Biology and Fertility of Soils
https://doi.org/10.1007/s00374-025-01976-4

ORIGINAL PAPER

Application of high carbon amendments stabilizes soil microbial community composition and improves microbial recovery after a late spring drought during winter wheat cultivation

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Received: 4 November 2025 / Revised: 23 December 2025 / Accepted: 27 December 2025
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Abstract

High-carbon amendments (HCAs) are increasingly recognized as important tools in agriculture for reducing nutrient losses, such as nitrate leaching resulting from nitrification. However, most studies have not considered the influence of climate change as a confounding factor. In this study, we took advantage of an extreme drought lasting over eight weeks in late spring 2023 in Southern Germany. We examined the effects of wheat straw application as an HCA, implemented in late autumn 2022, within a field trial of an oilseed rape–winter wheat rotation. Using a molecular barcoding approach, we analyzed bacterial and archaeal communities in soil samples collected six, nine, eleven, and twelve months after HCA application during the wheat-growing season. HCA application had a positive effect on the thousand-kernel weight and stabilized microbial community composition, as indicated by a greater shared core microbiome across all sampling time points. Members of the phyla *Acidobacteriota* (e.g., *Vicinamibacteraceae*, *Blastocatellaceae*) and *Bacteroidota* (*Chitinophagaceae*), known degraders of complex organic materials, benefited from HCA addition. In contrast, bacteria typically associated with the winter wheat rhizosphere, such as *Arthrobacter* and *Bradyrhizobium*, were not affected, suggesting that HCAs exerted a greater impact on the bulk soil microbiome than on the rhizosphere. Overall, HCA implementation enhanced the stability of the soil microbiome during drought and promoted faster recovery afterward, highlighting an additional ecological benefit of these amendments.

Keywords High carbon amendments · Soil microbial communities · Drought · Wheat rhizosphere · Nutrient retention

Introduction

The risk of high nitrogen (N) losses from applied fertilizer into groundwater bodies through nitrate leaching is especially high at sites under agricultural use, particularly when residues of N-rich crops like oilseed rape (*Brassica napus* L.) or legumes remain on the field (Sieling and Kage 2006; Yang et al. 2018). One strategy to mitigate the post-harvest N losses is to implement high carbon amendments (HCA) into topsoil layers of agricultural fields. Potential HCA are residues, such as sawdust, lignin or wheat straw, which are composed of complex, polymeric organic substances, i.e., cellulose, hemicellulose, and lignin (Carvalheiro et al. 2009), with a C: N ratio higher than 25 before application to soil. These residues induce a fast microbial N immobilization (He et al. 2015; Shindo and Nishio 2005) to compensate for the C surplus. Reichel et al. (2018) reported an

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Published online: 12 January 2026

increase of microbial biomass N (N_{mic}) of 42 kg N_{mic} ha^{-1} and a reduction of soil nitrate content of 69 kg N ha^{-1} based on a laboratory incubation experiment. This observation is supported by a 12-month field study, where wheat residues were used as HCA and a decline of taxa involved in nitrification (*Nitrospira*) was observed shortly after the incorporation of HCAs (Tardy et al. 2015), indicating a lower ammonium availability in the soil.

In addition to the positive effects of HCAs on nutrient retention, in a mesocosm experiment using wheat straw as HCA, an increase in soil microbial diversity was observed in a setting with a loamy, sandy textured soil and barley plants (Kamau et al. 2021).The stabilizing effect of HCAs might be due to their high content of complex organic carbon. This carbon supports microbes that degrade such compounds which are usually outcompeted by fast-growing copiotrophs when only easily available nutrients (like plant exudates) dominate and no HCA is added. In line with this argument, (Gladkov et al. 2022) isolated bacteria such as *Flavobacterium*, *Sporocytophaga* or *Cellulomonas* as members of cellulolytic consortia from oat straw. Furthermore, other authors have described an increase of typical K-strategists such as *Acidobacteriota* shortly after HCA application (Pascault et al. 2013). In addition, many studies were performed under controlled conditions in greenhouses or mesocosms (Kamau et al. 2021; Reichel et al. 2022), focussing on short-term effects of HCA. Thus, the role of weather conditions, such as variable precipitation, during the growing season as a modulating factor of HCA effects has not been considered in most cases.

However, considering the ongoing climate change, such issues are becoming increasingly important, especially if the consequences of HCA application for future agricultural management are to be considered. There are clear indications that drought has a strong effect on microbial communities, indicated by loss of biomass and different responses of microbial taxa (Hueso et al. 2012). An increase in the abundance of drought-tolerant bacterial taxa, such as *Chloroflexota*, *Bacillota* and *Actinobacteria*, and a decrease in the abundance of drought-sensitive taxa, such as *Bacteriodota*, *Planctomycetota* and *Acidobacteriota*, has been described (Naylor and Coleman-Derr 2018; Santos-Medellín et al. 2017). Especially *Actinobacteria*, e.g. *Streptomyces*, which are capable of degrading complex C as cellulose, are enriched under drought (Metze et al. 2023). The effects of HCA application may become more prevalent as a result of higher degradation rates during drought.

Here we investigated how HCAs influence the resilience of the soil microbiome towards drought stress. We made use of the extreme drought which occurred in late spring 2023 in Southern Germany, with precipitation rates of less than 40 mm from May to July. We investigated the effects of wheat straw implementation in autumn 2022 after the harvest of oilseed rape on nitrogen concentrations, microbial biomass and community composition in the soil during the subsequent growing season of winter wheat as affected by an extreme spring drought in an experimental field trial in 2023. We sampled in February, May (before drought), July (after drought) and August (harvest) 2023 and assessed both bacterial and archaeal abundance and community composition as well as nutrient dynamics. Further we measured consequences of the HCA application for plant yield. We expected that due to the presence of more bacteria with a more oligotrophic lifestyle in the HCA treatment, which are typically more resilient to environmental changes, that drought effects might be less pronounced in HCA treatments compared to controls without HCA implementation, resulting in increased yield values in the HCA treated plots.

Materials and methods

Experimental design and soil sampling

The study is based on a randomized plot-scale field experiment set up in 2022 and sampled in 2023. The field site was located at the experimental station of the Technical University Munich in Dürnast, Germany (48°40'57" N; 11°69'56" E; 477 m asl). The soil type is silty loam with condition level 3 (L3 D-67/60), represent medium water conditions and medium productivity in soil with a total organic carbon (C) content of 1.4% C, a total N content of 0.2%, a C: N ratio of 9.2, a pH of 6.8 and a maximum water holding capacity of 33.3 (method: Schinner et al. 1996). The experimental field was divided into 12 plots with a plot size of 8 m x 1.85 m. Between each treatment plot, a margin (8 m x 1.85 m) was established to reduce carryover and border effects and plots were arranged in for rows separated by a margin of 1.5 m.

In 2022, oilseed rape was grown (cultivar "Ludgar", sowing date: September 17th, 2021, harvest date: August 11th, 2022). The HCA treatment was applied on half of the plots on August 23rd, 2022. As HCA, chopped wheat straw (42.5% C, 0.5% N and a C: N ratio 78.5) with a particle size of ≤5 cm was incorporated to the first 10 cm of soil and mixed using a rotary harrow. Subsequently, the six plots for each treatment were treated as biological replicates. On November 2nd, 2022, winter wheat (*T. aestivum*), cultivar "*RGT Reform*", was sown using a plant density of 340 seeds m^{-2}. During the cultivation of winter wheat, plant protection and fertilization application were carried out as followed: On March 20th 2023 Atlantis® OD (1.0 l/ha), on March 22th 2023 Husar® OD (0.08 l/ha) and one May 4th 2023 Ascra® Xpro (1.5 l/ha) were applied for plant protection.

For fertilization YaraLiva® TROPICOTE® 15.5% N, 26% CaO was utilized three times with an application rate of 387 kg/ha at each time point one March 23th 2023, May 8th 2023 and June 1 st 2023. Information about plant protection and fertilization is also summarized in Table S1.

Winter wheat was harvested on August 11th, 2023. In 2023 annual mean temperature was 10.4 °C and annual precipitation was 899.3 mm (https://meteostat.net/de/). Daily temperature and precipitation data during the sampling period (February to August 2023) is summarized in Figure S1. From May 2023 to July 2023, the site was affected by a severe drought period with a total precipitation of only 40.7 mm and a mean temperature of 18.0 °C. Accordingly, the water content of the topsoil (0–10 cm layer) decreased in all plots from 27% in May to 7% in July, and increased again towards 19% in August (Fig. S2).

During the experiment, four soil sampling campaigns were carried out in 2023, namely in February (six months after HCA implementation), May (nine months after implementation, two weeks after second N-fertilizer treatment, starting point of the severe drought period), July (eleven months after implementation, end point of the severe drought period), and in August (twelve months after implementation, ten days before wheat harvest). Four soil samples per plot were taken from 0 to 10 cm depth, according to the HCA incorporation depths, with a soil auger (diameter 1 cm; 10 cm length) and pooled to form a composite sample. All soil samples were sieved to 2 mm. Samples for fumigation analysis were stored at +4 °C. Samples for molecular analysis were frozen at −80 °C directly until further use. Soil moisture content was analyzed by drying 1 g fresh soil for 3 days at 60 °C. At the time of harvest, grain subsamples were dried at 80 °C for 72 h and weighed to determine total solids (TS, %). Thousand-kernel weight was assessed with a grain counting device (Condator, Pfeuffer GmbH, Germany). Protein and starch concentrations (on a dry matter basis) were analyzed by near-infrared spectroscopy (Fox and Manley, 2014). Straw yield was calculated from plot-specific straw-to-grain ratios, with threshed ears and straw dried at 60 °C, weighed separately, and standardized to 86% dry matter.

Bulk soil properties

Microbial biomass C and N (Cmic and Nmic) were determined by the chloroform fumigation–extraction method (Joergensen, 1996), with 0.01 M $CaCl_2$ as the extractant. Measurements were done using a DIMATOC 2000 system (Dimatec Analysen GmbH, Germany). For the calculation of C_{mic} and N_{mic} data, values of extracted soil organic carbon and total nitrogen of the non-fumigated soil samples were subtracted from the fumigated samples and the correction factors k_{EC} 0.45 (Joergensen 1996) and k_{EN} 0.54 (Joergensen and Mueller 1996) for C_{mic} and N_{mic} were applied, respectively. $CaCl_2$ extracts of non-fumigated soil samples were further used to analyze ammonium and nitrate content using a Continuous Flow Analyzer SA5100 (Skalar® Analytical B.V., Netherlands) as well as dissolved organic carbon (DOC) and nitrogen content (DON) measured using DIMATOC 2000 system (Dimatec Analysen GmbH, Germany). For determination of pH values 5 g soil were shaken for 5 min in 25 ml 0.01 M $CaCl_2$ solution and stored at room temperature for 3 h. Afterwards, pH values were analyzed using a Lab pH level 1 sensor (WTW GmbH & Co. KG, Germany).

DNA extraction and quantitative real time PCR for bacteria and archaea

DNA was extracted from 0.3 g frozen bulk soil using the NucleoSpin Soil Kit (Macherey-Nagel GmbH & CO KG, Germany) following the user manual (Lysis Buffer SL1 was used). Blank extractions were processed as negative controls to exclude contaminations during DNA extraction. Quality of DNA extracts was analyzed using Nanodrop photometric system (Thermo Fisher Scientific, Germany) and DNA was quantified with Quan-iT™ PicoGreen dsDNA Assay Kit (ThermoFisher Scientific, Massachusetts, USA) using a SpectraMax Gemini EM Microplate Spectrofluorometer (Molecular Devices, CA, USA) at 520 nm. Extracted DNA was stored at −20 °C until usage. Absolute abundance of 16 S rRNA genes for bacteria and archaea were determined by qPCR assays on a 7300 Real-time PCR System (Applied Biosystems, USA). Amplification parameters, primers and standard plasmids are further described in Table S2. A dilution test identified a 1:32 dilution as sufficient to exclude inhibitory effects. Standard dilution curves were used in triplicates for bacterial and archaeal 16 S rRNA genes, based on plasmids containing the 16 S rRNA gene fragment of *Pseudomonas putida* for bacteria and *Methanobacterium sp.* for archaea. The final 25 µL PCR reaction mix contained 12.5 µL SYBR™ Green Master Mix (ThermoFisher Scientific), 0.5 µL of 3% BSA (bovine-serum albumin), 2 µL of DNA template, 0.5 µL primers (10 pmol μL^{-1}), and 9 µL DEPC water. No template controls were included using DEPC water. For verification of specificity of the PCR product a melting curve analysis and a 1.5% agarose gel was performed. The respective qPCR efficiency (calculated with the formula $Ef = [10^{(-1/slope)} - 1]$) ranged from 96.1% to 98.9% for bacteria and from 86.9% to 84.4% for archaea. R^2 values were above 0.99 for all runs.

16 S rRNA gene amplicon sequencing

16S rRNA gene fragments were amplified targeting the V4 region of the prokaryotic 16S rRNA gene using the primer pair 515 F (5' GTGYCAGCMGCCGCGGTAA 3', Parada et al. 2016) and 806R (5'GGACTACNVGGGTWTCTAAT 3', Apprill et al. 2015). We followed the quality guidelines of the 16 S rRNA Metagenomic Sequencing Library Preparation protocol (Illumina, San Diego, CA, USA) and Schöler et al. 2017). Sequencing was done on Illumina MiSeq using the MiSeq Reagent v3 (600 Cycle) kit. More information about the library preparation can be found in the Supplementary Material. Raw sequencing data is available at the sequencing read archive (SRA) under the BioProject ID PRJNA1128396 (SAMN42050636, SAMN42051589, SAMN42107788, SAMN42108963).

Bioinformatic processing

Raw sequences were uploaded to the U.S. Galaxy server (https://usegalaxy.org/), adaptors were removed using Adapt or-Removal version 2.3.1 (Schubert et al. 2016), trimmed by removing 20 bases from the start and end of the sequences, resulting in 14,352,310 raw reads. Subsequent datasets were processed using Qiime2 2019.10 pipeline (Bolyen et al. 2019) and the plugin DADA2 version 1.18 (Callahan et al. 2016). Sequencing primers were removed, and reads were quality filtered using a minimum length of 240 bp for forward reads and 200 bp for reverse reads. Only reads with less than 3 expected errors for forward and 4 for reverse were preserved. The taxonomy of the Amplicon Sequencing Variants (ASVs) was assigned based on the SILVA database (version 138.1 Nr. 99) applying default parameters (Quast et al. 2012). The downstream analysis of ASVs was executed in R version 4.3.1. Two samples of sampling date August (T4S3, T4S7) exhibit low read counts. Therefore, this samples were sequenced twice and received sequence reads were added up to the reads of the first sequencing run using "merge"-function of "dplyr" package (Table S3). R package "decontam" (Davis et al. 2018) was used to remove potential putative contaminant sequences (threshold: 0.05) as well non-bacterial ASVs assigned to chloroplasts and mitochondria. Vegan package (Oksanen et al. 2017) was used to draw rarefaction curves, to observe sequencing coverage, and to normalize number of reads using subsampling based on median sequencing depth in each sample with the rarefy function. Alpha diversity was estimated by counting the number of Observed ASVs using the vegan package. Beta diversity of samples at each sampling time was estimated using Non-metric multidimensional scaling (NMDS) and the Bray-Curtis dissimilarity index (Bray and Curtis 1957) using the "plot_ordination" function of the "phyloseq" package (McMurdie and Holmes 2013).

Statistical analyses

For statistical analyses of yield and plant parameters, t-tests were performed using the t.test-function of "stats" package. For all other statistical analyses, measured variables were log-transformed, to reach normal distribution and linearity (exception of qPCR data for bacteria) and fitted to a linear mixed-effects model using the "lme"-function of "nlme" package (Pinheiro et al. 2024), for investigating the effect of treatment and sampling on the measured variables (Observed Richness for alpha diversity, qPCR data for bacteria and archaea, C_{mic} and N_{mic} DOC and DON, as well as ammonium and nitrate content of soil). Furthermore, the position of plots inside the four rows of the experimental field was included into the model as random factor block. The fitting order of the model was: model← lme (log_variable ~treatment*sampling, random = ~1 |block). Distribution of residuals of models were plotted using the function plot(model). Post hoc comparisons were conducted using the Tukey´s method, exhibiting pairwise comparison between treatment and control separately for each sampling date.

Therefore, estimated marginal means (EMMS) were generated with "emmeans" package (Lenth 2024). Core prokaryotic community analyses were carried out with the "core_members" function (detection threshold = 0, prevalence threshold = 0.67) of the "microbiome" package and plotted with the "ggvenn" package version 0.1.16 (Yan 2023). Compositional differences between sites were calculated with the permutational multivariate analysis of variance (PERMANOVA) within the "adonis2"- function of the "vegan" package using 999 permutations. The results were visualized using packages "ggplot2" (Wickham 2011), "gghighlight" (Yuntani 2024), "scales" (Wickham et al. 2023), "ggrepel" (Slowikowski 2024) and "ggforce" (Pedersen 2024) and "ggbreak" (Xu et al. 2021).

Results

Plant yield

The implementation of HCAs had a significant positive effect on the thousand kernel weight of winter wheat, which was significantly higher in the plots treated with HCA compared to the controls at harvest (p-value = 0.03, Table S4/S5). However, no differences on total yield (p-value = 0.54), straw yield (p-value = 0.55), protein N (p-value = 0.09) and starch content (p-value = 0.14) of winter wheat were found.

Bulk soil properties

Overall, values of pH changed over the growing season, ranging from 7.04 in February to 6.68 in August in Control treatment and from 7.01 in February to 6.78 in August in HCA treatment. For both treatments especially a drop from February (HCA: 7.04, Control: 7.01) to May occurred (HCA: 6.42, Control: 6.50). Afterwards pH raised in both treatments ins July (HCA: 6.84, Control: 6.85) and stabilized until August (HCA: 6.78, Control: 6.869. However, pH value was not significantly affected by sampling time point and treatment (Table S6).

Sampling time had a significant effect on DOC (p-value = 0.0002), DON (p-value = < 0.0001), nitrate (p-value = < 0.0001) and ammonium (p-value = 0.0001) concentrations in soil (Fig. S3, Table S7/S8). DOC values were highest for both treatments in February (HCA = 83.93 µg g^{-1} dry soil, Control = 60.84 µg g^{-1} dry soil respectively), dropped in July to 46.79 µg g^{-1} dry soil in plots amended with HCA and to 43.64 µg g^{-1} dry soil for control plots and increased slightly in August to 47.47 µg g^{-1} dry soil for HCA amended plots and 55.75 µg g^{-1} dry soil for control plots (Fig. S3). Pairwise comparison between the sampling time points showed significant differences between the sampling time point in February and those in May (p-value = 0.01), July (p-value = 0.0045) and August (p-value = 0.0039) for the HCA treatment but not for the Control (Table S7/S8) as a matter of the high DOC values in February in the HCA treated soil.

For DON, pairwise comparisons of sampling time points showed a significant decrease from May (15.08 µg g^{-1} dry soil) to July (6.67 µg g^{-1} dry soil, p-value = 0.0036) in HCA amended and control plots (May = 13.97 µg g^{-1} dry soil, July = 4.47 µg g^{-1} dry soil, p-value = < 0001, Fig. S3, Table S8).

Nitrate values were highest in May (HCA = 3.64 µg NO_3^--N g^{-1} dry soil, Control = 3.21 µg NO_3^--N g^{-1} dry soil) as a matter of fertilizer application (see Table S1), while at all other sampling time points values were significantly lower, with values below 1.51 µg NO_3^--N g^{-1} dry soil in both treatments (Fig.S3). In contrast, ammonium values were highest in February (HCA = 0.32 µg NH_4^+-N g^{-1} dry soil, Control = 0.38 µg NH_4^+-N g^{-1} dry soil) and August (HCA = 0.33 µg NH_4^+-N g^{-1} dry soil, Control = 0.34 µg NH_4^+-N g^{-1} dry soil, Fig S4) in both treatments. Neither sampling time nor treatment had a significant effect on C_{mic} and N_{mic} values (Fig. S3, Table S7). Nevertheless, as expected lowest values for C_{mic} were measured in July after the drought period (mean-value HCA = 942.9 µg C_{mic} g^{-1}, Control = 916.4 µg C_{mic} g^{-1}, Fig S4). A comparable development was visible for N_{mic} values with highest values in February (mean-value HCA = 42.3 µg N_{mic} g^{-1}, Control 38.6 µg N_{mic} g^{-1}, Fig. S4) and a decrease in July (mean-value HCA = 21.6 µg N_{mic} g^{-1}, Control = 19.9 µg N_{mic} g^{-1}, Fig. S4) after the drought period.

Bacterial and archaeal abundance

Bacterial and archaeal abundance was significantly affected by sampling time (Bacteria: $p = 0.003$, Archaea: $p = 0.02$, Table S9) but not by HCA implementation (Bacteria: p-value = 0.05, Archaea: p-value = 0.61). Pairwise comparison of the different sampling time points revealed that the abundance of both groups between sampling time points was not significantly different in soil samples from the HCA treatment (Table S10) but in soil samples from the Control treatment where bacterial abundance was significantly lower in February (median value = 1.42×10^{11} copies per g^{-1} dry soil, Fig. 1) and May (median value = 1.23×10^{11} copies per g^{-1} dry soil, Fig. 1) compared to August (median value = 2.70×10^{11} copies per g^{-1} dry soil; Feb-Aug: p-value = 0.03; May-Aug: p-value = 0.0031, Fig. 1).

Prokaryotic diversity

13,676,175 paired end reads of the amplified V4 region of 16 S rRNA gene were obtained across 48 samples. After

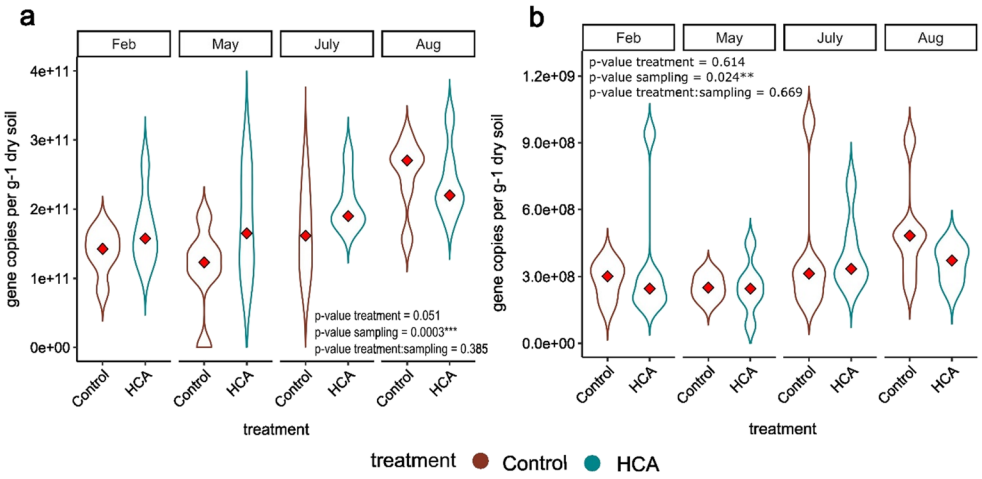

Fig. 1 Violin plots showing the results of qPCR quantification for **a** 16 S rRNA gene for bacteria and **b** archaea calculated as copies per g^{-1} dry soil. Red diamonds represent median value of 16 S rRNA gene of bacteria and archaea. dark red = Control, dark green = HCA

filtering, denoising, merging and chimera removal 8,702,361 reads remained, which were normalized to 52,699 reads per sample, which was sufficient to reach saturation in rarefaction curves (Fig. S4). Remaining reads resulted in 18,925 ASVs after taxonomic assignment. The sampling time point had a significant effect on Observed Species Richness ($p = 0.001$, Table S11) with highest values in February for the HCA treatment (median value = 1,977 ASVs) as well as for Control (median value = 1,838 ASVs), followed by values of the August sampling (median value HCA = 895 ASVs, Control = 826 ASVs). Lowest values were detected for both treatments in July (HCA = 405 ASVs, Control = 281 ASVs; Fig. 2a, Table S12).

As shown in the non-metric multidimensional scaling (NMDS) plot (Fig. 2b) and tested by PERMANOVA, prokaryotic community composition was significantly influenced by the sampling time (p-value < 0.001). Pairwise comparison between sampling time points revealed significant differences in community composition between samples from February vs. May (p-value = 0.006, Table S13), February vs. July (p-value = 0.006) and February vs. August (p-value = 0.01), as well as between July and August (p-value = 0.02, Table S13) in both treatments.

Prokaryotic taxonomical responses to HCA implementation and drought

The number of ASVs present at all sampling time points was with 93 ASVs (12.5% of all detected ASVs) remarkably higher in the plots treated with HCA compared to the Control treatment with 43 ASVs (6.1% of all detected ASVs) as indicated by the Venn diagrams (Fig. 3a-b). This finding correlates with the slightly higher bacterial diversity observed at all sampling time points in soil samples from HCA treated plots (Fig. 2a). Almost all "core ASVs" present in the Control treatment were also found in the HCA treatment, resulting in 35 overlapping ASVs (Fig. 3c). ASVs shared at all sampling time points in both treatments are dominated by ASVs assigned to the bacterial family *Vicinamibacteraceae* as well as members of the order *Gaiellales* (Fig. 3e). ASVs assigned to the genera *Arthrobacter*, *Bradyrhizobium* or *Arenimonas* were present in both treatments at all sampling time points but were lower in relative abundance in the HCA treated plots compared to soil samples from the Control treatment (Fig. 3e).

In soil samples treated with HCA, 58 ASVs representing the majority of "core ASVs" were not present in the core microbiome of soil samples from the Control plots (Fig. 3c). Differences in relative abundance of these 58 ASVs between sampling time points was thereby very low, indicating a stable abundance of ASVs over the growing season (Fig. 3d). These ASVs only present in the core microbiome of HCA treatment were again assigned to the families *Vicinamibacteraceae* as well as to the *Nitrosomonadaceae* and *Blastocatellaceae* (Fig. 3d). Only eight ASVs were unique for the core microbiome of the Control treatment (Fig. 3f) which were assigned to the genera *Blastococcus*, *Haliangium* and the family *Pedosphaeraceae*. Also, the number of unique ASVs for each sampling time point differed for HCA amended and Control plots (Fig. 3a-b). The number of unique ASVs thereby decreased from February (HCA: 420 ASVs, Control: 456 ASVs) to May (HCA: 5 ASVs, Control: 9 ASVs) to July (HCA: 5 ASVs, Control: 0 ASVs) and increased again in August (HCA: 19 ASVs, Control: 9 ASVs, Fig. 3a-b).

A comparison of unique ASVs derived from HCA and Control treatments in February resulted in 217 shared ASVs between both treatments (Fig. 4a). Furthermore, 203 ASVs were detected as unique in HCA amended plots and 239 ASVs as unique in the Control (Fig. 4a) indicating a significant effect of the HCA application in autumn on the bacterial

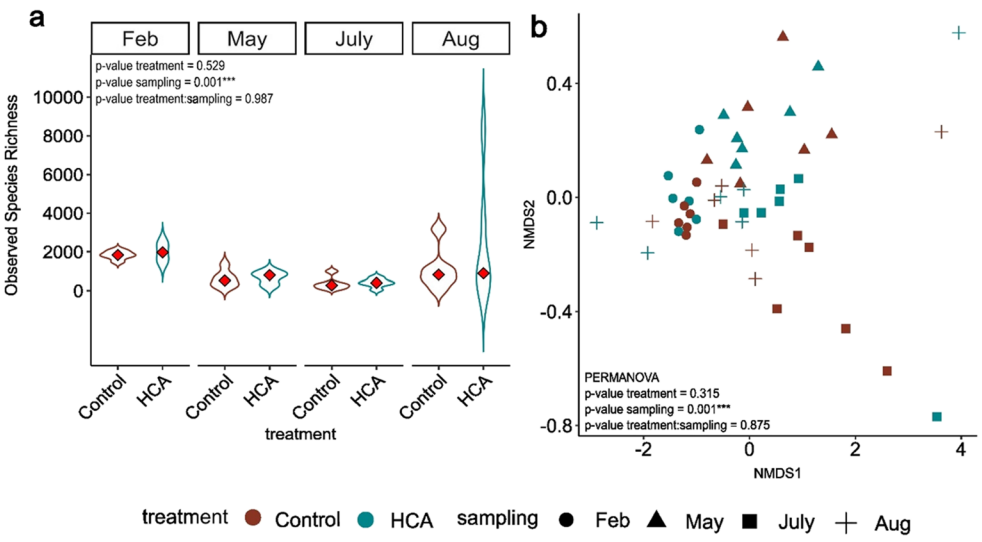

Fig. 2 Bacterial and archaeal alpha diversity, assessed by **a** Observed ASV richness. Red diamond represents mean value of Observed Species Richness. **b** NMDS plots using Bray-Curtis dissimilarity matrix based on ASVs level. Dissimilarity differences between HCA and Control for each sampling time point separately was calculated using PERMANOVA, p-values are presented in Fig. 2B. Dark green = HCA, dark red = Control, circle = sampling time point February, triangle = sampling time point May, quadrate = sampling time point July, cross = sampling time point August

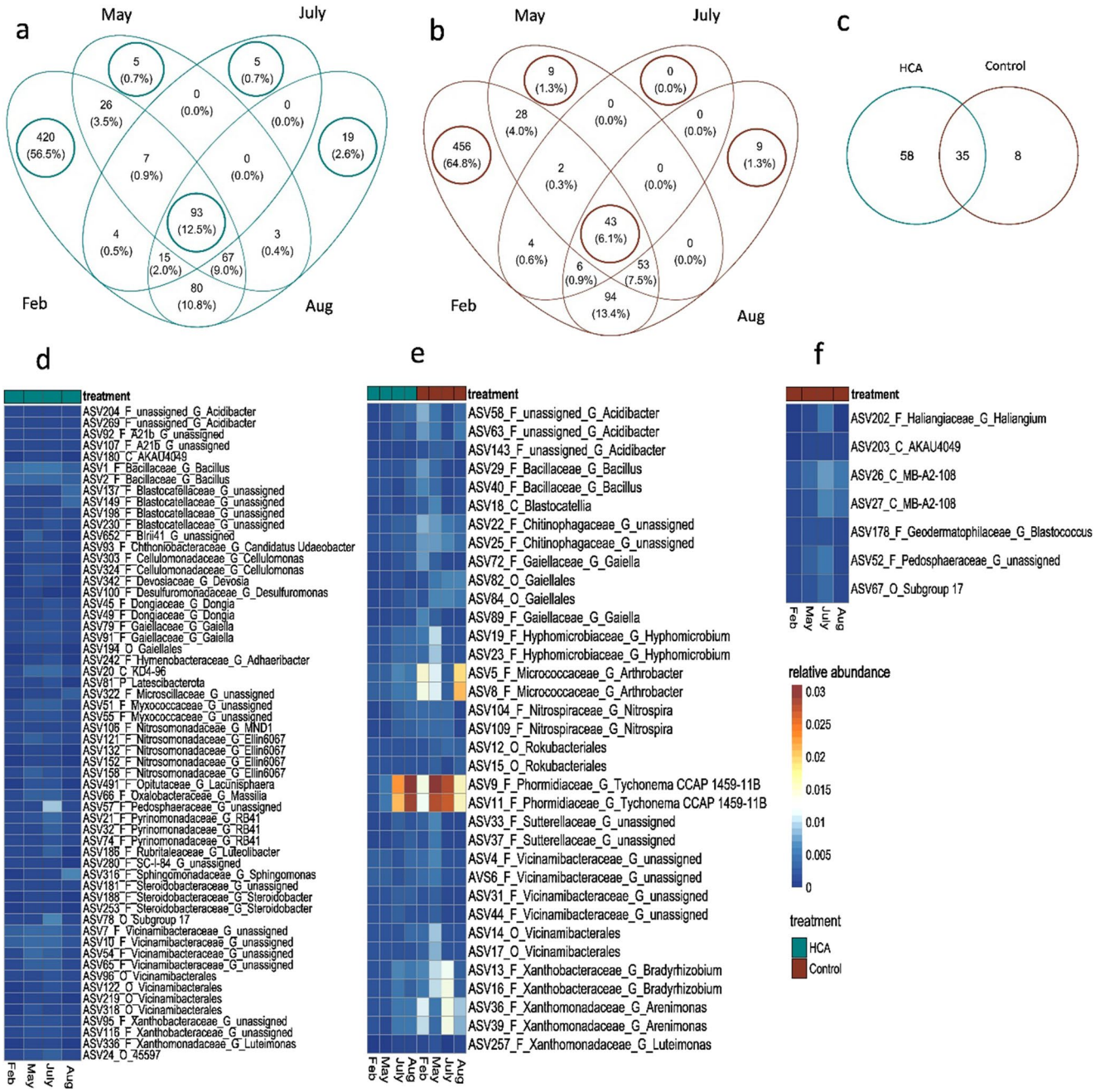

Fig. 3 Core microbiome analyses showing the number and proportion (%) of unique and shared ASVs. **a** Venn diagram showing shared and unique ASVs across season of HCA treatment and **b** of Control. **c** Venn diagram comparing ASVs detected across all sampling time points in HCA and Control (ASVs derived from core in **a** and **b**). Detection threshold = 0, prevalence threshold = 0.67. **d** Heatmap showing relative abundance of 58 unique ASVs in HCA treatment, derived from Venn diagram c, **e** Heatmap showing relative abundance of 35 shared ASVs between Control and HCA over all sampling time points, derived from Venn diagram c, **f** Heatmap showing relative abundance of 8 unique ASVs from Control treatment, derived from Venn diagram c. Taxa are annotated at family and genus level, if possible, otherwise last annotation level was presented. Dark green = HCA, dark red = Control. Unique and core ASVs from core microbiome analyses in panel **a** + **b** used in further analysis (Fig. 4) are marked with circles

community composition for the subsequent spring. Among the shared 217 ASVs, bacterial families such as *Vicinamibacteraceae* and *Gemmatimonadaceae* were dominating (Fig. 4b). From the 203 ASVs unique for the HCA amend plots also *Vicinamibacteraceae*, other observed ASVs were assigned as e.g. bacterial families *Flavobacteriaceae* or *Ilumatobacteraceae* (Fig. 4b). For the Control, unique ASVs were also dominated by *Vicinamibacterales*, furthermore

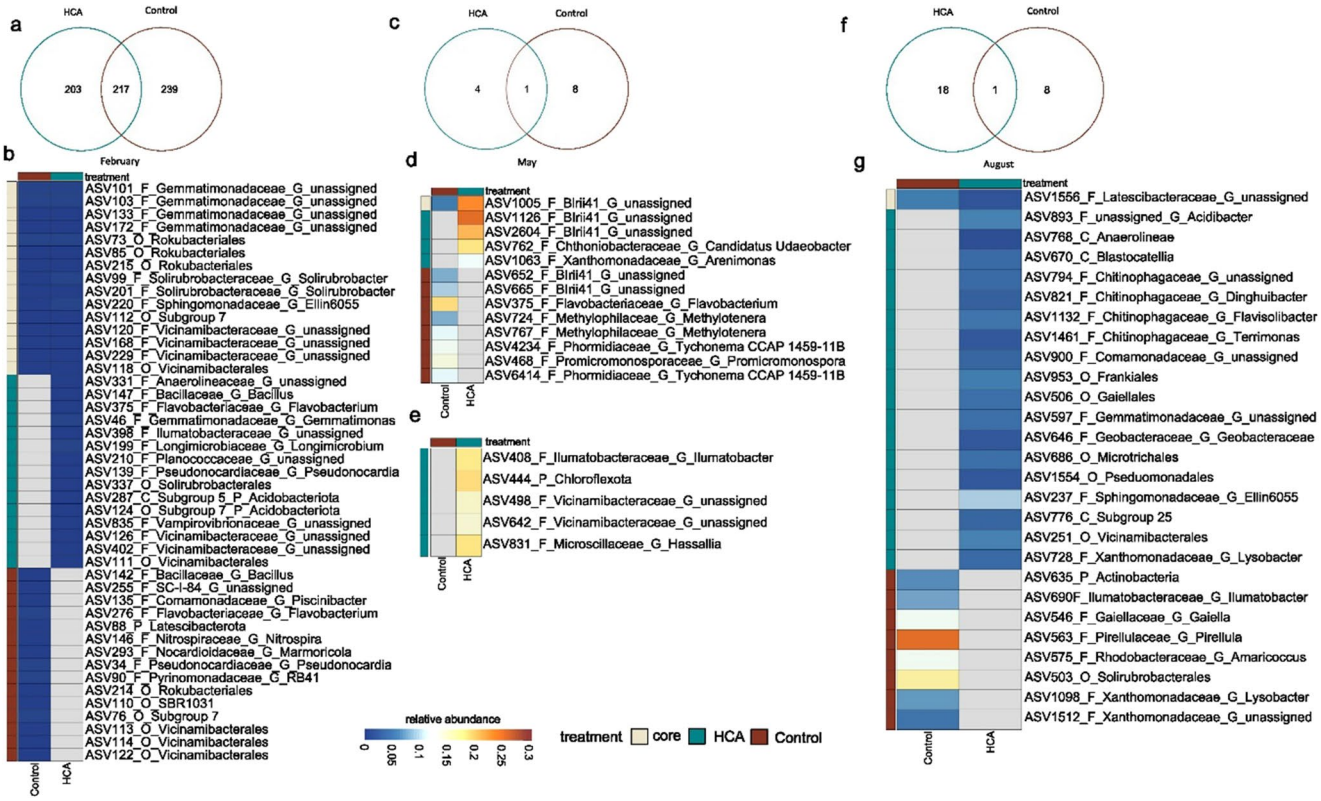

Fig. 4 Core microbiome analysis and heatmap showing relative abundance of shared and unique ASVs derived from core analysis in Fig. 3. **a** Venn diagram showing unique and shared ASVs from HCA and Control treatment at sampling time point February and **b** heatmap showing relative abundance of 15 core ASVs shared by unique ASVs derived from HCA and Control treatment, 15 ASVs unique in HCA treatment and 15 ASVs unique in Control at sampling time point February, **c** Venn diagram showing unique and shared ASVs from HCA and Control at sampling time point May and **d** heatmap showing relative abundance of unique and shared ASVs at sampling time point May, **e** heatmap showing relative abundance of 5 ASVs unique in HCA treatment at sampling time point July, **f** Venn diagram showing unique and shared ASVs from HCA and Control treatment at sampling time point August and **g** heatmap showing relative abundance of unique and shared ASVs from HCA and Control at sampling time point August

ASVs as bacterial family *Pseudonocardiaceae* or *Nitrospiraceae* were found in contrast to HCA implemented plots (Fig. 4b). In May only one ASV was shared between both treatments, which could be assigned to the family Blrii41 (order *Burkholderiales*, Fig. 4c/d). Four unique ASVs in HCA treatment were detected at sampling time point May, two were assigned to the family Blrii41, one to genus *Arenimonas* and one to genus *Candidatus Udaeobacter* (Fig. 4d). For the Control, unique ASVs for sampling time point May, were assigned to families Blrii41, *Phormidiaceae*, *Methylophilaceae* or *Flavobacteriaceae* (Fig. 4d). Under the drought conditions at the sampling time point July in HCA treated plots unique ASVs belonged to the families *Ilumatobacteraceae*, *Vicinamibacteraceae* and *Microscillaceae* as well as to phylum *Chloroflexota*, whereas no unique ASVs were detected for the Control treatment (Fig. 4e). For August, after the four-week rewetting phase, the number of unique ASVs increased in plots with HCA treatment in comparison to the sampling time point in July (Fig. 3a). Only one ASV, identified as *Latescibacteraceae*, was shared between unique ASVs from HCA and Control treatment of sampling time points August (Fig. 4f/g). In HCA treatment unique ASVs were dominated by family *Chitinophagaceae*. Other unique ASVs were assigned for to families as *Sphingomonadaceae*, *Comamonadaceae* or *Xanthomonadaceae* (Fig. 4g). In Control treatment, unique ASVs were assigned to the genera *Gaiella*, *Pirellula*, *Ilumatobacter* or family *Xanthomonadaceae* at sampling time point August (Fig. 4g). Furthermore, in HCA treatment 3 ASVs where exclusively shared between sampling time point before the drought in May and after rewetting in August (Fig. 3a), assigned to bacterial family BIrii41 and genus *Acidibacter*. The persistence of this bacterial taxa during drought could be based on HCA implementation, because this effect was not detectable in Control treatment (Fig. 3a-b).

Discussion

Overall, our study indicated that the implementation of HCA caused a stabilization of the microbiome composition and mitigated seasonal dynamics and drought effects during a growing season of winter wheat. This stabilization of the soil microbiome was linked to an increase in the thousand kernel weight of winter wheat. Thus, in addition to the well described effects of HCAs for nutrient retention mainly during the winter period (Reichel et al. 2018), HCA may also act as a useful mitigation strategy for climate-change-related effects on soil microbiota, mainly extreme drought periods.

Influence of HCA implementation microbial nutrients turnover in soil and plant development

The primary aim of incorporating wheat straw into the topsoil was to reduce nitrogen losses. However, neither soil nutrient content nor microbial biomass differed significantly between the HCA treatments and the Control. This finding contrasts with a laboratory incubation study using wheat straw in a nutrient-poor sandy soil under optimal moisture and temperature conditions (60% mWHC, 20 °C), where microbial biomass carbon (C_{mic}) increased by up to 100 kg ha^{-1} (Reichel et al. 2018). Similarly, a two-year field experiment in a wheat–rice cropping system on sandy loam soil reported increases in C_{mic} and N_{mic} of up to 21.5% and 40.4%, respectively (Zhao et al. 2019). In contrast to both of these studies, another two-year field trial conducted under a temperate continental monsoon climate on sandy clay loam soil found that incorporation of maize straw reduced both C_{mic} and N_{mic} relative to a straw-removal control (Ling et al. 2024). In our study, we assume that nitrogen immobilization stimulated by the additional carbon input likely occurred in autumn immediately after wheat-straw incorporation and therefore before the first sampling date. This interpretation is supported by the rapid decomposition of labile carbon fractions in wheat straw under aerobic conditions, which can result in a carbon loss of up to 69.9% within the first six months, accompanied by substantial declines in cellulose and hemicellulose content (Gao et al. 2016). Although HCA implementation did not alter soil nutrient concentrations, thousand-kernel weight of winter wheat was significantly higher in the HCA treatment. An increase in thousand-kernel weight reflects a greater proportion of endosperm relative to germ and pericarp tissues and is closely linked to milling quality (Ramirez-Wong et al. 2017). We therefore propose that during the drought period in July and the subsequent rewetting in August, the HCA treatment may have supported increased abundances of microbial taxa capable of degrading complex plant residues. The resulting nutrient release could have benefited winter wheat during the grain-filling stage (Rezaie et al. 2022), in contrast to the Control treatment.

Microbial responders to HCA application

Processes of microbial decomposition of organic material into plant-available inorganic nutrients drive global nutrient cycling and manage around 90% of global energy flux in soil (Saccá et al. 2017). Typical nutrient cycling process are nitrogen transformation process, for example nitrogen fixation carried out by plant-symbionts as *Rhizobium* or *Bradyrhizobium* (Canfield et al. 2010) or ammonia-oxidizing bacteria such as *Nitrosomomas* (Clark et al. 2021). Besides nutrient cycling several bacteria are characterized as plant-growth-promoting bacteria, such as *Pseudomonas*, *Bacillus* or *Arthrobacter* (Compant et al. 2019; Eichmann et al. 2021) and furthermore bacteria, such as genus *Bacillus*, hold the potential to act as pest and disease control (Shafi et al. 2017).

In our study, members of the phyla *Acidobacteriota* and *Bacteroidota* responded most strongly to HCA application and thus represent promising model groups for investigating how HCAs shape soil microbial community composition and function. Within these groups, for instance, *Vicinamibacteraceae* showed a distinct response to HCA addition. Whereas a few ASVs of this family were shared between all samples analysed, most ASVs responded either to the treatment or time point indicating a differentiation on the genus, species or strain level. *Vicinamibacteraceae* are a dominant and phylogenetically diverse bacterial group commonly found in soils, particularly in acidic and nutrient-poor soils (Kielak et al. 2016; Naether et al. 2012). Furthermore, *Vicinamibacteraceae* were described as keystone taxa in bulk soil samples from a soybean field trial treated with crushed maize straw and manure application (Ding et al. 2025). Despite their widespread distribution, they are considered being part of the "microbial dark matter" due to limited cultivability and genomic characterization, which constrains functional understanding (Ward et al. 2009). However, recent advances in metagenomics and single-cell genomics have provided insights into the potential ecological roles of *Vicinamibacteraceae*. Genomic reconstructions suggest that members of this family possess versatile metabolic capabilities, including the degradation of complex proteinaceous compounds and the utilization of diverse carbon sources, implying a role in soil organic matter (SOM) turnover (Dedysh et al. 2022; Huber and Overmann 2018). Additionally, some lineages exhibit genes associated with nitrogen cycling, including nitrate and nitrite reductases, suggesting involvement in denitrification processes under anoxic condition (Domeignoz-Horta et al. 2015). Continues presence of *Vicinamibacteraceae* in HCA implemented soil during

all sampling time points, including drought period in July, implies a potential mitigation of drought impact on microbial community in our study. In contrast, members of the family *Vicinamibacteracae* was observed as drought sensitive in African savannah soils (Huber et al. 2022), which might again point towards different ecotypes within that family.

Similar results like for *Vicinamibacteraceae* were obtained in our study for *Blastocatellaceae*, another family within the phylum *Acidobacteriota.* Members of *Blastocatellaceae* are consistently detected in a range of soil types, including desert, forest, and agricultural soils, and are often associated with oligotrophic, well-drained, and nutrient-poor conditions (Foesel et al. 2014). Their abundance has been positively correlated with dry, sandy soils and sparse vegetation cover, suggesting a niche specialization distinct from other soil bacteria (Navarrete et al. 2015). The ecological relevance of *Blastocatellaceae* lies in their metabolic adaptability and contribution to soil biogeochemical processes. Cultivated representatives, such as *Blastocatella fastidiosa*, display aerobic, chemoorganotrophic lifestyles with a preference for carbon compounds of medium complexity, indicating a role in the turnover of low-molecular-weight organic matter (Foesel et al. 2013), which again may explain their positive response to HCA implementation. In addition, family *Blastocatellaceae* was also described as a less drought-tolerant member of *Acidobacteriota*, underlying the positive impact of HCA on microbial community during drought (Huber et al. 2022).

Other bacteria responding to the HCA implementation were *Nitrosomonadaceae*, a family of chemolithoautotrophic bacteria within the class of *Betaproteobacteria*, and *Chitinophagaceae*, a family within the bacterial phylum *Bacteroidota. Nitrosomonadaceae* are primarily known for their role in ammonia oxidation, the first and rate-limiting step of nitrification in the nitrogen cycle. This transformation is critical for plant nitrogen availability, particularly in agricultural systems, where nitrate is the predominant form of nitrogen taken up by roots (Näsholm et al. 2009). Thus, the activity of *Nitrosomonadaceae* in soils directly influences nitrogen use efficiency (NUE) and plant productivity. By facilitating the conversion of ammonium-based fertilizers and organic nitrogen to nitrate, they enhance the bioavailability of nitrogen for crops, such as wheat and maize (Subbarao et al. 2006), explaining the positive correlation between relative abundance of *Nitrosomonadaceae* and the thousand kernel weight of winter wheat in our study. Wheat straw application has been shown to influence the abundance and activity of *Nitrosomonadaceae* in various agroecosystems. The addition of straw provides a carbon source that stimulates heterotrophic microbial populations, which can affect nitrogen cycling indirectly by altering ammonia availability and oxygen diffusion in the soil matrix (Zhang et al. 2011). While initial straw decomposition may suppress nitrification by increasing microbial competition for nitrogen (immobilization), longer-term effects often include enhanced ammonium turnover and a shift in nitrifier community composition (Chen et al. 2023). Furthermore, studies combining straw biochar implementation, respectively straw mulching reported increased *amoA* gene abundance and a shift of community composition of ammonia oxidizing bacteria from *Nitrosospira* to *Nitrosomonas* (Jia et al. 2024; Lin et al. 2017; Zhang et al. 2019).

Chitinophagaceae were reported to be able to degrade complex plant material (Eichorst et al. 2013; Funnicelli et al. 2021; Huang et al. 2023), were described as common members of wheat-associated microbiomes (Dai et al. 2020; Kavamura et al. 2021) and were isolated from soil-rice straw mixtures (Hui et al. 2019). Furthermore, *Chitinophagaceae* were also observed as responding taxa in several wheat straw application experiments, including mesocosm and field trials (Jarrige et al. 2025; Kamau et al. 2021; Yang et al. 2019).

No response of potential plant-promoting bacteria to HCA implementation

In contrast to bacterial groups which are often detected in bulk soil, which responded clearly to the application of HCA to soil, taxa often associated with the rhizosphere of cereal crops, such as *Arthrobacter*, *Bradyrhizobium or Arenimonas*, were not affected by the application of the HCA, mostly as they depend more on easily degradable carbon sources like root exudates. While the functional importance of *Bradyrhizobium* as a plant growth-promoting bacterium has been well described (Matilla and Krell 2018), for the other taxa just recently data on their importance as members of the plant microbiome became clear (Mongodin et al. 2006; Shan et al. 2023). *Arthrobacter* is widely distributed in soils and known for its remarkable metabolic versatility, stress tolerance, and ecological resilience with observed dominance in the rhizosphere and emerging importance as a plant growth-promoting rhizobacterium (PCPR) in unmanaged and agricultural ecosystems (Jones and Keddie 2006; Mongodin et al. 2006).

Arenimonas is a genus within the family *Xanthomonadaceae*, that has been increasingly recognized for its presence and potential ecological functions in soil and rhizosphere environments (Shan et al. 2023). Originally described as a genus of aerobic, Gram-negative, rod-shaped bacteria capable of degrading complex organic compounds, *Arenimonas* species are now frequently detected in the rhizosphere of diverse crops, including rice and maize (Aslam et al. 2009; Kwon et al. 2007; Li et al. 2014). Several studies described

strains of the genus *Arenimonas*, which inhibit growth of Gram-positive bacteria and harbour genes for metallo-β-lactamases (Fang et al. 2022; Zayulina et al. 2020). The latter ability may also explain, that in our study we did not observe an increase of bacterial wheat pathogens, although a number of studies have described a risk of transferring wheat pathogens from one to the other generation, based on accumulation as well as providing favourable growth conditions for wheat pathogens (Zhen et al. 2009).

Conclusion

Overall, our data demonstrates that the positive effects observed for wheat plant performance, demonstrated by the thousand kernel weight, after a long drought period in response to HCA application are mostly modulated by changes in classical bulk soil-associated bacteria, which indirectly support plant growth mainly under drought. In contrast, bacteria frequently detected in plant rhizospheres were not affected by the HCA treatment. Thus, it can be speculated that the observed effects might also be observed for other plant-associated microbiota. However, the soil type as well as the agricultural management (e.g., tillage intensity) might strongly modulate the outcomes of this study. Therefore, more research is needed on the sustainability of the observed effects, particularly regarding the question whether a repeated application of the HCAs is needed to sustain the beneficial effect of HCA. In view of the potentially problematic effect of wheat straw addition prior to wheat cropping in terms of accumulating pathogens (although we did not observe it in our study), the question whether other residues or HCAs with different properties induce comparable effects should be addressed in the future. The same applies also to effects of HCAs on fungal communities, although their resilience after drought might be more pronounced due to the formation of spores.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-025-01976-4.

Acknowledgements Many thanks to Wolfgang Heer for granting access experimental field station and to Susanne Walch from the Research Unit Comparative Microbiome Analysis for the Illumina MiSeq sequencing.

Author contributions Experimental design and funding acquisition was performed by Nicolas Brüggemann, Michael Schloter, Sabine von Tucher, Rüdiger Reichel and Stefanie Schulz. Sampling was carried out by Nora Bissinger, Hannah Anzenberger and Rüdiger Reichel. Chloroform fumigation was performed by Nora Bissinger and Hannah Anzenberger. Sample processing and sequencing was carried out by Nora Bissinger and Hannah Anzenberger. Bioinformatic processing and data analysis were carried out by Nora Bissinger. The first draft of the manuscript was written by Nora Bissinger, Michael Schloter, Stefanie Schulz. All authors have read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. The project was funded by the German Federal Ministry of Education and Research (BMBF) as part of the BonaRes initiative in the project INPLAMINT: "Increasing agricultural nutrient-use efficiency by optimizing plant-soil-microorganism interactions" (grant numbers 031B1062A and 031B1062C).

Data availability Raw amplicon sequences can be found at the NCBI Sequence Read Archive (SRA) under the BioProject ID PRJNA1128396 (SAMN42050636, SAMN42051589, SAMN42107788, SAMN42108963).

Declarations

Competing interests The authors declare no competing interests.

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