



Impact of high carbon amendments and pre-crops on soil bacterial communities

Catherine W. Kamau¹ · Richard van Duijnen² · Christoph A. O. Schmid¹ · Helga E. Balázs^{1,3} · Julien Roy^{4,5} · Matthias Rillig^{4,5} · Peter Schröder¹ · Viviane Radl¹  · Vicky M. Temperton² · Michael Schloter^{1,6}

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Abstract

A 2-year outdoor mesocosm experiment was carried out to determine the effects of high C amendments (HCAs; wheat straw and sawdust) compared to a control with no addition of HCAs (no-HCA) and 2 different crop rotation systems (spring barley/winter barley and faba bean/winter barley) on soil bacterial communities using a molecular barcoding approach. Samples were analyzed after pre-crop harvest (T1) and harvest of winter barley (T2). Our data demonstrate a clear drop in bacterial diversity after winter barley harvest in the no-HCA and wheat straw treatment compared to the pre-crops. Sawdust application had a stabilizing effect on bacterial diversity compared to the pre-crops and induced an increase in carbon (C) stocks in soil which were however negatively correlated with yields. Main responders in the no-HCA and wheat straw treatment compared to the pre-crops were bacteria of the phyla Actinobacteria and Bacteroidetes which were enriched and bacteria belonging to Firmicutes, Gemmatimonadetes, Proteobacteria, and Gemmatimonadaceae which were depleted. Overall differences between wheat straw-amended and no-HCA control samples were small and included single ASVs from various phyla. In sawdust-amended samples, only a shift of some Proteobacteria families was observed compared to the no-HCA control. Overall, pre-crop plant species had small influence on the observed response pattern of the soil microbiome towards the amendments and was only visible for wheat straw.

Keywords High carbon amendment · Crop rotation · Soil microbiome · Soil multifunctionality

✉ Viviane Radl
viviane.radl@helmholtz-muenchen.de

¹ Research Unit for Comparative Microbiome Analysis, Helmholtz Zentrum München GmbH, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

² Institute of Ecology, Leuphana University of Lüneburg, Universitätsallee 1, 21335 Lüneburg, Germany

³ Botanical Garden “Alexandru Borza”, 42 Republicii St, 400015 Cluj-Napoca, Romania

⁴ Institute for Biology, Ecology of Plants, Freie Universität Berlin, 14195 Berlin, Germany

⁵ Berlin-Brandenburg Institute of Advanced Biodiversity Research, 14195 Berlin, Germany

⁶ Technical University of Munich, Chair of Soil Science, Emil-Ramann-Straße 2, 85354 Freising, Germany

Introduction

The growing human population in the last century all over the world has necessitated an increase in agricultural productivity resulting in higher demands for fertilizer (Galloway and Cowling 2002; Smil 2002). However, the use efficiency of many fertilizers is still low. Consequently, the increased fertilizer application rates have induced high nutrient levels not only in agroecosystems but also in many other parts of our environment, resulting in negative consequences for many ecosystems and also affecting human health (Di and Cameron 2002; Lu and Tian 2017). For example, as a result of nitrogen (N) surplus and low N use efficiency, nitrate leaching and denitrification rates have increased, and negative feedback loops have been reported. These include soil acidification as well as reduced soil microbial diversity and activity, leading to reduced crop yields and quality as well as to reduced C sequestration rates (Goulding 2000; Guo et al. 2010; Ramirez et al. 2012; Tian et al. 2012). As possible, mitigation strategies and management practices such as crop

rotation or the use of high carbon amendments (HCAs) (Congreves et al. 2013; Malcolm et al. 2019) have been proposed.

Crop rotation enhances soil quality, influences N uptake and nutrient use efficiency, and increases soil microbial biomass and diversity (Garbeva et al. 2004; Ma et al. 2003; O'Donnell et al. 2001). Rotations differing in functional plant types, e.g., legumes or cereals, induce the development of different microbial communities in the soil (Larkin 2015). This is mainly as a result of differences in root development, plant-microbe interactions, exudation patterns, and rhizodeposition (Bakker et al. 2013; Santoyo et al. 2017). Here, differences have been mainly described for a number of microbial taxa, namely, r-strategists, including many Proteobacteria (Fierer et al. 2007).

HCAs, with a C:N ratio higher than 25–30, promote microbial N immobilization by stimulating microbial N uptake from the soil to maintain the stoichiometric demands for nutrients of the soil microbiome (Kuzyakov et al. 2000). Consequently, HCAs induce a fast immobilization of mineral N in the soil (Shindo and Nishio 2005) (up to 42 kg N ha⁻¹), by increasing microbial N pools (Reichel et al. 2018). In addition, HCAs increase the organic matter content and availability of C in the soil, which in turn increases microbial biomass and activity (Larkin 2015). HCAs mainly stimulate microbes which are involved in the degradation of complex C-rich compounds like cellulose and lignin, often characterized as k-strategists, for example, Firmicutes and Acidobacteria (Pascault et al. 2013). Interestingly, some authors also report an increase in microorganisms capable of suppressing soilborne plant pathogens in soils following HCA application (Larkin 2015).

Despite the fact that crop rotation and HCA amendments induce positive feedback loops in soil by stimulating different microbial groups, a combination of both forms of management has rarely been considered. Most studies on the combined effects of both forms of management have focused on the influence of incidence of soilborne pathogens (Bernard et al. 2012; Su et al. 2020) or addressed the effects of organic fertilizer (Ashworth et al. 2017; Soman et al. 2017). Still, the combined effects of management strategies for N retention on the soil microbial communities remain unclarified (Siedt et al. 2021). Therefore, in the present study, we simulated a management practice based on crop rotation and HCA application in a mesocosm experiment. We focused on the legacy effect of the crops in the first crop cycle (pre-crop effect) as well as the effects of HCAs. We used 2 pre-crops, namely, faba bean and spring barley, which were selected based on their symbiotic traits (mycorrhizal and rhizobial and only mycorrhizal, respectively), in the first crop cycle and winter barley in the second cycle. Sawdust and wheat straw were used as HCAs and were applied 1 week after harvesting of the pre-crops. A molecular barcoding approach was used to analyze bacterial

communities in the original soil as well as in the cropped soil at two sampling time points, i.e., after pre-crop harvest in the first crop cycle and after winter barley harvest in the second crop cycle, following the recommendations for soil microbiome analyses (Nannipieri et al. 2019).

Materials and methods

Site characteristics and experimental design

The mesocosm experiment was carried out in the experimental garden at the Leuphana University of Lüneburg (Lüneburg, Germany, 53°14' 23.8" N 10°24' 45.5" E). The mean annual rainfall and temperature were 718 mm and 9.2 °C, respectively. The mesocosm experiment was carried out in 2016 and 2017 and has been described in detail by van Duijnen et al. (2018). In short, square mesocosms with an edge length of 37.5 cm (top) and 26.5 cm (bottom), a height of 37 cm, and a volume of 38 L were used. The mesocosms were filled to a bulk soil density of ~ 1.1 g cm⁻³ using soil with a loamy, sandy texture characterized as a Cambic Luvisol from an experimental farm (upper 30 cm) in Kiel, Germany (54°19' 05.6" N 9°58' 38.8" E). The soil was homogenized by passing it through a 1-cm sieve before filling the pots. A mixture of catch crops (clover and lupine) was grown without fertilization in the growing season before the start of the experiment. In the previous season, maize fertilized with 40 m³ slurry (1.8% P, 3% N) and 100 kg/ha triple superphosphate (20% P) had been cultivated. At the start of the experiment, the soil had a total organic C content of 1.26%, a total N content of 0.14%, a C:N ratio of 9.2, and a pH of 6.0.

Mesocosms were sown with spring barley (*Hordeum vulgare* cv. Barke, Saatzeit Breun) and faba bean (*Vicia faba* cv. Tiffany, NPZ) in May 2016 and harvested in early autumn of the same year. Winter barley (*Hordeum vulgare*, cv. Antonella, Nordsaat Saatzeit) was sown in October 2016 in the same pots with the same soil and harvested in July 2017. To simulate a typical agricultural practice in Germany, plant densities per pot were adapted to 300 seeds/m² for spring barley, 45 seeds/m² for faba bean, and 240 seeds/m² for winter barley. The mesocosms were fertilized in accordance to standard agricultural practices in Germany on the sowing dates for spring barley (N: 75 kg/ha, K₂O: 130 kg/ha, P₂O₅: 40 kg/ha, MgO: 35 kg/ha, S: 98 kg/ha) and faba bean (N: 0 kg/ha, K₂O: 50 kg/ha, P₂O₅: 115 kg/ha, MgO: 35 kg/ha, S: 60 kg/ha). For winter barley, the fertilizer application (N: 160 kg/ha, K₂O: 100 kg/ha, P₂O₅: 70 kg/ha, MgO: 50 kg/ha, S: 86 kg/ha) was split and applied in March 2017 (40% of the total amount), beginning of May (40% of the total amount) and mid of May (20% of the total amount). All mesocosms were treated against slugs in June 2016 with 0.8 g Schneckenkorn (9.9 g/kg iron(III)-phosphate, Neudorff GmbH) and against aphids

in July 2016 with 200 mL diluted Spruzit Schädlingfrei per mesocosm (45.9 mg/L pyrethrin, Neudorff GmbH) to prevent plant damage. At the time point of pre-crop harvest, only above ground parts of the plants were removed from each mesocosm.

The HCAs, spruce sawdust and air-dry wheat straw, were applied 1 week after pre-crop harvest in September 2016 at a rate of 8.6 t/ha (137.6 g/mesocosm). Spruce sawdust was composed of 51% C, 0.1% N, and a C/N ratio of 539. Wheat straw contained 46% C, 0.7% N, and a C/N ratio of 71. The particle sizes of the wheat straw and sawdust were 5–10 cm and 1–2 cm, respectively. The HCAs were added to the top 10 cm of the soil, mixed into the soil and watered slightly to enhance incorporation. Further a control treatment (from now known as no-HCA) without the amendments was established, where only the watering and mixing was performed as described above. Harvesting was also performed as described above. As the mesocosms were placed outdoor, no additional watering of the mesocosms was performed during the experimental period.

Overall, 30 mesocosms were established, 15 for each pre-crop. Sampling was performed after pre-crop harvest (T1). Six soil samples per mesocosm were taken with a soil core (diameter 1 cm; 10 cm length) and pooled to form a composite sample. Each of the 15 pots per pre-crop were treated as true replicates ($n = 15$). All 15 samples per pre-crop were tested for homogeneity before HCAs were applied, and winter barley was cultivated. For each of the two HCAs as well as the no-HCA control, 5 mesocosms were established ($n = 5$). Sampling was performed after the harvest of winter barley (T2) as described for T1. In addition, six soil samples ($n = 6$) were taken from the original soil before the mesocosms were filled and labelled as T0. All soil samples were sieved at 5 mm and stored immediately at $-80\text{ }^{\circ}\text{C}$ until further use. Plant yields as well as C and N contents together with basic soil characteristics at the time points of sampling have been published by van Duijnen et al. (2018), and those used in our analysis are summarized in Supplementary Table S1.

DNA extraction and bacterial barcoding

DNA was extracted from 0.25 g of the pooled soil cores using the PowerSoil DNA extraction kit (MoBio Laboratories Inc., Carlsbad, California, USA) according to the manufacturer's instructions. Negative extraction controls were included using empty extraction tubes, to check for contamination during the procedure. The DNA concentration was quantified in duplicates using the Quant-iTTM PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, USA) following the manufacturer's protocol. The concentrations were measured at 520 nm using a SpectraMax Gemini EM Microplate Spectrofluorometer (Molecular Devices, CA, USA). DNA extracts were stored at $-80\text{ }^{\circ}\text{C}$ for further use.

Amplification of the V1–V2 region of the 16S rRNA gene was carried out using primers S-D-Bact-0008-a-S-16 (5'-AGAGTTTGATCMTGGC-3') and S-D-Bact-0343-a-A-15 (5'-CTGCTGCCTYCCGTA-3') described by Klindworth et al. (2013). PCR amplifications were carried out in triplicates. The reaction mix contained 1x NEBNext High Fidelity Master Mix (New England Biolabs, Ipswich, USA), 5 pmol of each primer, 0.3% bovine serum albumin (BSA), 5 ng of template DNA, and DEPC water to a final volume of 25 μL . The PCR conditions were the following: initial denaturation at $98\text{ }^{\circ}\text{C}$ for 30 s, followed by 25 cycles each at $98\text{ }^{\circ}\text{C}$ for 10 s (denaturation), $60\text{ }^{\circ}\text{C}$ for 30 s (annealing), and $72\text{ }^{\circ}\text{C}$ for 30 s (elongation), followed by $72\text{ }^{\circ}\text{C}$ for 5 min (final elongation). Negative controls containing DEPC water instead of template DNA were included and amplified using the same PCR conditions. The quality of the PCR amplicons was analyzed using 1% agarose gels. Triplicate PCR products were pooled and purified using the Nucleospin Gel and PCR Cleanup Kit (Macherey- Nagel, Düren, Germany) following the manufacturer's instructions (with the modification of an extended elution time of 10–15 mins). Presence of primer-dimers and amplicon sizes was checked using the DNF-473 Standard Sensitivity NGS Fragment Analysis Kit (1–6000 bp) on a Fragment Analyzer Instrument (Agilent Technologies, California, USA) and quantified using the Quant-iTTM PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, USA) and SpectraMax Gemini EM Microplate Spectrofluorometer (Molecular Devices, California, USA).

The indexing PCR was performed using 10 ng per sample of the 16S rRNA gene amplicons using the Nextera XT Index Kit v2 (Illumina, Inc., San Diego, USA) according to the manufacturer's instructions. For the indexing PCR, 8 cycles were used. The PCR products were purified and both quality and quantity checked as described above. Purified PCR products were diluted to a concentration of 4 nM, pooled equimolar, and sequenced using the MiSeq Reagent kit v3 (600 cycles) (Illumina Inc., San Diego, USA) for paired-end sequencing.

Bioinformatic and statistical analysis

Analysis of the sequence data was done using the QIIME 2 (V. 2018.8.0) software package (Caporaso et al. 2010). Primers and adaptors were removed from the de-multiplexed raw sequence data using AdapterRemoval (V. 2.1.7) (Lindgreen 2012). DADA2 plugin (V. 1.8.0) (Callahan et al. 2016) was used to quality and length filter the reads. The forward and reverse reads were trimmed at 10 and 212 bp and 10 and 170 bp, respectively. PhiX contamination and chimeric sequences were also removed during the quality filtering. The reads were denoised, merged, and inferred into amplicon sequence variants (ASVs) at $\geq 99\%$ similarity. The

ASVs were taxonomically annotated against the SILVA database (V. 132) (Quast et al. 2013).

In total, 11,190,949 raw sequence reads were obtained. The number of reads varied from 66,919 to 411,421 reads per sample. After pre-processing during taxonomy assignment, a total of 6,494,342 reads remained. Further analysis of the raw sequences was done in R (V. 3.6.0). ASVs (99% sequence identity) assigned to chloroplasts and mitochondria were removed. ASVs present in the negative controls and those identified as singletons were also removed, resulting in 19,181 ASVs. The remaining reads were rarefied to a sampling depth of 33,614 (lowest read count) using the rarefy function of the vegan package (V. 2.5-6) (Oksanen et al. 2019), resulting in a total of 18,834 ASVs. This sampling depth was sufficient to cover bacteria diversity as shown by the rarefaction curves (Supplementary Figure S1).

Sequence data was imported to R (V. 3.6.0) (R Core Team 2020) using the phyloseq package (V. 1.28.0) (McMurdie and Holmes 2013). Shannon diversity was used as a measure of α -diversity. ASVs evenness was calculated using Pielou's evenness index. Statistical significance of α -diversity measures between the experimental groups was determined by pairwise comparisons of the means using Kruskal-Wallis test (p value < 0.05) and Wilcoxon test (p value < 0.05), as Shapiro-Wilk test revealed a non-normal data distribution of the samples. Differences in bacterial community composition were visualized by ordination using principal coordinate analysis (PCoA) with Bray-Curtis dissimilarity matrix. To compare the effects of crop rotation and the pre-crops on the bacterial community composition, a permutational multivariate analysis of variance (PERMANOVA: adonis) was performed using the R package vegan (V. 2.5-6) (Oksanen et al. 2019) at 999 permutations. PCoA, PERMANOVA, and alpha diversity tests were performed using subsets of the generated data to better evaluate the effects of the pre-crops and HCAs at the various sampling time points. First, an overall analysis (including all 3 time points, pre-crops, and HCAs) was done. This was followed by an analysis of time point T1 to evaluate the pre-crop effect after the first crop harvest. Finally, the analysis of time point T2 was carried out to determine the combined effect of pre-crops and HCA addition.

Canonical analysis of principle components (CAP) was carried out using the R package vegan (Oksanen et al. 2019) on the samples at T2. The analysis was constrained to soil N content, soil C content, and soil C:N ratio while conditioning on the other parameters. A permutation-based ANOVA test at 999 permutations was done to calculate the significance of the effect of experimental factors on the bacterial communities. We also ran mantel tests to determine the correlations between the soil properties and bacterial community composition at T2.

Differential abundance analysis was also carried out using the R package DESeq2 (V. 1.24.0) (Love et al. 2014) in R. Differentially abundant taxa between the HCA samples at T2

and the same samples at T1 (before amendment) were determined. The Benjamini-Hochberg method (Benjamini and Hochberg 1995) was used to adjust the p values for multiple testing. Only ASVs with a \log_2 -fold change > 2.0 and an adjusted p value < 0.001 were considered to be significantly differentially abundant. The results of this analysis were visualized using volcano plots generated using the EnhancedVolcano (V. 1.5.4) package (Blighe et al. 2020) in R. ASVs exclusively enriched and depleted in the wheat straw and sawdust treatments and not in the no-HCA control were also determined (Supplementary Tables S2 and S3, respectively).

Results

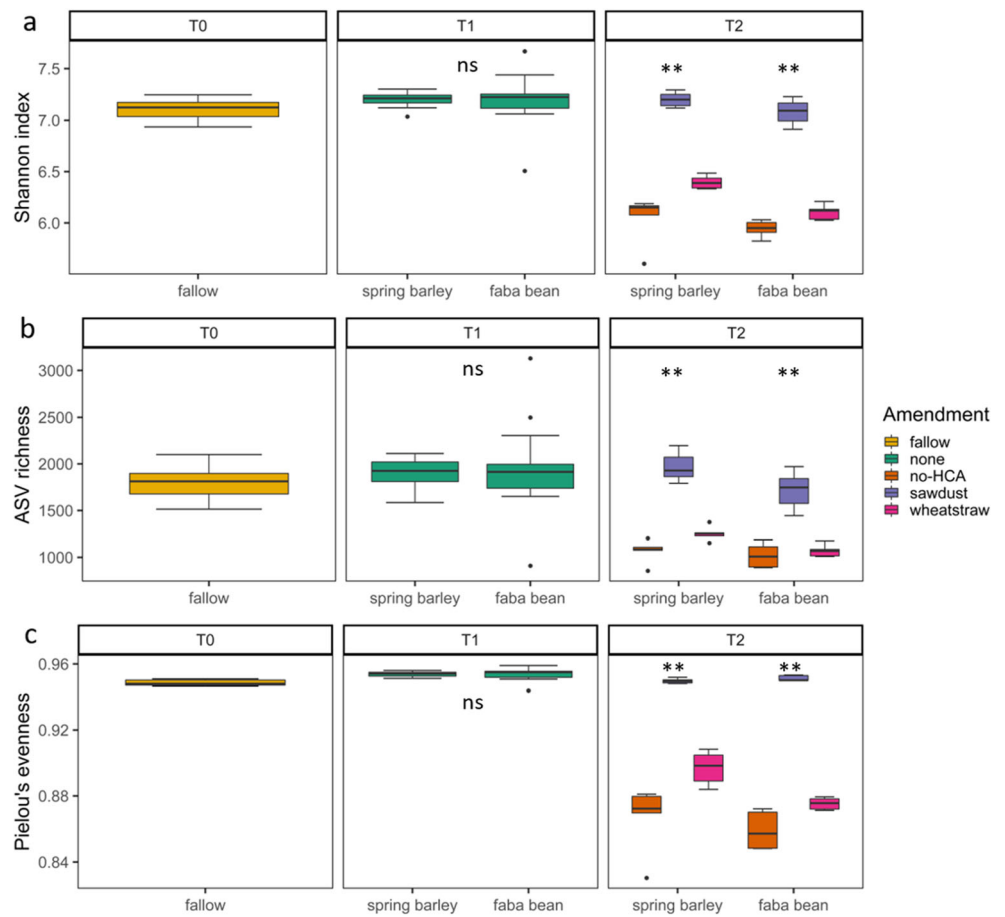
Impact of crop rotation and HCAs on the bacterial diversity and community composition

Bacterial α -diversity was analyzed using Shannon index, ASV richness, and Pielou's evenness index. α -Diversity measures at T1 did not differ for faba bean or spring barley and were comparable to T0 (Kruskal-Wallis, $p > 0.05$). At T2, a significant drop in α -diversity (Kruskal-Wallis, $p < 0.01$) was observed in samples where wheat straw was added as well as in the no-HCA treatment independent of the pre-crop (Fig. 1). In contrast, the application of sawdust resulted in a preservation of the high α -diversity observed for the pre-crops and at T0 with significantly higher values compared to the no-HCA and wheat straw treatment (Kruskal-Wallis, $p < 0.01$) (Fig. 1).

Bray-Curtis PCoA analysis was carried out to investigate the effects of the treatments on bacterial community composition (Fig. 2; Supplementary Fig. S2). PERMANOVA using distance matrices revealed that effects of HCAs on bacterial community composition ($R^2 = 0.176$; $p = 0.001$) were higher than that of the pre-crops ($R^2 = 0.078$, $p = 0.011$). The cultivation of the pre-crop did not change bacterial community composition significantly at T1 compared to T0. However, the addition of the amendments induced significant shifts mainly in the no-HCA controls and the samples where wheat straw had been applied at T2 compared to T1 ($R^2 = 0.565$; $p = 0.0001$). The sawdust-amended samples at T2 still clustered closely together with samples from T0 and T1. Overall, at both sampling time points, little variation was explained by the pre-crops ($R^2 = 0.105$; $p = 0.001$ and $R^2 = 0.066$; $p = 0.058$ for T1 and T2, respectively).

We utilized CAP analysis at T2 to determine the interaction of soil parameters, i.e., soil C and N content, as well as soil C:N ratio on the bacterial community composition (Fig. 3). Permutational multivariate analysis of variance (PERMANOVA: adonis) revealed that the soil C content ($R^2 = 0.3237$; $p = 0.001$) and C:N ratios ($R^2 = 0.3889$; $p = 0.001$) were strongest correlated with bacterial community

Fig. 1 Boxplot showing the influence of the pre-crops ($n = 15$) and addition of high C amendments ($n = 5$) at T1 and T2, respectively. Alpha diversity evaluated using Shannon index (a), ASV richness (b), and Pielou's evenness index (c). Further diversity levels at T0 (fallow) is presented ($n = 6$). Statistical significance was calculated using Wilcoxon test (between 2 groups) and Kruskal-Wallis (between 3 groups) and is illustrated with ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$



composition at T2 leading to a separation of bacterial communities from soil samples with sawdust as an amendment and the other treatments (no-HCA and wheat straw). Mantel tests based on Spearman's rank correlation confirmed this observation (soil C content: mantel statistic $r = 0.4228$; $p = 0.001$; C:N ratio: mantel statistic $r = 0.6145$;

$p = 0.001$). The influence of the soil N content was minor and negatively correlated (mantel statistic $r = -0.02551$; $p = 0.584$) with soil C content and soil C:N ratio. In addition, the winter barley yield was negatively correlated (mantel statistic $r = -0.1031$; $p = 0.974$) with soil C content and soil C:N ratio.

Fig. 2 Principal coordinate analysis (PCoA) ordination plot based on Bray-Curtis dissimilarities showing the impact of sampling time points: T0 ($n = 6$), T1 ($n = 15$ per pre-crop), and T2 ($n = 5$ per pre-crop-amendment combination). The filled and empty symbols at T1 and T2 represent faba bean and spring barley pre-crops, respectively. Analysis was done on the whole dataset. Results on permutational multivariate analysis of variance (PERMANOVA) are illustrated with * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for all treatments

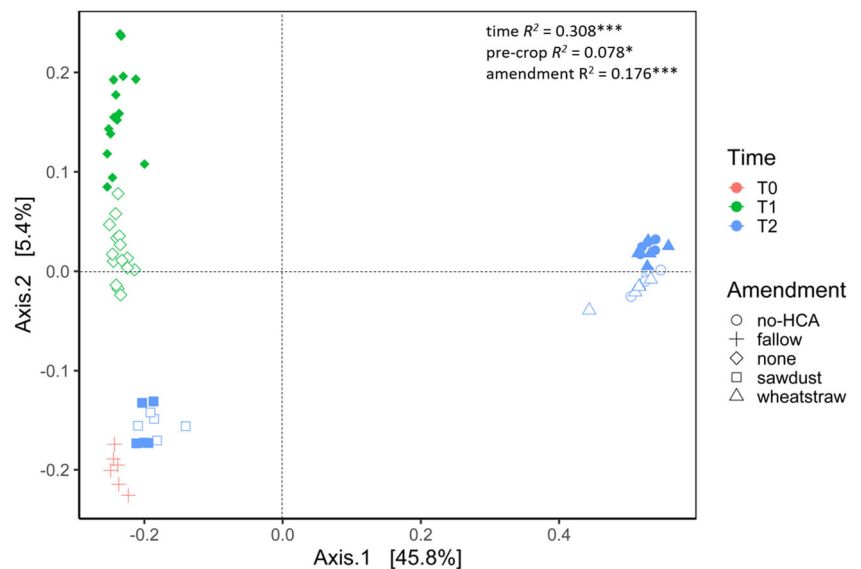
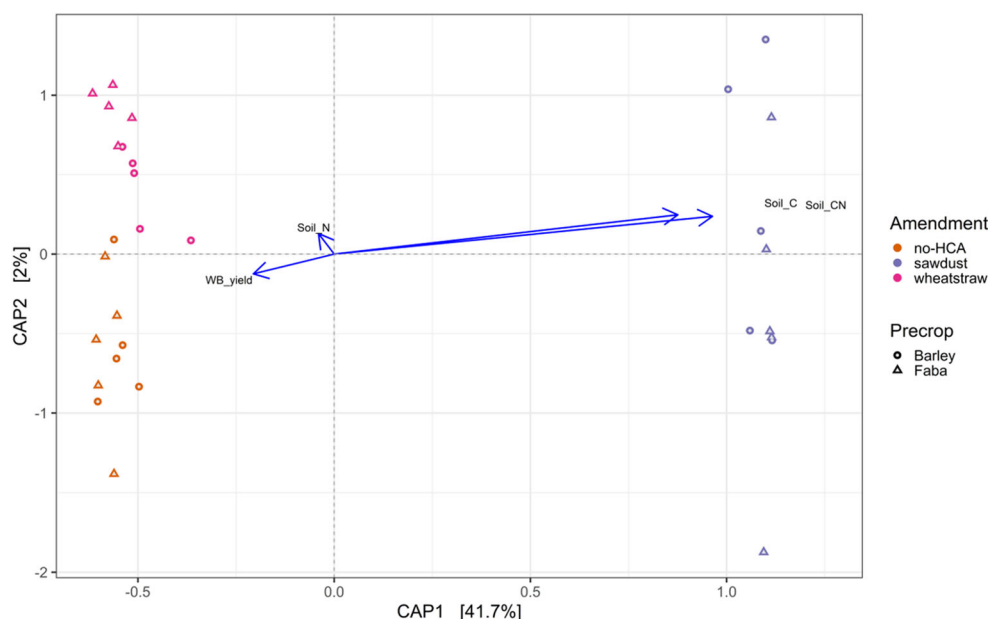


Fig. 3 Canonical analysis of principle components calculated using Bray-Curtis distances showing the effect of soil C content, soil N content, C:N ratio, and winter barley (WB) yield on bacterial community composition at T2 ($n = 5$ per pre-crop amendment combination). Axes values indicate % of total variation explained by the corresponding axis



Microbial responders to addition of HCAs

Based on the analysis of the obtained ASVs, a total of 29 bacterial phyla, 78 classes, 125 orders, 249 families, 414 genera, and 320 assigned species were detected. The most dominant phyla across all samples were Actinobacteria (39.4%), Proteobacteria (26.5%), Bacteroidetes (6.6%), Acidobacteria (6.3%), Chloroflexi (5.8%), Gemmatimonadetes (5.8%), Firmicutes (4.8%), Saccharibacteria (1.7%), and Cyanobacteria (1.2%). The relative abundance profiles of the 9 most abundant families at T1 was similar for both pre-crops (Fig. 4) including ASVs linked to Streptomycetaceae, Nocardioidaceae, Pseudonocardiaceae and Micromonosporaceae (Actinobacteria), Bradyrhizobiaceae and Xanthobacteriaceae (Proteobacteria), Bacillaceae (Firmicutes), Chitinophagaceae (Bacteroidetes), and Gemmatimonadaceae (Gemmatimonadetes). At T2 in the no-HCA and wheat straw-amended samples, an

increased relative abundance of ASVs linked to all major families belonging to Actinobacteria as well as for Chitinophagaceae was observed, whereas mostly ASVs belonging to the families of Gemmatimonadaceae Xanthobacteriaceae and Bacillaceae were reduced in relative abundance. In contrast, sawdust-amended samples exhibited comparable profiles to those observed in the pre-crops.

Differential abundance analysis was conducted to determine ASVs which were strongly influenced by the addition of HCAs at T2. Each amendment treatment was compared, and differences between time point T1 (before HCA addition) and time point T2 (after harvest of winter barley) were calculated (Fig. 5). Enriched (eASVs) and depleted (dASVs) ASVs were identified, representing ASVs that increased and decreased significantly in relative abundance. Overall, 203 and 182 eASVs and 151 and 101 dASVs were detected as

Fig. 4 Heatmap showing the relative abundance of the 9 most abundant bacterial families at T1 ($n = 15$ per pre-crop) and T2 ($n = 5$ per pre-crop and amendment combination)

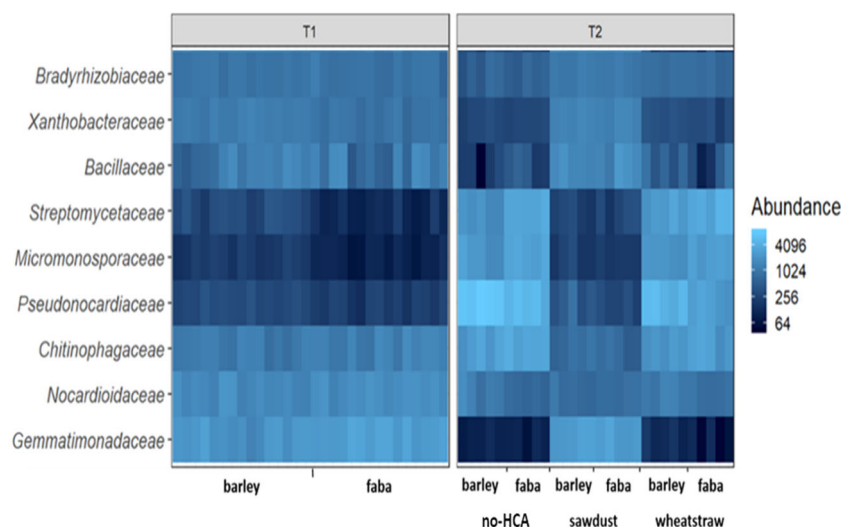
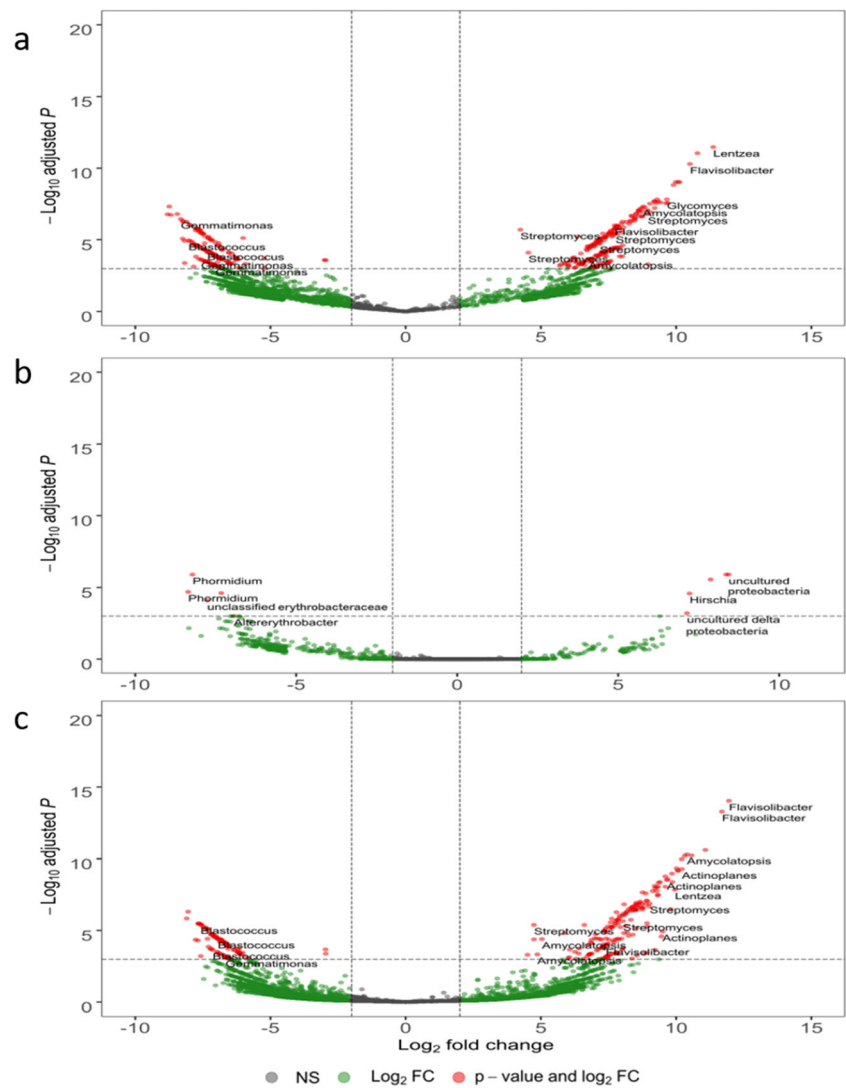


Fig. 5 Volcano plots showing differentially abundant ASVs (99% similarity) significantly enriched and depleted after addition of: **a** wheat straw, **b** sawdust, and **c** no-HCA at time point T2 compared to the same samples at T1 using DESeq2. For each treatment, samples from both pre-crops were merged as no significant differences were found for the pre-crops resulting in $n = 10$ per treatment. Each point represents an ASV, x-axis shows the abundance fold change, and the y-axis shows the $-\log_{10}$ of the adjusted p value. Gray dotted lines highlight cutoff thresholds of log fold changes of < -2 and > 2 , and p_{adj} of 0.001. Significantly enriched ASVs meeting both cutoff thresholds are in red. ASVs meeting only log fold change cutoff are in green. Nonsignificant ASVs are in gray. Taxonomic affiliation of major differentially abundant ASVs were added to the plots. The complete list containing all differentially abundant ASVs strongly influenced by wheat straw and sawdust treatments is available in Supplementary Table S2



responders in wheat straw-amended samples and controls (no-HCA), respectively. Interestingly, most ASVs responding with an increase or decrease in abundance could be linked to Actinobacteria. Whereas ASVs related to the genera *Lentzea*, *Amycolatopsis*, *Glycomyces*, *Actinoplanes*, and *Streptomyces* were positively influenced by the two treatments, ASVs linked to *Gemmatimonas*, *Phycococcus*, *Blastococcus*, and *Microthricum* were depleted. However, the most pronounced increase in abundance compared to T1 was found for a single ASV, which was linked to the genus *Flavisolibacter* (Chitinophagaceae). Although most of the taxa enriched in the wheat straw treatment at T2 compared to T1 were also enriched in the no-HCA control, single ASVs assigned to Proteobacteria (*Sphingomonas* and *Pseudoxanthomonas*), Actinobacteria (*Promicromonospora* and *Curtobacterium*), Firmicutes (*Bacillus*), and Chloroflexi (*Roseiflexus*) showed a positive response to wheat straw addition (Supplementary Table S2).

Fewer ASVs (5 eASVs and 5 dASVs) were enriched and depleted by the sawdust treatment compared to T1. Here, the most responding ASVs were mainly Proteobacteria. An ASV belonging to the genus *Hirschia* and an unclassified ASV from the order *Myxococcales* were enriched as a result of the sawdust application, while ASVs linked to *Skermanella* and *Phormidium* were depleted. Interestingly, Cyanobacteria of the genus *Phormidium* responded to the sawdust amendments.

A comparable analysis was done to assess the effects of the pre-crops at T2. As expected based on the performed PCoA (Fig. 2), no differentially abundant ASVs were detected in sawdust-amended samples, when the two pre-crops were compared. However, a number of ASVs were observed, which were significantly enriched when faba bean was used as a pre-crop in the wheat straw-amended samples (Fig. 6). These ASVs are assigned to Actinobacteria (unclassified Pseudonocardiales and unclassified Micromonosporaceae), Saccharibacteria, and Proteobacteria (*Altererythrobacter*).

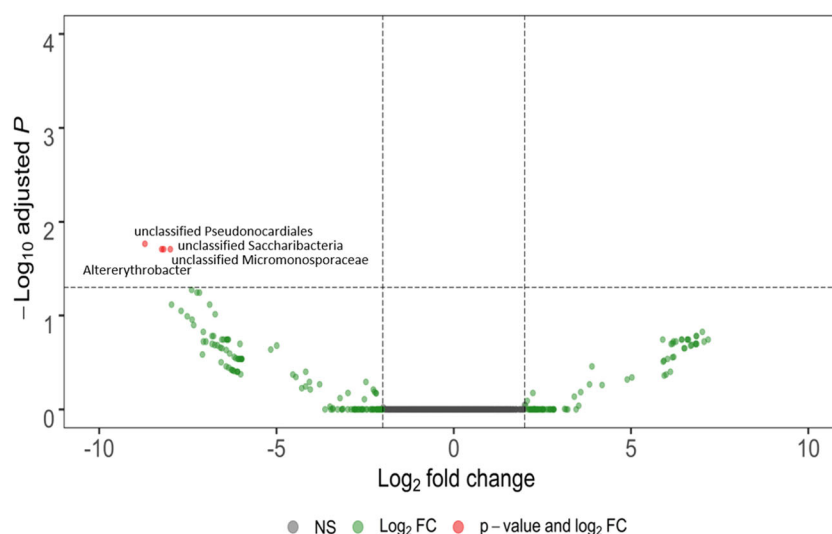


Fig. 6 Volcano plots showing differentially abundant ASVs (99% similarity) significantly enriched and depleted after wheat straw application at T2 comparing the two pre-crops (faba bean and spring barley) using DESeq2 ($n = 5$). Each point represents an ASV, x-axis shows the abundance fold change, and the y-axis shows the $-\log_{10}$ of the adjusted p value. Gray dotted lines highlight cutoff thresholds of log

fold changes of < -2 and > 2 and p_{adj} of 0.05. Significantly enriched ASVs meeting both cutoff thresholds are in red. ASVs meeting only log fold change cutoff are in green. Nonsignificant ASVs are in gray. Taxonomic affiliation of significant differentially abundant ASVs were added to the plots

Discussion

In the present study, we investigated the combined effects of crop-rotation and HCAs with different C quality and C:N ratios, which are used as measures to reduce N losses in agriculture ecosystems, on soil bacterial communities. HCAs are made up of different quantities and qualities of slow, moderate, and fast degradable organic fractions (Plante and Parton 2007). The fast degradable fraction contains easily accessible sugars, cellulose, and hemicellulose (Mueller et al. 1998; Van Der Wal et al. 2007) which are favorable for rapid growth of zymogenous microorganisms (Kuz'yakov and Blagodatskaya 2015).

Effects of wheat straw as an amendment on soil bacterial diversity

We observed in wheat straw-amended samples a clear reduced diversity compared to T1. It could be assumed that this indicates a stimulation of copiotrophic fast-growing bacteria species that utilize easily decomposable C rich compounds which outcompete slow-growing bacteria more adapted to environments with lower nutrient content (Pascault et al. 2013; Tardy et al. 2015). Shifts in bacterial community composition occur within days after wheat straw amendment addition (Bastian et al. 2009), supporting the hypothesis that wheat straw stimulates the growth of r-strategists. This development might have been favored by the high levels of fertilizer applied in combination with the wheat straw amendments. As shown by Banerjee et al. (2016), the addition of wheat straw in combination with N fertilization

induced a short-term increase in microbial biomass and a decrease in bacterial richness and diversity. A reduction of soil bacterial diversity due to wheat straw amendment was also observed for long-term field experiments (Sun et al. 2015). In our study, the high fertilization levels used for winter barley cultivation ensured enough amounts of bioavailable N or P for the soil microbiome to maintain high activities and a stable stoichiometry of nutrients despite the transformation of easily degradable C. The lower C content in soils with wheat straw used as HCA compared to samples from soils where sawdust has been applied is a strong indicator for a faster turnover of wheat straw and higher respiration rates of the soil microbiome compared to sawdust as HCA. The degradation of HCAs is a very complex process, which depends on many factors, including soil moisture and temperature (Maenhout et al. 2018). An extensive investigation of the degradation rates of sawdust or wheat straw amendments was out of the scope of the present study. However, previous studies carried out under controlled laboratory conditions (Reichel et al. 2018) demonstrated that 113 days after HCAs application, on average 65% and 30% of the added C was decomposed for wheat straw and sawdust, respectively. Therefore, we assumed that in the period of our experiment, C added in form of wheat straw was decomposed, whereas a great part of the sawdust C remained in the soil. Both previous experiments and total C contents support this hypothesis. Finally, as an agricultural soil was used for the experiment, it is likely that the soil microbiome was already well adapted to the applied wheat straw and had a high potential for degradation of the major constituents being present, which was stimulated by the wheat straw addition.

Similar response patterns were also observed in the no-HCA controls, including a reduction in bacterial diversity compared to T1, indicating the overarching negative impact of the high levels of fertilizer applied to the diversity of the soil microbiome. Obviously, the quality of C in root residues left in the pots after pre-crop harvest was comparable to the introduced C of the wheat straw amendments and was still high enough to trigger comparable responses of the soil microbiome in the no-HCA control treatment compared to the wheat straw-amended samples at T2. However, in the no-HCA control, the bacterial abundance was reduced as indicated by a 16S rRNA gene based qPCR compared to the wheat straw-amended samples, indicating that the comparable C quality in the no-HCA treatment, which is however present in lower amounts (only root residues), induced mostly differences in microbial biomass but not in the diversity (Fig. S3).

Interestingly in our study, the most pronounced difference in samples from no-HCA and wheat straw addition compared to T1 was found in ASVs linked to the genus *Flavisolibacter*, which belongs to the Chitinophagaceae family. Chitinophagaceae were shown to be predominantly abundant in extracts from soils amended with different organic C sources, such as rice straw (Hui et al. 2019). Bacteria of this genus have been described previously as a major part of the wheat-associated microbiome (Dai et al. 2020). Therefore, we assume that plant derived C originated from both pre-crop residues and wheat straw might have supported the growth of bacteria belonging to this family. Further, Streptomycetaceae, Micromonosporaceae, and Pseudonocardiaceae families were increased in abundance in the wheat straw treatment and the no-HCA samples at T2 compared to T1 and were also not stimulated by sawdust application. Bacteria of these families exhibit hydrolytic activity and are capable to degrade polysaccharides including carboxymethyl cellulose, chitin, and xylan (Yeager et al. 2017), which can be considered as typical constituents of wheat straw.

Effects of sawdust as an amendment on soil bacterial diversity

In general, fungi are considered to play a key role in the degradation of sawdust. However, as shown in a recent study published by Clocchiatti et al. (2020) who did an extensive investigation on the effects of HCAs on the abundance and diversity of saprophytic fungi in arable soils, effects of sawdust amendments on fungal diversity clearly depend on its origin, with an increase of fungal biomass only for deciduous wood but not for coniferous wood (also used in the present study). This indicates that here bacteria might play an important role.

As indicated by the C content data from soils at T2, samples from sawdust treated soils showed that more recalcitrant C remained in the soil, most likely in the form of lignin and polyphenol content (Kostov et al. 1991; Thomas

and Spurway 1999). This newly introduced C pool with differing C quality compared to root residues apparently induced a stabilization of the diversity pattern observed at T1 even in the presence of the high fertilizer application rates during the second vegetation cycle. Thus, sawdust may provide niches for more oligotrophic bacteria, which have been outcompeted in the no-HCA control and wheat straw treatment by fast-growing copiotrophs. Moreover, the need for tight interacting network structures of microbes as prerequisite for the degradation of complex materials like lignin (Louis et al. 2016) might have further induced the stabilization of the high diversity of soil bacteria in sawdust-amended samples at T2.

Sawdust maintained the high relative abundance in soil of the families Gemmatimonadaceae, Bacillaceae, and Xanthobacteraceae at T2, which were observed after the pre-crop harvest at T1, despite the high levels of fertilizer applied. Our results are in line with previous studies which have reported an increase in the abundance of members of Gemmatimonadetes in soils treated with highly complex, heavily degradable organic materials like biochar (Xu et al. 2014) and a reduction in soils treated with wheat residues (Bernard et al. 2007). Biochar, like sawdust, contains high C:N ratios and a high content of recalcitrant material, indicating specific pathways of Gemmatimonadetes to break down complex organic matter (Whitman et al. 2016). Many bacteria belonging to the family Bacillaceae also have the ability to degrade lignin and other complex materials and thus might benefit from the application of sawdust to soil (Brink et al. 2019; Janusz et al. 2017).

Effects of the amendments on plant yield

Many strains of the bacterial groups which remained high in relative abundance after sawdust application are known to promote the growth of plants (De-Bashan et al. 2020; Kloepper et al. 2004). For example, some members of the family Xanthobacteraceae have the ability to promote plant growth and performance (Suarez et al. 2017). The fact that this increased presence of plant growth promoting rhizobacteria in the sawdust treated samples did not induce an improved plant growth of winter barley but the opposite compared to the other treatments points to the presence of allochemicals which may have impaired the growth of the main crop (Saha et al. 2018) and, hence, masking positive effects observed on soil microbial diversity.

In contrast to the sawdust treatment, the wheat straw amendment had no negative influence on the growth of winter barley in comparison to no-HCA treatment. This might be a result of the high levels of fertilizer present in soil, which may have resulted in a decoupling of microbiome and plants in soil, as the plant does not need

the support of the microbiome and thus does not invest into its microbiome. In our study, it is most likely that other factors like light or water might be the growth limiting factors and conditions that cannot be complemented by soil bacteria. In order to cope with water stress, soil fungi might have been of importance although they were not included in this study. Thus, reducing fertilization levels might reduce the plant yield in the no-HCA control compared to the soils with wheat straw amendment as many of the genera that were enriched in wheat straw treatment but not in no-HCA soils at T2 might have plant growth promoting properties. Many of these ASVs belonged to the genus *Bacillus* (Santoyo et al. 2012) and were detected at an 8.5 log₂-fold change in comparison with T1. A long-term field experiment carried out by Sun et al. (2015) supports this assumption, as they showed that repeated application of wheat straw actually improved crop yield in comparison to non-amended soils.

Effects of the pre-crops

The pre-crops used caused no significant differences in bacterial diversity and only little variation in the soil bacterial community composition at T1. These results confirm many studies, where responses of the soil microbiome towards different plants species were most distinct during the vegetation period and not during or after harvest, as it is well-known that plants affect microbial communities, particularly at the plant-soil interface in the rhizosphere as a result of different root exudation pattern and root morphology (Berg and Smalla 2009). However, our study samples were taken after harvest, where only decaying root materials are still present and differences in the quality of the provided organic material are low. One might speculate about an increase in abundance of Rhizobia in the soils which were cultivated with faba bean; however, the used primer system did not allow for the discrimination between many soil-borne α -Proteobacteria like *Agrobacteria* and the rhizobial symbionts of faba bean.

Based on the little shifts in the soil microbiome induced by faba bean and spring barley, only few modulations of the general responses of soil bacteria towards the amendments, which could be tracked back to the pre-crops, were expected. Indeed, the most pronounced effects at T2 are caused by the applied amendments. When sawdust was added to soil, no pre-crop-based effects were visible. In contrast, in the no-HCA (control) soils and soils which were amended with wheat straw, some responding bacteria were detected. Actinobacteria were detected here as main responders. Interestingly also Saccharibacteria, a bacteria from the candidate division TM7 which parasites Actinobacteria, was enriched (Kindaichi et al. 2016).

Conclusion

Our results indicate that HCAs rather than pre-crops were major drivers of the composition of the soil bacterial communities. Even though sawdust fostered a high soil bacterial diversity, it did not show short-term positive effects on crop yield. On the contrary, plant material composed of more easily degradable C, such as wheat straw, was shown to boost the growth of bacterial taxa, mainly from the phylum Actinomycetes, which might positively affect the growth of crop plants and at the same time improve the incorporation of N in the soil. However, our data only considered short-term effects, and it might be assumed that a continuous application of amendments, mainly sawdust, might trigger higher soil C contents resulting in positive feedback loops in soil, which might induce sustainable improvements of soil quality. Furthermore, in this study, we used one soil type, which was already well adapted to good management and had a balanced soil texture. Thus, in other situations, the application of the amendments might induce more positive effects, and the role of wheat straw as an amendment might be more pronounced than in our study. Consequently, a generalization of the obtained data mainly related to plant performance and yield is not possible. Furthermore, this study only considered bacterial responses to pre-crops and HCA although it is well accepted that also fungi play a very important role in agroecosystems both as plant beneficials like arbuscular mycorrhiza or plant pathogens (e.g., *Fusarium* species), and several studies have indicated that fungi strongly respond to HCA amendments. Fungal responses should, therefore, be considered in future studies. Finally, previous work by van Duijnen et al. (2018) indicated changes in N fluxes and amounts of available N as a result of HCA amendments. To understand the link to microbial activities, in depth transcriptomic studies, would be needed, which, however, would require a much denser sampling design compared to this study, considering temporal and spatial heterogeneities.

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Data availability The raw sequence data were submitted to the Sequence Read Archive (SRA) in NCBI (<https://submit.ncbi.nlm.nih.gov/sra/>) under the project accession number PRJNA635677.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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