 ab
CT-Int-R	2.44 ± 0.58 cd	0.98 ± 0.02 cd
CT-Ext-R	3.81 ± 0.95 bc	1.48 ± 0.13 bc

roles in oxidative and biotic stress signaling pathways was performed. PERMANOVA analysis showed that fertilization intensity followed by the last standing field crop significantly influenced gene expression and explained 52% and 19.8% of data variation, respectively. Tillage practice had a minor effect and accounted for 3.7% of the variance (Table S11, Supporting Information). A pairwise comparison of gene expression revealed that eight out of 20 genes with known and putative functions in abiotic and biotic stress regulation pathways (*PR1*, *PDF1.2*, *WRKY25*, *MYC2*, *SEN1*, *RbohF*, *HSP70*, *MYB15*) showed significantly enhanced expression in plants from CT-Ext soils compared to CT-Int soils in both last standing field crops W and R according to Tukey's HSD analyses (Fig. 6A). This effect was more pronounced in plants growing in MP soils, where 16 out of 20 genes examined showed significantly enhanced expression in MP-Ext compared to MP-Int in both last standing field crops (Fig. 6B). When we compared the gene expression levels between CT-Ext and MP-Ext, only a few genes showed significant differences between expression levels (data not shown).

DbRDA analysis showed that fertilization intensity (Int, Ext) had the highest influence on the gene expression patterns and was associated with the first axis that separated samples (64% explained variance). The last standing field crops (W or R) were related to the second axis, that explained 24% of variance. The differences observed in plant gene expression profiles were correlated with the relative abundance of major rhizosphere bacterial and archaeal taxa (Fig. 7). Expression profiles of lettuce grown on Ext-W significantly correlated with OTUs which had the closest similarity to *Methylobacillus* sp. (OTU_105; *Methylophilaceae*) and *Rhizobium mesoamericanum* (OTU_8493; *Rhizobiaceae*). Gene expression profiles of plants from Ext-R soils showed a significant correlation to the relative abundance of bacterial OTUs identified as *Acidovorax radicis* (OTU_11657; *Comamonadaceae*), *Dyadobacter endophyticus* (OTU_54; *Cytophagaceae*), and *Rhizobium nepotum* (OTU_11818; *Rhizobiaceae*). The predominant Int-R correlated OTUs were identified as *Rhizobacter gummiphilus* (OTU_35; Burkholderiales), or *Nitrososphaera* sp. (OTU_2742; *Nitrososphaeraceae*). In contrast, the Int-W gene expression profiles showed a positive association with the relative abundance of OTUs identified as *Lacisediminimonas profundi* (OTU_10235; *Oxalobacteraceae*), *Pseudarthrobacter phenanthrenivorans* (OTU_51; *Micrococcaceae*), and *Ohtaekwangia kribbensis* (OTU_329; *Fulvivirgaceae*; Fig. 7).

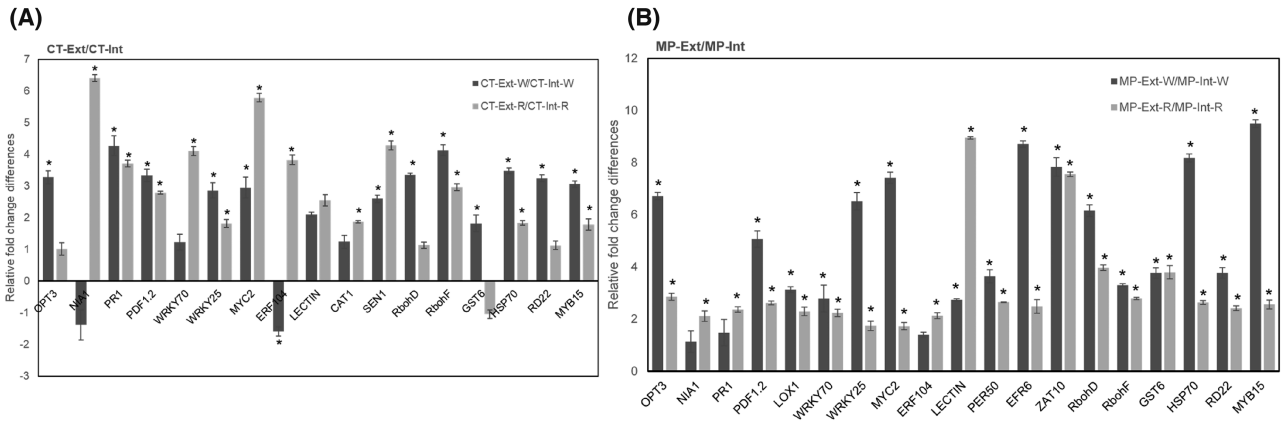

Figure 6. Log fold change of gene expression (ΔΔCt) in lettuce (cv. Tizian) grown in extensive (Ext) *vs* intensive (Int) fertilized soils with last standing field crops wheat (W) or rapeseed (R) under **A**) cultivator tillage (CT) or **B**) mouldboard plough (MP). Asterisks indicate significant differences in ΔCt according to pairwise Tukey's HSD test ($P < 0.05$). Error bars indicate standard errors of means ($n = 4$).

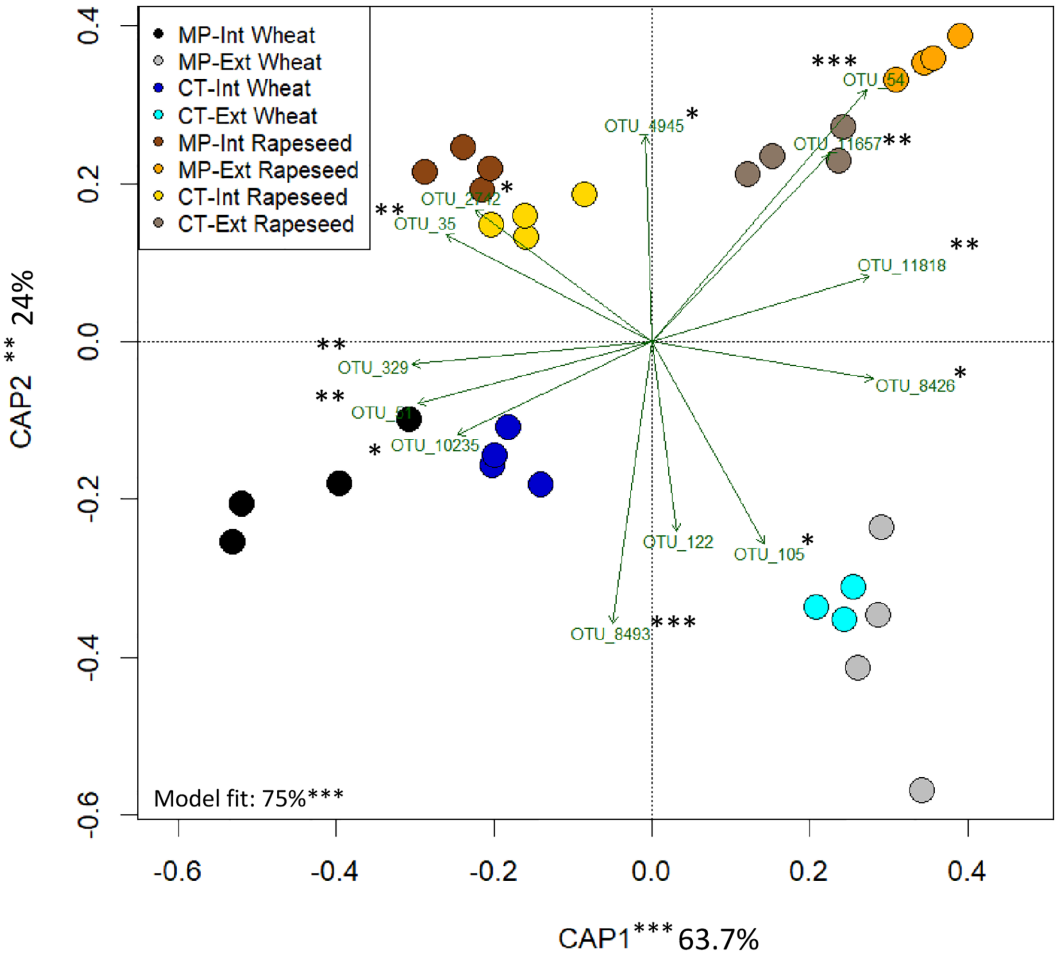

Figure 7. Distance-based redundancy analysis (dbRDA) based on the expression of 20 selected lettuce genes (ΔCt values) associated with oxidative and biotic stress signaling pathways using the relative abundance of the most abundant bacterial and archaeal OTUs as explanatory variables. Lettuce (cv. Tizian) was grown for 10 weeks in different long-term soil treatments from LTE Bernburg. MP- mouldboard plough, CT- cultivator tillage, Ext- extensive N-fertilization without fungicides/growth regulators, Int- intensive N-fertilization with pesticides/growth regulators. Tentative taxonomic identification of OTUs based on most similar BLAST hit: OTU_11657- *Acidovorax radicis* (100% similarity), OTU_54- *Dyadobacter endophyticus* (100%). OTU_11818- *Rhizobium nepotum* (99%), OTU_8426- *Sphingobium boeckii* (99%), OTU_105- *Methylobacillus rhizosphaerae* (95%), OTU_122- *Thermomarinilinea lacunifontana* (84%), OTU_8493- *Rhizobium mesoamericanum* (100%), OTU_10235- *Lacisediminimonas profundi* (98%), OTU_51- *Pseudarthrobacter phenanthrenivorans* (100%), OTU_329- *Ohtaekwangia kribbensis* (98%), OTU_35- *Rhizobacter gummiphilus* (100%), OTU_2742- *Nitrososphaera viennensis* (95%), OTU_4945- *Hydrogenophaga palleronii* (100%). Significant codes: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Management practice shaped soil and lettuce macro- and micro-nutrient contents

The impact of the tillage practice, fertilization intensity and the last standing field crop on the content of macro- and micro-nutrients in BS and in the shoot of lettuce grown in the respective soils was analyzed by three-way ANOVA. A significant effect of tillage practice was detected on all examined macro- and micro-nutrients in BS with the exception of Na (Table S12A, Supporting Information). For instance, CT soils exhibited higher C_{org} and K contents than MP. In addition, tillage practice affected pH in BS. Soils from long-term CT had a lower pH as compared to MP (7.57 *vs* 7.72; Table S12B, Supporting Information). Fertilization intensity had a significant impact on the pH and content of P, K, Cu, and Mn. Significant changes in the content of C_{org}, N_{total}, P, K, Na, Mg, Cu, Fe, Mn, and Zn were caused by the last standing field crop. The pH and the content of P, K, Na, Fe, Mn, and Zn were altered when considering the interaction of all three factors (Table S12A, Supporting Information). Macro- and micro-nutrient levels were sufficient and caused no deficiencies for the plants (Table S12B, Supporting Information). Only the content of Mn in MP-Ext-W was with 33.5 mg kg^{-1} soil below the recommended concentration (40 mg kg^{-1} soil; LUFA North Rhine-Westphalia). No impact of management practices as well as no significant differences between the treatments were found for C/N ratios.

Regarding lettuce shoots sampled after 10 weeks of cultivation, three-way ANOVA revealed that tillage practice significantly affected the content of all analyzed macro- and micro-nutrients except for Fe and Mn (Table S13A, Supporting Information). Fertilization intensity influenced the shoot nutrient contents significantly as well, except for Mg, Na, Cu, and Zn. A significant effect of the last standing field crop was only found for the micro-nutrients Cu and Zn. Most of the macro- and micro-nutrients were above the requirements for lettuce. However, a deficiency in shoots was found for N_{total}, P and K in all treatments which was most likely a consequence of limited soil amounts in the pot experiments (Table S13B, Supporting Information). In addition, Ca levels were close or below deficiency except for soils with MP-Int history (independent of the last standing field crop). When lettuce was grown in MP-Ext-R and MP-Ext-W soils, a low Fe content was found in shoots. Lettuce plants cultivated in CT soils exhibited a Zn content close or below the requirement level (except for CT-Ext-R; Table S13B, Supporting Information).

DISCUSSION

In the present study we investigated to which extent agricultural management practices (tillage practice, fertilization intensity, last standing field crop) used in the LTE Bernburg affect microbiota assemblages in soil and rhizosphere microhabitats and the performance of the model plant lettuce grown under controlled growth chamber conditions.

Agricultural legacy is reflected in soil and influences rhizosphere microbiota assemblage

The microbial communities in bulk soils sampled at LTE Bernburg were previously characterized (Sommermann *et al.* 2018; Babin *et al.* 2019). Despite differences in sampling year/time point, annual weather conditions, and sampling procedure (here: growth chamber incubation *vs* direct field sampling), we found similar major archaeal, bacterial (e.g. *Nitrososphaera*, *Bacillus*, acidobacterial Gp4 and Gp6) and fungal (*Chaetomium*, *Fusarium* and *Gibellulopsis*) taxa in bulk soils (Figs 4 and 5) as in the previous studies. This indicates that these taxa belong to the native soil microbiota in the LTE Bernburg. Focusing on results of previous studies conducted with soils from LTE Bernburg (Sommermann *et al.* 2018; Babin *et al.* 2019; Nelkner *et al.* 2019), we could corroborate the effects of tillage, fertilization intensity and cropping history on bulk and root-associated soil microbiota (Table 1, Fig. 2; Fig. S1, Supporting Information). However, the relative proportion of each factor in shaping the soil microbiota differed. We conclude that the soil microbiota in LTE Bernburg were constantly reshaped by the recurrent changes according to the long-term agricultural management practices, but that similar abundant taxa constituting the major fraction of the native microbiota can be detected at consecutive sampling time points.

In the present study, we aimed to unravel whether the agricultural legacy is reflected also in the assemblage of the rhizomicrobiota of lettuce. We observed that these microbiota clearly differed from bulk and root-associated soil and typical taxa were *Pseudomonas*, *Rhizobium*, Candidatus Saccharibacteria, and *Devosia* (Schreiter *et al.* 2014; Chowdhury *et al.* 2019; Fig. 4). However, only a few studies have addressed the fungal community in the rhizosphere of lettuce (Adesina *et al.* 2009; Debode *et al.* 2016). Our sampling technique allowed to differentiate between fungal communities distant and in close proximity to the root. We showed a strong effect of the sampled microhabitat on the fungal community composition. In line with Debode *et al.* (2016) we detected similar taxa, such as *Pseudogymnoascus*, *Pseuderotium*, *Mortierella* in the rhizosphere of lettuce (Fig. 5). We could attribute the strong effect of the fertilization intensity on the fungal communities to the higher relative abundance of *Olpidium brassicae* (potentially also infectious to lettuce) in extensive compared to intensive fertilization treatments (Fig. 5, Tables 3 and 4; Table S9, Supporting Information). This difference was most remarkable in the rhizosphere (Fig. 3B, Table 1; Table S3, Supporting Information). The presence of *O. brassicae*, a known pathogen of *Brassicaceae*, in Bernburg soils is likely due to the crop rotation including rapeseed. *O. brassicae* has been described as member of the core microbiome of soils, rhizosphere and roots of rapeseed (Neupane *et al.* 2013; Bennett *et al.* 2014; Lay *et al.* 2018; Sommermann *et al.* 2018; Floc'h *et al.* 2020). Lay *et al.* (2018) found a decreased relative abundance of *O. brassicae* in canola roots when grown in densely seeded plots, which was explained by a higher concentration of fungicides since seeds were fungicide etched. This could explain why we observed a higher relative abundance of *O. brassicae* in extensive treatments where no fungicides are applied in contrast to intensive treatments. However, the lettuce plants grown in extensive soils were healthy and exhibited no visible disease symptoms. They also showed higher biomass than lettuce grown in intensive treatments. One reason might be that the expected pathogenic effect of *O. brassicae* was balanced by the higher relative abundance of Glomeromycota (Table S5, Supporting Information), which are well-known plant growth-promoting (PGP) arbuscular mycorrhizal fungi (Begum *et al.* 2019), in extensively as compared to intensively treated soils. This might be similarly promoted by the absence of fungicides in extensive soils (Trappe, Molina and Castellano 1984). Another reason might be related to the host specificity. Hartwright *et al.* (2010) showed that brassica-infecting *Olpidium* isolates differed phylogenetically from lettuce-infecting isolates and could not infect lettuce plants. Therefore, we conclude that the dominance of *O. brassicae* in the rhizosphere of lettuce grown in extensively

fertilized soils was not detrimental for the plants. It should be noted that the fungal rhizosphere community in our study partially displayed a very low diversity, which was probably an artefact from root washing prior to the Stomacher treatment. It is likely that this washing step removed most of the fungal cells except those strongly attached to the roots, such as *Olpidium* zoospores. Therefore, the root-associated soil, collected before washing the roots, is likely more suitable to assess the effect of agricultural management practices on fungal communities.

The tillage practice and last standing field crop were identified as the main drivers for fungal community compositions in root-associated soils (Table 1). The treatment MP-Ext (in R and W) exhibited, besides *Olpidium*, also a high relative abundance of Glomeromycota in root-associated soils (Table S5, Supporting Information). The genus *Mortierella*, a common saprotrophic soil fungus, was found as one of the dominant taxa in root-associated soils of intensive treatments (Table S5, Supporting Information), which is in line with previous studies (Antweiler *et al*. 2017; Sommermann *et al*. 2018; Grządziel and Gałązka 2019). Detheridge *et al*. (2016) found, similar to our study, a negative correlation of N-levels in soils with arbuscular mycorrhizal fungi but a positive correlation with *Mortierella*. Biocontrol activity and PGP traits have been shown for *Mortierella* strains (Alström 2000; Osorio and Habte 2001; Tamayo-Velez and Osorio 2017; Ozimek *et al*. 2018). We could identify OTUs strongly related to *Trichoderma petersenii, T. paraviridescens* (both <0.5% relative abundance) and *Mortierella elongata* as indicators for cultivator tillage treatments in root-associated soils (Table 3). These genera are known for their PGP properties and inducing systemic resistance against plant pathogens (Harman *et al*. 2004; López-Bucio, Pelagio-Flores and Herrera-Estrella 2015; Li *et al*. 2018; Bahramisharif and Rose 2019; Zhang *et al*. 2020). Therefore, they might have contributed to the observed higher lettuce biomass in cultivator tillage compared to mouldboard plough treatments (except for CT-Ext-R *vs* MP-Ext-R; Table 5). However, to which extent these OTUs contributed to plant growth remains unexplained and should be further investigated in studies comprising microbial cultivation and isolation.

In contrast to fungal communities, rhizosphere bacterial and archaeal communities were primarily shaped by tillage (Table 1, Fig. 3A). Taxonomic assignment revealed that the difference was mainly based on the enormous enrichment of *Pseudomonas* (Fig. 4), especially two OTUs with closest affiliation to *Pseudomonas brassicacearum* (OTU_10699) and *P. corrugata* (OTU_11269) in CT-Int-W, CT-Ext-R and CT-Int-R (Table S4, Supporting Information). In another growth chamber experiment that we conducted simultaneously, we detected OTU_11269 in a higher relative abundance in the rhizosphere of lettuce when cultivated in mineral *vs* organic fertilized soils (Windisch *et al*. 2021). A positive correlation between OTU_11269 relative abundance and root exudation of succinic acid, yet a negative correlation with fumaric acid and various amino acids was revealed. The present study used soils with different agricultural history and from a different site, however, we postulate that lettuce grown in CT-Int-W, CT-Ext-R, CT-Int-R had a higher exudation of certain dicarboxylates, such as succinic or malic acid which have been shown to attract *P. fluorescens* resulting in its enrichment in the rhizosphere (Oku *et al*. 2014). Species-level identification of *Pseudomonas* based on 16S rRNA gene sequences is difficult. Nevertheless, OTU_11269 and OTU_10699 were both placed with more than 99% similarity into the *P. corrugata*-subgroup which is part of the *P. fluorescens*-complex harboring biocontrol and PGP activity (Garrido-Sanz *et al*. 2016). The 'cry-for-help' hypothesis suggests that stressed plants assemble beneficial microbes in the rhizosphere by changing their root exudate composition (Pascale *et al*. 2019; Rolfe, Griffiths and Ton 2019). Accordingly, the high relative abundance of *Pseudomonas* in the rhizosphere of lettuce plants grown in CT-Int-W, CT-Ext-R and CT-Int-R could indicate that plants were facing abiotic or biotic stresses. An increased resource investment in defense reactions requires a tradeoff elsewhere (Huot *et al*. 2014) which could explain the reduced growth of these lettuce plants compared to CT-Ext-W. An alternative explanation could be that the reduced growth and altered gene expression observed in plants grown in CT-Int-W, CT-Ext-R and CT-Int-R compared to CT-Ext-W was a direct response to the relative dominance of *Pseudomonas* in the rhizosphere. There is a narrow gap between PGP and pathogenic bacteria especially in the genus *Pseudomonas*, as for instance similar strategies with respect to establishment in the rhizosphere and effects on plant immunity are employed which might cause deleterious plant effects even by non-pathogenic strains (Brader *et al*. 2017; Passera *et al*. 2019; Yu *et al*. 2019). However, since we do not have any chronological information about colonization events in the rhizosphere, it remains beyond the scope of this study to elucidate the actual reason behind the observed enrichment.

Taken together, regarding the rhizosphere, we could confirm our hypothesis that the last standing field crop, tillage and fertilization intensity significantly affected the rhizomicrobiota assemblage. This supports the idea that soil microbiota shaped by long-term farming practices form a soil-borne legacy (Bakker *et al*. 2018) influencing the rhizosphere microbiota assemblage in this case of lettuce. The present results complement our previously published study about the effect of long-term fertilization practice on the rhizosphere microbial community of lettuce (Chowdhury *et al*. 2019). Our results are also in accordance with a recent study on the rhizosphere of barley grown under greenhouse and field conditions in soils of LTE Bernburg (Bziuk *et al*. 2021). Since we performed a controlled growth chamber experiment with sieved soils and adjusted N concentration before cultivation, we speculate that the soil legacy is mainly conveyed via soil microorganisms to the rhizosphere. However, further tests are needed to better distinguish between the biotic and abiotic agricultural legacy. We found *Sphingomonas* and acidobacterial subgroup Gp4 as indicators for mouldboard plough in the rhizosphere which were previously reported also by Babin *et al*. (2019) in bulk soils of LTE Bernburg under mouldboard plough suggesting that the effect of agriculture extends also to the rhizosphere of plants not included in the crop rotation. Interestingly, the treatment-dependent indicator OTUs found in this study in the rhizosphere were different from the indicators in bulk soil. This points to an influence of the plant possibly via management-dependent changes in root exudation (Chaparro, Badri and Vivanco 2014; Neumann *et al*. 2014; Windisch *et al*. 2021). The strong response of the plant to contrasting fertilization intensities observed in this study (next section) might therefore interfere with the assemblage of the rhizosphere microbiota explaining why bacterial and archaeal communities were affected by fertilization intensity only in rhizosphere and not in bulk soils.

Agricultural legacy affects lettuce performance

After cultivation of lettuce for 10 weeks in soils from different long-term agricultural management in the growth chamber, we observed treatment-dependent differences in lettuce growth, especially between intensive and extensive treatments (Table 5). In accordance to plant growth, we found that also plant gene

expression was mainly affected by fertilization intensity (Figs 6 and 7; Table S11, Supporting Information). Higher expression of genes with putative functions in Fe transport, N assimilation, and (a)biotic stress regulation coupled with higher biomass in plants grown in extensively compared to intensively fertilized soils could indicate a higher tolerance or an induced physiological status (defense priming) (Martinez-Medina *et al.* 2016). The analyzed responses in shoots indicate a systemic, rather than a local induction and this might be a reflection of combinations of belowground interactions of the plant roots with a multitude of microbes in the rhizosphere. The crucial role of soil microorganisms for pathogen control, plant nutrition and performance is well known (Mendes, Garbeva and Raaijmakers 2013; Berg *et al.* 2014). The relative abundances of 13 major rhizosphere OTUs were significantly correlated to the gene expression patterns observed (Fig. 7). Most of the OTUs linked to gene expression of lettuce grown in extensive treatments have been previously isolated from different plant species and have been shown to possess PGP properties. For example, *Acidovorax radicis*, has been isolated as an endophyte and can induce systemic responses in barley (Han *et al.* 2016). Similarly, *Dyadobacter endophyticus* was isolated from maize as endophyte (Gao *et al.* 2016). *Rhizobium nepotum* and *R. mesoamericanum* have been described as nodule forming, N-fixing bacteria in legumes (Moulin *et al.* 2013). On the other hand, the OTUs showing significant correlations to the plant gene expression profiles in intensive treatments have been described as typically soil-associated microbes. For example the genus *Ohtaekwangia* has been detected as an indicator in *Pennisetum setaceum* rhizosphere in semiarid soils (Rodríguez-Caballero *et al.* 2017). *Ohtaekwangia* has been described previously to produce marinoquinolines—chemical compounds with antibiotic, antifungal, and insecticidal properties, which could play an important role in the rhizosphere (Okanya *et al.* 2011). However, as studies elucidating molecular defense mechanisms in lettuce are lacking, it is difficult to relate the observed relative abundances of particular microbial taxa directly to the gene expression in the leaves. Nevertheless, the results support our hypothesis that long-term agricultural management practices could have affected lettuce performance via changes in the soil microbiota (biotic legacy).

A lack of macro-nutrients (N, P, K) was detected at the end of the experiment in plant tissues in all treatments (Table S13, Supporting Information), although bulk soil nutrient analyses confirmed sufficient starter nutrient levels for lettuce cultivation (Table S12, Supporting Information). Since the respective macro-nutrient deficiencies were recorded in all treatments, and were not related to growth differences between treatments with particularly high or low biomass production (e.g. CT-Ext-W *vs* MP-Int-W or MP-Ext-R *vs* MP-Int-R), we propose that the observed lack after 10 weeks of cultivation was not responsible for differences in lettuce growth. This may point towards the involvement of the biotic legacy. However, long-term pesticide and growth regulator application in the intensive treatments could have had a negative impact on lettuce growth. Lettuce is known to be a sensitive plant species that might be affected by pesticides that persist in soil and exhibit toxicity to non-target organisms (Silva *et al.* 2019). A similar situation may apply for sulfonylurea herbicides applied exclusively for the last standing field crop wheat, which can cause re-plant problems in dicotyledonous plants due to delayed microbial degradation depending on soil pH, soil moisture and temperature (van Acker 2005). An increase in organic matter in soils under cultivator tillage, as reported from LTE Bernburg (Deubel, Hofmann and Orzessek 2011), can lead to a higher persistence of pesticides. We observed higher microbial biomasses in soils under cultivator tillage compared to mouldboard plough (data not shown). Therefore, pesticide persistence in cultivator tillage soils might have been compensated by a higher microbial activity and thus higher pesticide degradation capacity (Alletto *et al.* 2010). This might explain why we observed a better lettuce growth among the intensive treatments under cultivator than under mouldboard plough and similarly better growth in MP-R than in MP-W.

Despite technological progress regarding soil-microbe-plant interactions over the last years, our understanding of the complex processes in the rhizospheres of crop plants is still in its infancy. For instance, we observed here different responses of the plant and the associated microbiota to tillage in dependence on the last standing field crop and/or fertilization intensity. Furthermore, the analysis of the effect of the last standing field crop was hindered by interference with fertilization and pesticide application since every crop has different requirements. This underlines that interactions and synergies between agricultural management practices render agro-ecosystems highly complex environments.

CONCLUSIONS

In conclusion, by cultivating lettuce under controlled conditions in a growth chamber, we have shown that the long-term agricultural legacy affects both the soil microbiota and the assemblage of the plant rhizomicrobiota as well as the lettuce performance. The results from the growth chamber experiments cannot be translated directly to the field where the effects of biotic and abiotic factors are much more complex. However, our experimental setup provides a snapshot of a multitude of responses, interactions and synergies taking place between agricultural practice, soil (abiotic and biotic components) and the plant. In order to identify causal relationships at the bottom of this entangled interplay, a large amount of more simplified experiments is needed. The rhizosphere is a highly important zone for plant performance due to interactions with soil microorganisms. Changes in agricultural management will affect soil and rhizosphere microbiota assemblage resulting in differences in plant performance. Our work adds to the increasing knowledge of how soil microbiota can be managed by agricultural practices which could be harnessed for sustainable crop production.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Conflicts of interest. None declared.

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