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Journal

Phytobiomes Journal, 9(2)

ISSN

2471-2906

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Publication Date

2025-06-01

DOI

10.1094/pbiomes-08-24-0080-r

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A Transdisciplinary Journal of Sustainable Plant Productivity

RESEARCH

Legacy Effects of Cropping System and Precipitation Influence the Core *Camelina sativa* Microbiome

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Accepted for publication 6 December 2024.

Abstract

Camelina (*Camelina sativa* L.) is a potential biofuel crop and beneficial rotation crop in dryland cropping systems. Little is known about camelina microbiota or the legacy effect of soil origin/cropping system zones on camelina-associated microbiome assembly. To explore camelina-microbe associations, we grew camelina in the greenhouse using soil transplanted from 33 locations in the dryland wheat production area of eastern Washington. Bacterial, archaeal, and fungal communities from bulk soil, rhizosphere, and endosphere were characterized with 16S rRNA and internal transcribed spacer amplicon sequencing and were analyzed alongside site-specific climatic and edaphic data. We found that soil from the highest precipitation zone had higher alpha diversity than soil from the driest zone, but this effect was not seen in the greenhouse rhizosphere or endosphere. Plant compartment, cropping system zone, and soil origin all significantly influenced microbial composition, with soil pH and organic matter, as well as precipitation at origin, as major predictors. Analysis of abundance–occupancy distributions showed that the Actinobacteriota *Aeromicrobium* and *Marmoricola* and the fungus *Pseudogymnoascus* in the rhizosphere were plant-selected, while the endosphere was characterized by a number of Actinobacteriota, *Rhizobium*, and *Clostridium*. *Sphingomonas* amplicon sequence variants were also consistently enriched in the rhizosphere, suggesting that they are present in soils collected throughout eastern Washington and may represent good candidate biostimulants. Several lignin decomposing fungi had site-specific rhizospheric distributions, suggesting that they may be dispersal-limited or result from the legacy effect of long-term wheat cropping. Overall, this study contributes to our understanding of microbiome assembly in and on camelina roots while also highlighting the potential impact of cropping history on soil- and plant-associated microbiomes.

Keywords: camelina, cropping system, microbiome, plant compartment, precipitation

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E. M. Barnes and C. Yin contributed equally.

Author contributions: C.L., S.G.T., and T.C.P. designed the study. C.Y., H.P., D.S., and C.W. conducted the study. E.M.B. and C.Y. analyzed the data. E.M.B., C.Y., and T.C.P. wrote the manuscript. C.L. contributed to funding and overall concepts of the project. All authors contributed to editing and revising the manuscript.

Funding: This work was supported by a grant from the U.S. Department of Energy, Office of Science, Biological and Environmental Research (DE-SC0021369). The work (proposal: 10.46936/10.25585/60001387) conducted by the U.S. Department of Energy Joint Genome Institute (https://ror.org/04xm1d337), a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy operated under Contract DE-AC02-05CH11231.

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e-Xtra: Supplementary material is availables online.

The author(s) declare no conflict of interest.

Camelina (*Camelina sativa* L.), also known as false or wild flax, is a broadleaf member of the Brassicaceae family (Sydor et al. 2022). Although produced in Europe for over 3,000 years, camelina production declined in favor of other crops with higher yield (Larsson 2013). Only in the last few decades has there been a resurgence of interest in this plant as a biofuel, oilseed, and rotation crop in dryland farming systems (Neupane et al. 2018) due to its short growth season (85 to 100 days), resistance to disease and pests

(Séguin-Swartz et al. 2009), and tolerance to drought and frost (Gao et al. 2018; Zanetti et al. 2017). Further, camelina can be grown under low input conditions, which is unusual for an oilseed crop (Budin et al. 1995; Matteo et al. 2020; Neupane et al. 2018, 2022), and is a promising feedstock for generating low-carbon-emission biofuels (Sanehkoori et al. 2021; Schillinger 2019; Stefanoni et al. 2020). The beneficial agronomic characteristics of camelina have been extensively investigated in the Inland Pacific Northwest (Schillinger 2019; Schillinger et al. 2012; Wuest and Schillinger 2013; Wysocki et al. 2013), Montana (McVay and Khan 2011), and the Mediterranean (Italy) (Matteo et al. 2020). Because camelina oil features substantial industrial potential and extensive applications, it would be important to optimize its production potential through a deeper understanding of factors that contribute to camelina's environmental resiliency.

Studies have shown that several plant species' growth success under low input conditions is partially attributable to their rhizosphere and endosphere microbiomes (Azarbad et al. 2020; Schlatter et al. 2019; Wassermann et al. 2023; Yang et al. 2021). To recruit these microbes, plants export a large amount of carbon to the soil via root exudation into the rhizosphere, including organic acids, amino acids, sugars, carbohydrates, flavonoids, fatty acids, and lignins (Badri and Vivanco 2009), and may also serve as food sources via sloughed off root cells, mucilage, and polysaccharides. The rhizosphere microbiome has been shown to provide numerous benefits to plants, including suppression of plant pathogens and enhancement of plant immunity (Mendes et al. 2011, 2013; van Loon et al. 1998), increased nutrient uptake and availability (Jacoby et al. 2017; Richardson et al. 2009; Rodríguez and Fraga 1999), growth promotion (Hayat et al. 2010), production of phytohormones to stimulate plant growth (Egamberdieva et al. 2017), and tolerance of abiotic stresses, including drought and heat (Lau and Lennon 2012; Rodriguez et al. 2008; Rolli et al. 2015). In addition to the rhizosphere, there is a less diverse community of microbes that colonizes the interior of the root (the endosphere community), which includes fungal endophytes, that also benefits the plant through similar functions (Fitzpatrick et al. 2018). Research suggests that this process of plant-associated microbiome assembly is not static and can be influenced over time via plant–soil feedback and soil legacy effects (Hannula et al. 2021; Kostenko et al. 2012). However, predicting when and where legacy effects occur and how persistent they might be, especially in agricultural systems where soil and plant microbiomes are highly diverse, is still a challenge. In these cases, defining core microbial taxa (i.e., taxa that consistently associate with a plant host) and identifying the assembly mechanisms guiding their distributions over space and time can reveal plant-associated microbes that may be sensitive or indifferent to soil legacy effects and thus relevant for managing phytobiomes.

Despite the potential importance of the rhizosphere and endosphere microbiomes for camelina's growth success, there have been no reports to date about the diversity and composition of the camelina microbiome or the factors shaping its assembly from the soil reservoir. This study fills this gap by describing the alternative microbial community states that can arise when camelina is grown in soils originating across a 200-km gradient in the dryland wheat production area of eastern Washington, United States. We grew camelina in these diverse soils under controlled greenhouse conditions and performed amplicon sequencing on soil, rhizosphere, and endosphere samples. We hypothesized that edaphic and environmental factors would exert a legacy effect on the soil microbiome, resulting in distinct camelina-associated microbiomes by soil origin. We also predicted that the strength of this soil legacy effect would decrease from the rhizosphere to the endosphere due to stronger selection by the plant host. Finally, we identified the core soil and camelina-associated microbiome using an abundance–occupancy model and correlated enrichment of certain microbes with environmental factors.

Materials and Methods

Soil collection and analysis

Soil was collected from 33 sites (locations) across a 200-km gradient in the dryland wheat production area in eastern Washington, United States. In this area, there are gradients in precipitation (lowest precipitation at the western edge in the rain shadow of the Cascades, increasing to the east) and temperature (cooler in the north, warmer in the south). Sample sites were chosen that included four main cropping zones based on precipitation and temperature: low precipitation (wheat/summer fallow, <300 mm/year, 15 to 16°C), intermediate precipitation (300 to 450 mm/year, 15 to 16°C), high precipitation (annual cropping zone, 450 to 600 mm/year, 13 to 14°C), and south (400 to 500 mm/year, 17 to 19°C) (Fig. 1B; Supplementary Fig. S1). Soils were collected on October 15 (low and intermediate precipitation zones), October 16 (high precipitation zones), and October 29, 2020 (south). At each location, soil was harvested from the top 20 cm of each site using a clean spade, from three locations in the field within 10 m of each other. The soil was transported to the laboratory in a cooler and stored at 4°C until used in the greenhouse experiment. Despite the potential impact of transplantation on the soil microbiome, we determined that the best way to standardize our experiment (e.g., to easily sample at the same developmental stage) to focus on legacy effects was to perform a greenhouse experiment. In addition, camelina is a potential crop in eastern Washington but is currently not grown widely in this region.

To relate soil and environmental factors to community composition, soil samples were submitted for testing at Northwest Agricultural Consultants (Kennewick, WA, U.S.A.). The physicochemical variables measured included NH_4^+, NO_3^-, pH, soluble salts, organic matter (OM), N, P, K, Na, Ca, Mg, Fe, Mn, Zn, and B.

Greenhouse experiment

To determine the potential effect of soil origin on the assembly of the camelina microbiome, we performed a greenhouse experiment in April 2021 (Fig. 1). Soils were collected from across eastern Washington and then *Camelina sativa* cultivar Suneson was grown in these soils under uniform environmental conditions in the greenhouse, allowing us to associate variation in camelina microbial communities with differences in the soil reservoir.

The camelina seed was obtained from the Eastern Ag Research Center, Montana State University, Sydney, MT. Soil from each site (approximately 1 kg) was mixed and sieved with a 2-mm mesh to screen plant debris and loaded in three plastic conetainers (approximately 179 g/conetainer, 4 cm diameter and 21 cm long; Stuewe & Sons, Corvallis, OR), and three camelina seeds were planted in each cone. Cones were arranged in a randomized complete block design in plastic racks in the greenhouse. The greenhouse temperature was maintained at 16°C in 16:8 h light/darkness. Each cone received 10 ml of water twice a week. Some of the soils had residual Group 2 herbicides such as imazamox (Beyond) that are widely used in wheat production as part of the Clearfield technology with herbicide-resistant wheat. However, *Brassica* crops are sensitive to this herbicide, and many of the soils caused herbicide damage to the camelina plants in the greenhouse. Plants from these soils were not used in further analysis, resulting in 24 unique soil origins used in this study.

After 6 weeks of growth, camelina plants were removed from each cone. Five grams of bulk soil was collected from the conetainer and stored at −20°C in 50-ml centrifuge tubes. For the rhizosphere

sample, plant roots were shaken vigorously to remove loose soil and were added to a sterile 50-ml centrifuge tube with 10 ml of sterile ddH$_2$O. Then, the 50-ml centrifuge tubes with plant roots were vortexed at maximum speed for 1 min and sonicated for 1 min to release rhizosphere soil. After sonication, the heavy soil was allowed to settle to the bottom of the tube. Samples were taken from the liquid remaining at the top of the tubes, dispensed into 2-ml Eppendorf tubes, and frozen at −20°C. This suspension contained microbes that were adhering to the root, along with some colloidal particles. Plant roots were removed from tubes, rinsed with sterile ddH$_2$O, and frozen in 50-ml centrifuge tubes at −20°C.

DNA extraction and sequencing

DNA was extracted from bulk and rhizosphere soil using a DNeasy PowerSoil kit (Qiagen, Carlsbad, CA, U.S.A.). For rhizosphere soil samples, 1 ml of rhizosphere solution was centrifuged at 15,000 × g for 2 min. The pellet was suspended in PowerSoil buffer and homogenized on a FastPrep bead beater (MP Biomedical, Santa Ana, CA, U.S.A.) using the "soil" program, then processed according to the manufacturer's instructions. Plant root DNA (endosphere) was extracted using a DNeasy Plant Pro Kit (Qiagen). One hundred milligrams of plant roots was pulverized on a FastPrep bead beater (MP Biomedical) using the "plant root" program, then processed according to the manufacturer's instructions. Negative controls were included in both the DNA extraction (no soil or root samples) and PCR amplifications (PCR-certified water) for sequencing. The DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and submitted to the University of Minnesota Genomics Center for amplification and sequencing on the MiSeq platform using standard protocols. Briefly, two-step, dual-indexed PCR amplifications of bacterial/archaeal 16S (V4 region; 515F [5′-GTGCCAGCMGCCGCGGTAA-3′] and 806R [5′-GGACTACHVHHHTWTCTAAT-3′]; Caporaso et al. 2012) and fungal internal transcribed spacer (ITS) (ITS1 region [Smith and Peay 2014]; ITS1F [5′-CTTGGTCATTTAGAGGAAGTAA-3′] and ITS2 [5′-GCTGCGTTCTTCATCGATGC-3′]) regions were conducted with an initial 20-cycle PCR followed by a second 10-cycle indexing PCR on 1:100 dilution of the initial PCR (Gohl et al. 2016a, b). Amplicons were then pooled at equal molarity, denatured, and sequenced in a single run for each target using an Illumina MiSeq (2 × 300 v3 chemistry). The raw sequence data were deposited in the NCBI Sequence Read Archive under the project accession number PRJNA1015676.

Bioinformatic and statistical analysis

Sequences were demultiplexed and processed through QIIME2 v. 2022.2 (Bolyen et al. 2019). Primers and adapters were trimmed using cutadapt (Martin 2011) for bacterial/archaeal 16S rRNA sequences and ITSXpress (Rivers et al. 2018) for fungal ITS1

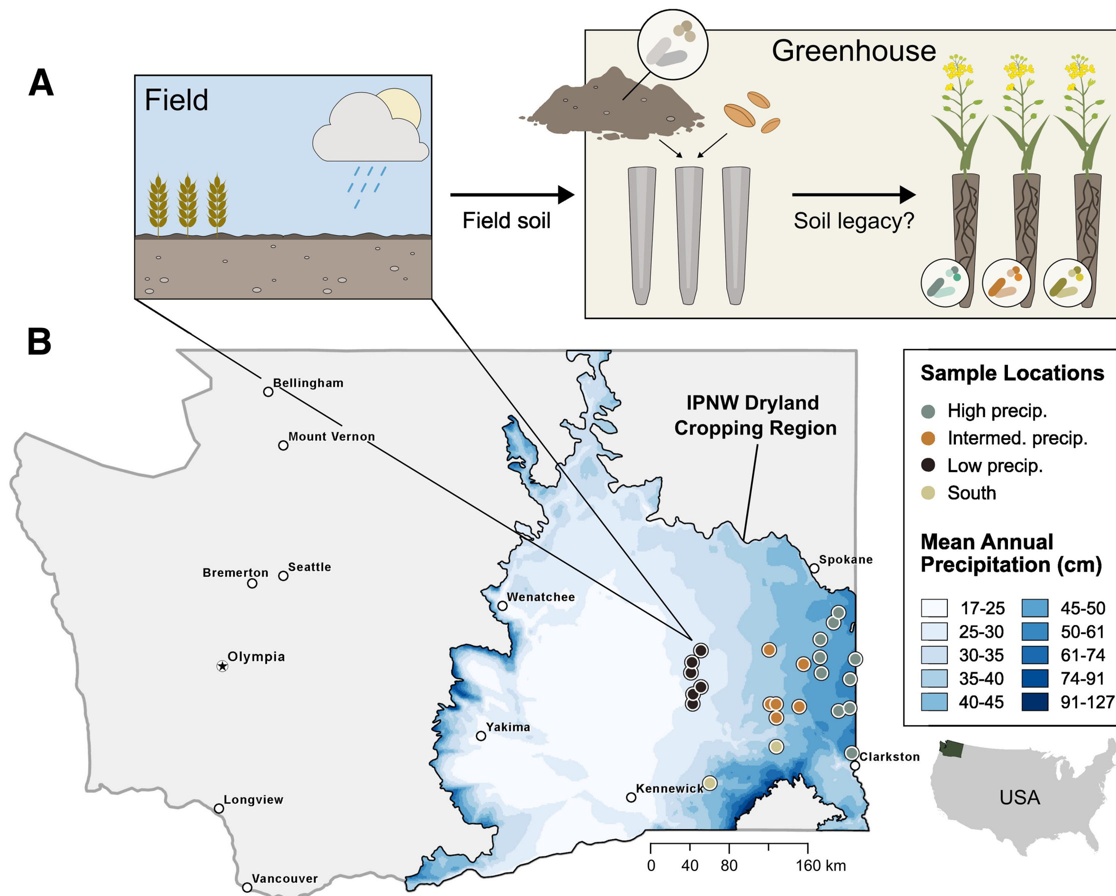


Fig. 1. Experimental design from field to greenhouse. **A,** Methodology of the camelina greenhouse experiment using soils collected from the field sites noted on the map. **B,** Map of sampling sites within the Inland Pacific Northwest dryland cropping region across a gradient of precipitation based on USDA 30-year averages. Thirty-year normal (1961 to 1990) precipitation (continuous variable but shown here as categorical for simplicity) for January through December is from the NRCS PRISM dataset (https://data.nal.usda.gov/dataset/prism). The map was created using ESRI ArcGIS Pro v. 3.1.0. A map of 30-year temperature can be found in Supplementary Figure S1.

sequences. Low-quality bases ($q < 30$ upon first encounter) were truncated from the ends of both the forward and reverse reads. Reads were denoised and merged, and consensus amplicon sequences were assigned to amplicon sequence variants (ASVs) at 100% sequence similarity in DADA2 (Callahan et al. 2016). Bacterial and archaeal taxonomy was assigned using the SILVA release 138.1 (Quast et al. 2013), and fungal taxonomy was assigned using UNITE v. 8.3 (Abarenkov et al. 2021). After SILVA or UNITE assignment, data from all MiSeq runs for a given amplicon type were combined, and 16S amplicon data were filtered for ASVs identified as chloroplasts and mitochondria. All further analyses were conducted in R v. 4.3.2 (R Core Team 2024). ASV and taxonomy tables were merged using the *phyloseq* package v. 1.46.0 (McMurdie and Holmes 2013). This resulted in 16,459 bacterial and 16 archaeal ASVs from 203 samples and 3,019 fungal ASVs from 194 samples after pruning samples with <2,000 reads for bacteria/archaea and <500 reads for fungi. Archaeal sequences were retained, although the primer set is not expected to have unbiased coverage among archaea.

We measured alpha diversity via ASV richness and Shannon diversity and tested the effect of plant compartment (bulk soil, rhizosphere, and endosphere), cropping zone (with soil origin nested within zone), and previous rotation crop via analysis of variance. We identified ASVs that were differentially abundant by plant compartment and cropping zone using DESeq2 v. 1.42.0 with a Wald test, local fit, and geometric means to estimate size factors (Love et al. 2014). We also compared community composition (after rarefying samples to the lowest sampling depth) between these treatments by calculating weighted Unifrac distances on $\log(n + 1)$ transformed ASV counts and performing comparisons of homogeneity of group dispersions (with *betadispers*) and permutational multivariate analyses of variance using the vegan package v. 2.6-4 (Lozupone and Knight 2005; Oksanen et al. 2024). We visualized these community composition patterns using principal coordinates analysis. We also performed SourceTracker analysis (v. 1.0.1) to estimate the proportion of bacteria, archaea, and fungi in each plant-associated compartment that come from zone-specific bulk soil sources (Knights et al. 2011).

Next, we calculated the core microbiome separately for each compartment using an abundance–occupancy model defined in Shade and Stopnisek (2019) to determine the microbial taxa that reproducibly associate with camelina across multiple cropping zones. As those authors mentioned, abundance–occupancy models offer an ecologically relevant approach to identifying core membership by considering not only ASVs' proportional contribution to community composition but also their detection rate (i.e., number of samples in which an ASV is found) across samples. This method allowed us to identify not only those taxa that are dominant in camelina-associated habitats but also those that may be rare (i.e., low occupancy) but functionally important to microbiome dynamics and plant health, which we can then target in follow-up studies. First, ASVs were ranked by occupancy (i.e., the total number of samples in which an ASV is found), and then we determined the contribution of these ranked ASVs to beta diversity by calculating the proportion of Bray-Curtis similarity explained for a set of core ASVs versus all ASVs in the dataset (using the R script available at https://github.com/ShadeLab/PAPER_Shade_CurrOpinMicro). Core ASVs represented those ASVs that each contributed to a >2% increase in Bray-Curtis similarity. In addition, fitting a neutral model of community assembly to our distribution gave us insight into the ecological mechanisms (e.g., determinism versus neutrality) responsible for patterns at both the population (ASV) and community (microbiome) levels. We fit Sloan's neutral model of community assembly (Sloan et al. 2006; model designed specifically for microorganisms) to our abundance–occupancy distribution using a custom R script from Burns et al. (2016) and interpreted the results based on recommendations in that paper. The model assumes a continuous cycle of immigration, reproduction, and death (i.e., local communities are saturated, and deaths are uniformly distributed in time) and does not incorporate microbial diversification. Microbial diversification is undoubtedly important at the regional scale but is unlikely to have substantially impacted diversity between plants in this greenhouse experiment (i.e., assembly over the course of a single plant generation). We decided to agglomerate fungal ASVs to the genus level before identifying the core because of (i) the limited ability of short ITS reads to distinguish fungi beyond the genus level (Kauserud 2023) and (ii) the greater dissimilarity between communities at the ASV level, which made it more difficult to identify a core. All visualizations were created using ggplot2 v. 3.4.4 (Wickham 2016).

We extracted 30-year averages for precipitation, temperature, relative humidity, and solar radiation for each soil origin at a 4-km scale using Climate Toolbox (https://climatetoolbox.org/). We then performed a canonical correspondence analysis (CCA) on samples for each compartment to assess the climatic and physicochemical variables' fit with community composition. The ordiR2step function from the *vegan* package was used for forward model selection with 999 permutations to identify soil and environmental factors that significantly explained bacterial and fungal composition in the bulk soil (with *P* values false discovery rate-corrected for multiple comparisons). Variance inflation factors for each constraint were calculated to identify and eliminate factors that covary with another factor to simplify the model and remove redundancies. The resulting subset of factors was also used to analyze relationships in the rhizosphere and endosphere. Permutation tests via the anova.cca function in *vegan* were conducted to confirm that each model, the terms, and axes explained more variation than expected by chance ($P < 0.05$). Finally, we performed Mantel tests using Pearson's correlations of ASV (Bray-Curtis dissimilarities) and soil/environmental matrices (Euclidean distances) for all compartments. Correlations were visualized using the *ggcor* v. 0.9.8.1 (Huang et al. 2020) and *linkET* v. 0.0.7.4 (Huang 2021) packages. *P* values were false discovery rate-corrected for multiple comparisons.

Results

Cropping zone, crop rotation, and plant compartment shape the camelina microbiome

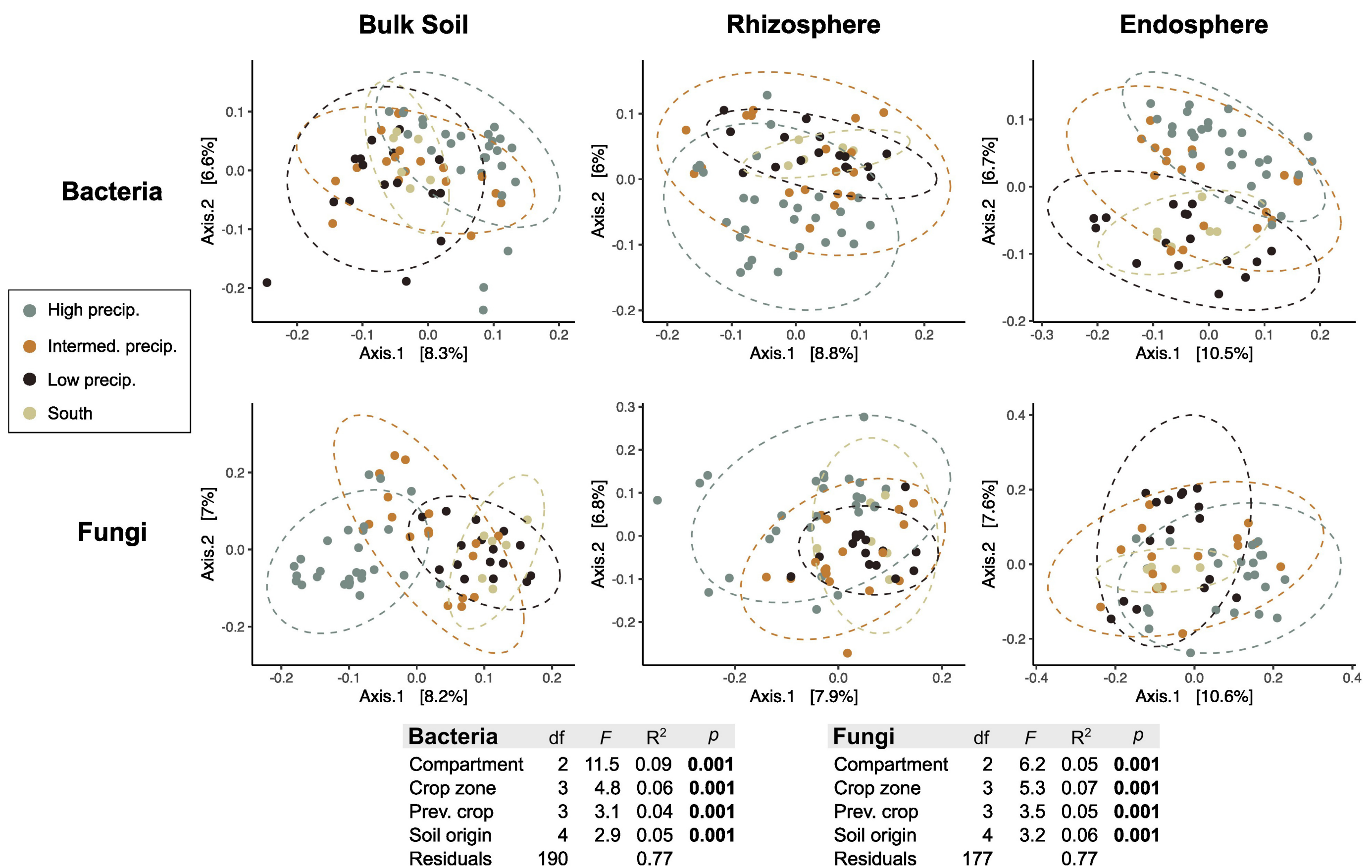
After filtering, our dataset generated 3.0×10^6 bacterial and archaeal sequences from 203 *Camelina sativa* root, rhizosphere, and bulk soil samples and 4.8×10^5 fungal sequences from 194 samples. Bacterial/archaeal ASV richness differed by compartment (with the lowest diversity in the endosphere), cropping zone, and most recent crop grown at soil origin ($P < 0.01$ for all factors; Supplementary Fig. S2A and C; Supplementary Table S1). Differences by cropping zone were most pronounced in the bulk soil, with the largest difference between high precipitation and low precipitation sites ($\text{mean}_{\text{High}} \pm \text{SD} = 460.9 \pm 121.3$, $\text{mean}_{\text{Low}} \pm \text{SD} = 328.8 \pm 109.6$). Fungal ASV richness differed by compartment, with no differences by cropping zone or previous recent crop (Supplementary Fig. S2B; Supplementary Table S1).

Compartment, cropping zone, soil origin, and previous crop grown at the soil origin all had a significant effect on bacterial/archaeal and fungal community composition, with compartment having the largest effect on bacteria/archaea and zone having the largest effect on fungi (Fig. 2; Supplementary Table S2). When we subset the data by compartment and then reanalyzed, zone and

soil origin each explained a comparable amount of variance between samples (e.g., $R^2_{\text{soil origin}} = 0.08$ to 0.10; $R^2_{\text{zone}} = 0.09$ to 0.12; Supplementary Table S2). We identified several bacterial and fungal ASVs that were significantly more abundant in plant-associated compartments as compared with the bulk soil (and no ASVs differentially abundant in bulk soil), most of which belonged to the phylum Actinobacteriota (Fig. 3; Supplementary Fig. S3). These included genera abundant regardless of cropping zone: *Aeromicrobium* in the rhizosphere and *Actinoplanes, Promicromonospora, Streptomyces*, and *Umezawaea* in the endosphere ($P_{adj} < 0.01$). In contrast, there were several ASVs from the genera *Clostridium* (phylum: Firmicutes) and *Kineosporia* (phylum: Actinobacteriota) and the family *Pseudonocardiaceae* (phylum: Actinobacteriota) that were more abundant in the endosphere of plants grown in soil originating from the high and intermediate precipitation zones than from the other two zones ($P_{adj} < 0.01$).

The largest differences in microbial composition by cropping zone (regardless of plant compartment) were found between plants grown in soils from sites in the high and low precipitation zones (Fig. 2). ASVs from the bacterial genera *Catenulispora, Microlunatus*, KD4-96 (Chloroflexi), *Clostridium*, and *Sphingomonas*, as well as the fungal family Hydnodontaceae, were particularly strongly associated with the high precipitation zone (l2FC > 20, $P_{adj} < 0.001$; Fig. 3; Supplementary Fig. S4) in addition to several other genera. ASVs from the bacterial genera *Amycolatopsis* and *Nonomuraea*; the bacterial families *Streptomycetaceae, Gemmatimonadaceae*, and *Beijerinckiaceae*; and the fungal genera *Hohenbuehelia, Macrocystidia*, and *Typhula* were more abundant in the low precipitation zone (l2FC > 20, $P_{adj} < 0.001$; Fig. 3; Supplementary Fig. S4).

To estimate the strength of soil legacy effects on camelina-associated bacteria, archaea, and fungi, we performed SourceTracker analysis by cropping zone (Supplementary Fig. S5). As expected, we found a stronger signature of bulk soil source on the rhizosphere than the endosphere. In the rhizosphere, a greater number of samples from the low precipitation zone had a high proportion of microbes that could be traced from the bulk soil of that zone, compared with other zones (Supplementary Fig. S5A). This trend was also seen in the intermediate precipitation cropping zone, and the trend was weakest in the high precipitation zone. This suggests that there is a specificity to the cropping zones (i.e., taxa in that cropping zone are more likely to colonize the rhizosphere than taxa from other cropping zones). However, we also found a signature of microbes from other zones in samples that would otherwise not mix in the field due to geographic separation. This could be due to many factors, such as possible contamination/mixing of soil from different zones in the greenhouse, intra-zone variability, or the differential abundance of a cosmopolitan ASV in a specific source. We found no trend of a cropping zone preference for the endosphere, and most of the source was identified by an "unknown" fraction, which may in part represent microbes associated with camelina seed or the greenhouse, or taxa found in all zones and therefore could not be confidently assigned to a single zone. In contrast to bacteria, the rhizosphere effect with fungi was much weaker (i.e., a smaller proportion of taxa could be traced to the bulk soil; Supplementary Fig. S5B). The strongest cropping zone preference was found in



Bacteria	df	F	R^2	p
Compartment	2	11.5	0.09	**0.001**
Crop zone	3	4.8	0.06	**0.001**
Prev. crop	3	3.1	0.04	**0.001**
Soil origin	4	2.9	0.05	**0.001**
Residuals	190		0.77	

Fungi	df	F	R^2	p
Compartment	2	6.2	0.05	**0.001**
Crop zone	3	5.3	0.07	**0.001**
Prev. crop	3	3.5	0.05	**0.001**
Soil origin	4	3.2	0.06	**0.001**
Residuals	177		0.77	

Fig. 2. Principal coordinates analyses of bacterial and archaeal (top row) and fungal (bottom row) community composition by compartment and cropping zone. Tables below the plots show the results of a permutational multivariate analysis of variance of weighted Unifrac distances with soil origin nested within cropping zone. Full statistical results can be found in Supplementary Table S2.

the high precipitation zone, and the proportion of taxa traced to the bulk soil was similar in the rhizosphere and endosphere.

Physicochemical factors associated with soil origin influence variation of camelina microbiomes in the greenhouse

To further explore which aspects of soil origin contribute to legacy effects in the greenhouse, we performed CCA on bulk soil samples. We then used that model to estimate the fit of those significant edaphic and environmental factors with the soil and camelina-associated microbiome (Fig. 4). After removing redundant factors, we found a significant potential effect of seven soil physicochemical factors on microbiome composition in all compartments, explaining between 15 and 17% of the total variation among greenhouse samples (with 50 to 57% of constrained variation explained by the first three axes; $P < 0.001$). Soil bacterial/archaeal communities originating from the high precipitation zone were most separated from the other zones in ordination space, associated mainly with 30-year average temperature, precipitation, and OM (Fig. 4A). Variation among communities within each cropping zone was associated with soil P, Zn, pH, and Mg. Similar trends were seen for fungi, except that variation among communities within each cropping zone was associated with soil pH, P, Ca, and Mg (Fig. 4B).

Overall, pH and P were the strongest predictors of bacterial/archaeal composition in soil ($r = 0.54$ and 0.57, respectively) followed by Ca and Mg ($r = 0.45$ and 0.38, respectively), which were both positively correlated with pH (Supplementary Fig. S6A; comparisons considered significant if $P < 0.05$). However, bacterial/archaeal composition was not significantly correlated with average precipitation, temperature, and humidity but was with solar radiation ($r = 0.22$). Similar trends were observed with

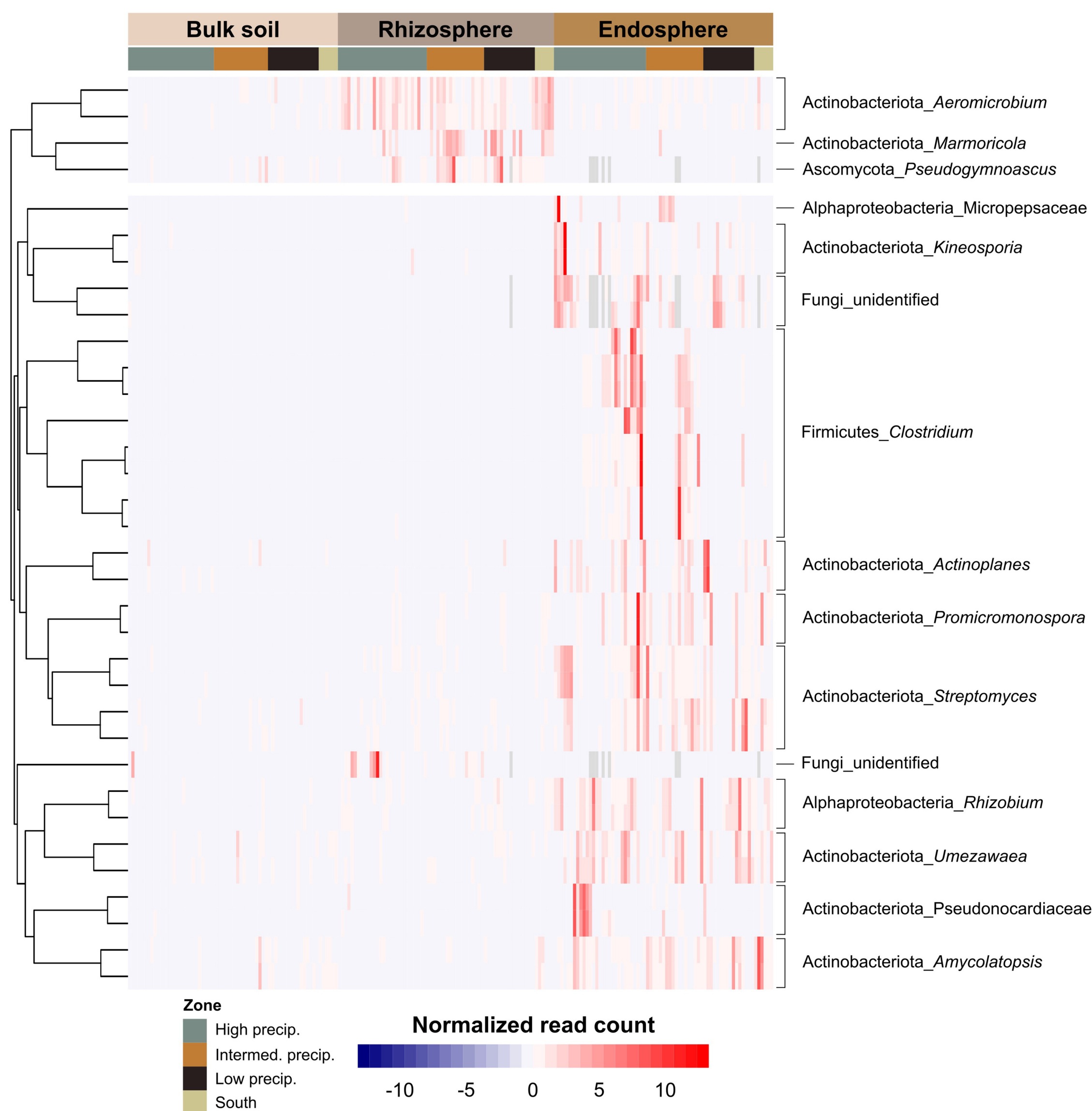


Fig. 3. Heatmap of bacterial and fungal amplicon sequence variants (ASVs) identified as differentially abundant in plant-associated compartments (rhizosphere and endosphere). Only ASVs that were differentially abundant based on DESeq2-normalized read counts with false discovery rate correction for multiple comparisons in one compartment as compared with all other compartments at $P_{adj} < 0.01$ are shown (e.g., ASVs significantly more abundant in the rhizosphere as compared with both the bulk soil and endosphere). Abundances shown are z-scores of deseq2-normalized abundances. Dark gray heatmap cells indicate samples that were excluded from fungal analysis due to low fungal read counts and thus do not have an available normalized read count. Taxonomy to the right of the heatmap shows phylum (or class for Proteobacteria) and the lowest identified taxonomy (family or genus).

bacterial/archaeal rhizosphere and endosphere communities. In many cases, the strength of the association between environmental variables and microbial composition decreased from the soil to the endosphere (Supplementary Table S3). Even still, we found significant correlations between endosphere composition and soil nitrate, OM, Fe, and Mn ($r = 0.19$, 0.18, 0.20, and 0.22, respectively), of which Fe and Mn were inversely correlated with pH. Shifts in endospheric bacterial/archaeal communities were also associated with precipitation and solar radiation ($r = 0.23$ and 0.14, respectively), unlike in the other compartments. Unlike bacteria, fungi were not significantly correlated with pH (Supplementary Fig. S6B). Soil fungal composition was correlated with 30-year temperature, precipitation, humidity, and solar radiation ($r = 0.23$, 0.26, 0.19, and 0.24, respectively). Soil and endosphere fungi, but not rhizosphere fungi, were also correlated with OM ($r = 0.21$ and 0.16, respectively). The only soil nutrients consistently correlated with fungi in all three compartments were K and Fe.

Defining a core microbiome of camelina

Environmental microbial communities are inherently diverse, which can make identifying and quantifying the importance of specific assembly processes difficult (e.g., we are unable to observe the interactions of these taxa directly, nor can we characterize most of their traits; Burns et al. 2016). By assessing the fit of our communities to a neutral model, we were able simplify this analysis and parse the influence of stochastic versus deterministic processes. Our analysis revealed 80 core bacterial ASVs and 85 core fungal genera; no archaeal ASVs were part of the core (Supplementary Data S1; Fig. 5A). Most of the core community was shared across at least two compartments (Fig. 5A). The most abundant core bacterial order in plant-associated compartments, in terms of both total reads and number of ASVs, was Sphingomonadales ($\text{mean}_{\text{Rhizosphere}} \pm \text{SD} = 12.6 \pm 4.9\%$, $\text{mean}_{\text{Endosphere}} \pm \text{SD} = 7.2 \pm 3.8\%$; Fig. 5B), followed by Rhizobiales and Micrococcales. Notably, the relative abundance of Sphingomonadales decreased, whereas that of Rhizobiales and Streptomycetales increased, in the endosphere compared with the rhizosphere (Supplementary Fig. S7). We compared the occupancy and abundance of ASVs from these orders with the neutral model of community assembly and found that they fall outside the 95% confidence interval of the model, suggesting that these taxa are deterministically selected for in these habitats (Fig. 6; Supplementary Data S1). Six of these ASVs, identified as *Sphingomonas, Sphingomonadaceae*, and *Devosia*, were overrepresented in the rhizosphere and endosphere, suggesting that they may be strongly plant-selected (higher occupancy than expected by their mean abundance; Fig. 6). Mantel correlations further revealed that plant-associated

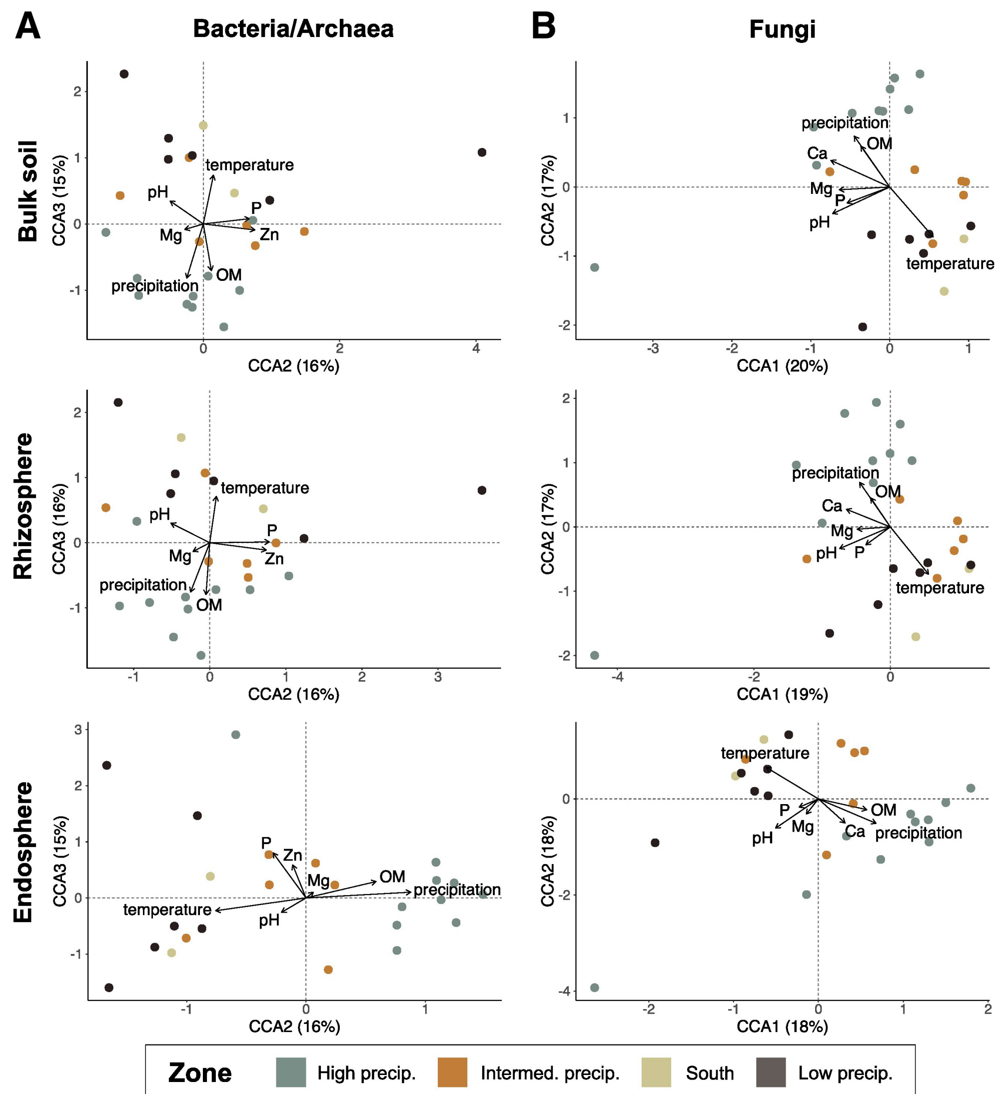


Fig. 4. Canonical correspondence analyses depicting the relationship between community composition of **A,** bacteria and archaea or **B,** fungi and environmental factors for each plant compartment. Forward model selection with 999 permutations was used to identify soil and environmental factors that significantly explained bacterial and fungal composition in the bulk soil, after which variance inflation factors for each constraint were calculated to eliminate covariates and simplify the model. Significant factors identified for the bulk soil were then applied to analyze relationships in the rhizosphere and endosphere to determine if these same factors also fit with plant-associated microbiomes. Each model, the terms, and axes explained more variation than expected by chance and suggest significant relationships between datasets ($P < 0.05$).

Sphingomonadales and Rhizobiales were significantly correlated with soil pH, Ca, and Mg (as well as Sphingomonadales with precipitation and Rhizobiales with nitrate), resulting in persistence and/or enrichment of these orders in the rhizosphere/endosphere of camelina grown in soils with these traits. It is worth noting that, as expected, correlations between microbes and physicochemical factors were generally stronger in the bulk soil than in plant-associated compartments (Supplementary Table S4). One exception to this was Streptomycetales, which were not significantly correlated with any factors in soil samples but whose abundance was weakly correlated with precipitation, OM, and Fe in plant-associated samples. Thus, unlike other taxa whose enrichment might be heavily influenced by soil legacy effects (i.e., if it is abundant in soil, it will also be abundant in plant-associated habitats), Streptomycetales recruitment may be influenced more by host selection.

The most abundant fungal orders were Pleosporales, Chaetothyriales, and Sordariales (Fig. 5B; Supplementary Fig. S8). Several genera from these orders appear to be deterministically selected, including *Chalastospora, Cladophialophora, Equiseticola, Exophiala, Knufia*, and *Paraphoma*, which were overrepresented (plant-selected) in the rhizosphere and endosphere (Fig. 6; Supplementary Data S1). Host-driven selection for certain core fungal groups is further supported by our Mantel correlations, which revealed either weak or nonsignificant correlations between edaphic factors and fungi in soil or plant-associated samples (Supplementary Table S4). Of course, we cannot rule out the possible effect of transportation from the field to the greenhouse, which could have impacted fungi more than bacteria. Still, Sordariales, Chaetothyriales, and Pleosporales were significantly correlated with environmental factors, including 30-year averages of precipitation and/or

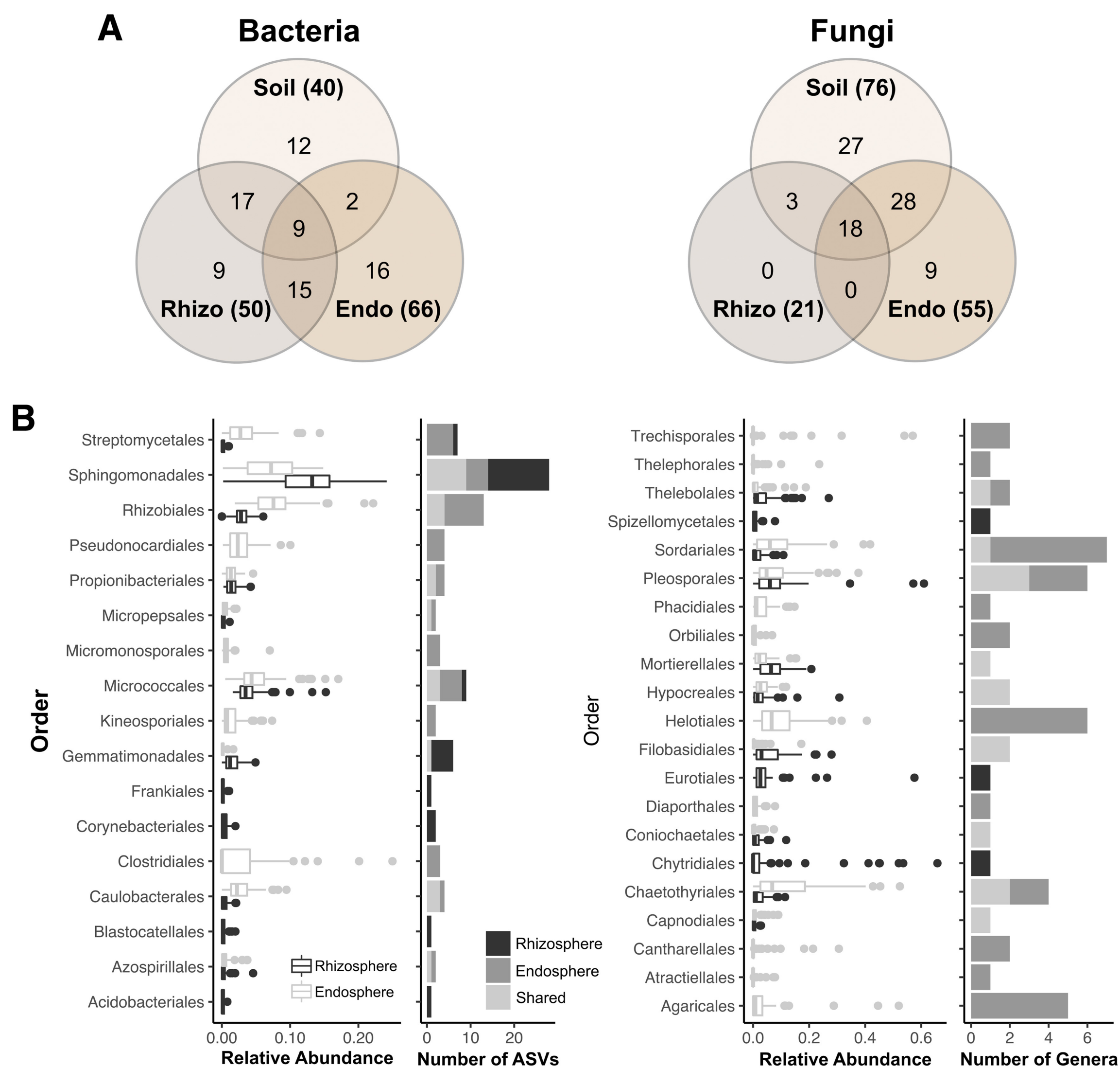


Fig. 5. Core bacterial and fungal communities of *Camelina sativa*. **A,** Venn diagram of the bulk soil, rhizosphere (rhizo), and endosphere (endo) core communities identified using an abundance–occupancy model. Numbers in parentheses represent the total number of core amplicon sequence variants (ASVs) or genera in that plant compartment. **B,** Relative abundance of core bacterial or fungal ASVs grouped by order in plant-associated compartments. The panel to the right of relative abundance depicts the number of core ASVs (or genera) represented in the relative abundance panel. Core microbiomes were identified following the methods of Shade and Stopnisek (2019). Briefly, occupancy and abundance were calculated for each taxon, and then taxa were ranked based on these metrics to estimate their contribution to compositional similarity (beta diversity). A core member had to have both high occupancy and abundance and contribute significantly to Bray-Curtis similarity in context with all the other microbes in the community.

temperature. Except for Pleosporales, most fungi were not correlated with pH or OM. Thus, assembly of core camelina-associated fungi seems to be driven mainly by host factors (and possibly transplantation), with historical patterns of precipitation and temperature driving variability rather than homogeneity among individuals.

Discussion

This paper represents the first detailed report of the composition and diversity of the camelina microbiome, including both bacterial/archaeal and fungal communities. Camelina has attracted increasing interest as a biofuel crop, in both Europe and North America. As a rotation crop, it can provide many benefits to existing cropping systems, including breaking disease cycles and reducing N requirements, especially in more marginal dryland areas (Neupane et al. 2022). The benefits of the root microbiome to the plant include enhanced nutrient uptake (Jacoby et al. 2017), increased phosphorus availability (Bargaz et al. 2021), reduction of pathogen effects (Bakker et al. 2020), and tolerance to drought (Ma et al. 2022). However, understanding these benefits hinges on characterizing the plant-, microbe-, and environment-mediated factors responsible for structuring the camelina rhizosphere and endosphere microbiome.

Legacy effects of environment on the camelina-associated microbiome

Although it is well known that environmental and edaphic properties can be major drivers of soil bacterial and fungal communities (Fierer 2017; Tan et al. 2020) in natural ecosystems such as grasslands and forests, fewer studies have been done in soils with significant agricultural history, and those that have been done focused primarily on the bulk topsoil (Bahram et al. 2018; Ochoa-Hueso et al. 2018; Yao et al. 2017). Previous environmental patterns can impact current community composition and function through legacy effects (Cuddington 2011). Monger et al. (2015) note that these legacy effects can be particularly important in dryland ecosystems, where cropping must contend with spatial and temporal variability in soil moisture. Thus, for farmers to optimize plant health, they must understand how potential legacy effects in their area influence plant microbiome assembly.

The design of our study, namely that camelina plants were grown in soils from different locations within the Inland Pacific Northwest dryland cropping region under controlled greenhouse conditions, was particularly well suited for investigating how the legacy of different environments and cropping zones affects the diversity of microbes that assemble in the camelina rhizosphere and

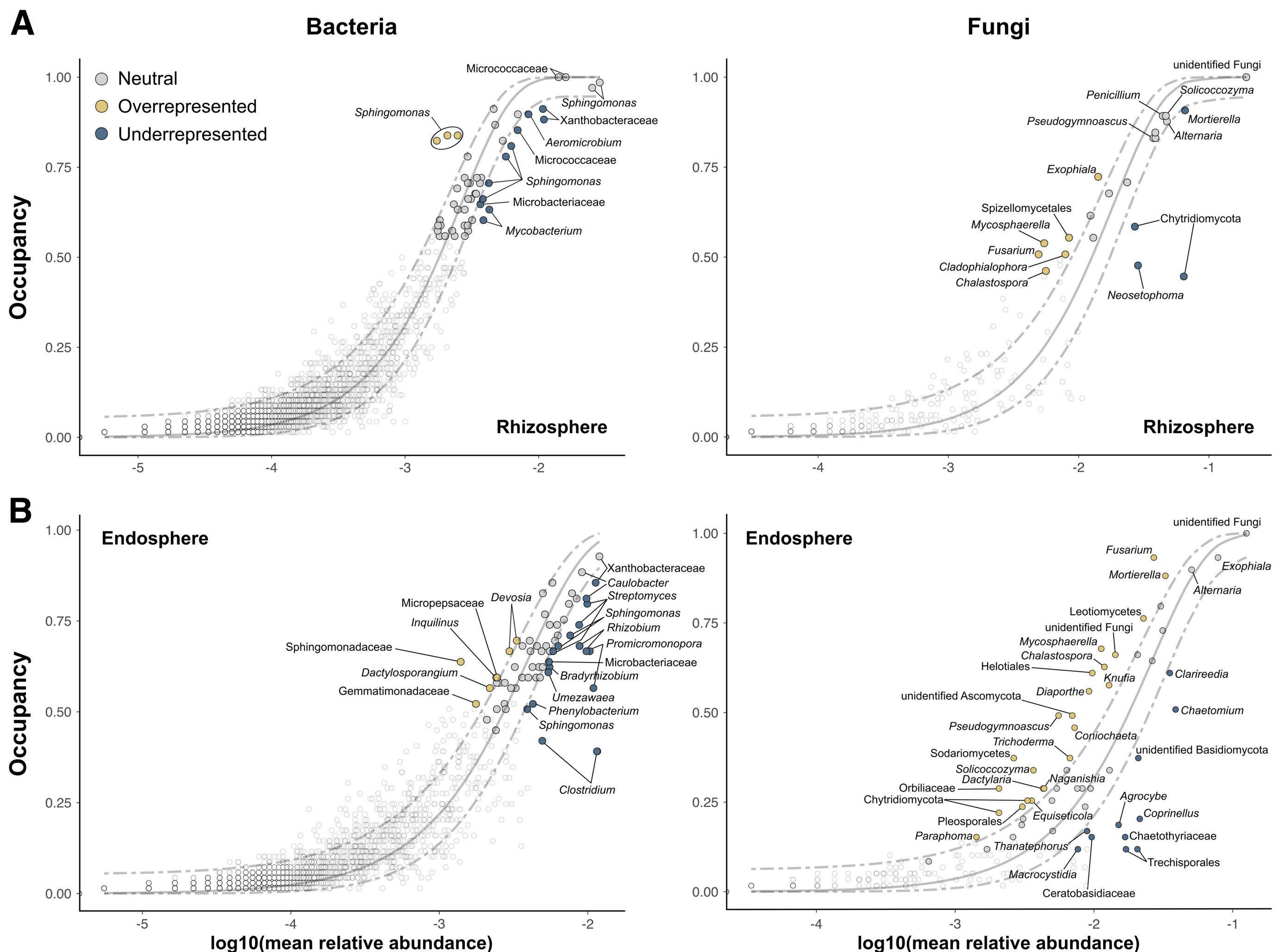


Fig. 6. Sloan's neutral model of community assembly fit to our abundance–occupancy distribution for core bacteria and fungi in the camelina **A,** rhizosphere or **B,** endosphere. The solid line represents the neutral model, and the dashed lines represent 95% confidence intervals around the model. Each point represents an amplicon sequence variant (for bacteria) or genus (for fungi) and is labeled with its lowest identified taxonomy. Core taxa are colored based on whether they fell within (neutral) or outside (deterministic) the confidence intervals.

endosphere. As expected, we found that plant compartment exerted the strongest effect on overall diversity, with the lowest diversity in the endosphere, as has been observed for the core microbiome of wheat (Schlatter et al. 2020). After controlling for plant compartment, we found a strong effect of environment/cropping zone on bulk soil diversity, with the lowest diversity in soils originating from the driest areas in the low precipitation zone, where a crop is only grown every other year due to limited soil water availability.

Although environment and cropping zone did not have a strong effect on microbial diversity of the rhizosphere and endosphere, they did have a significant effect on microbial community composition. Some of the abundant bacteria and fungi in the camelina microbiome have the potential to degrade plant biomass, including cellulose-degrading bacterial genera *Clostridium* and *Actinomycetes* and fungal lignin decomposers such as Sordariales and Agaricales (Datta 2024; Janusz et al. 2017). We believe that the abundance of wheat straw in these cropping systems may exert a legacy effect on the camelina microbiomes assembled from these soils favoring these groups. In addition, we found several microbial taxa that were associated only with specific zones. *Clostridium* was only found in the endosphere of camelina grown in soils from the high and intermediate precipitation areas but not in the low precipitation areas. *Clostridium* is a spore-forming Firmicute known to degrade fungal cell walls (Ueki et al. 2017), promote plant growth (Doni et al. 2014), and grow under anaerobic conditions (such as in the human gut). Its absence in certain camelina endospheres may reflect the lack of micro-anaerobic sites in the drier and sandier soil of the low precipitation region. Chytrids, including Spizellomycetales, were unexpectedly abundant and seemed to be found exclusively in the rhizosphere, not the endosphere, in camelina grown in soils from the higher precipitation zones rather than the drier low precipitation zone. Although *Spizellomyces* is a common terrestrial fungus (Lozupone and Klein 2002), other chytrids can parasitize fungi, algae, and higher plants and could be parasitic to other rhizosphere fungi.

Both plants and microbes can alter the abiotic and biotic characteristics of the soil environment, and these impacts can persist for multiple seasons or years (Jing et al. 2022). The presence of soil legacy effects has been studied in various systems (Mariotte et al. 2018) and is linked to overall plant performance and coexistence (Heinen et al. 2020). Further, there is growing interest in taking advantage of these microbial legacy effects in agriculture, where inoculants of plant growth-promoting rhizobacteria may provide lasting beneficial effects on plant growth by conditioning or priming the plant surface for further microbial colonization (Mallon et al. 2018; Moore et al. 2022). Given this, our study makes an important contribution to understanding how the environment and previous cropping systems have lasting effects on the microbial communities in agricultural soils.

Hallmarks of the camelina microbiome

Several taxa identified as part of the core camelina microbiome have been identified as core members of the microbiomes of other crops, such as *Arabidopsis* (also in the Brassicaceae family), wheat, barley, and canola (Bergelson et al. 2019; Lundberg et al. 2012; Lupwayi et al. 2004; Moreira et al. 2021; Schlatter et al. 2020), where they have been linked to plant growth-promoting effects including drought stress tolerance (Asaf et al. 2017; Halo et al. 2015; Khan et al. 2016), free-living N fixation in nonleguminous plants (Nag et al. 2019; Yoneyama et al. 2019), and phosphorus solubilization (Djuuna et al. 2022). These taxa include ASVs from the bacterial orders Sphingomonadales, Streptomycetales, Rhizobiales, Pseudonocardiales, Caulobacterales, and Micrococcales. Although the enrichment of these taxa appears common across agricultural crops, confirming their enrichment in “real” camelina microbial communities was critical to our future experiments using cultivated microbes for plant growth promotion.

By fitting Sloan’s neutral model of community assembly to our entire camelina microbiome abundance–occupancy distribution, we were able to provide additional insights into the potential ecological mechanisms driving the assembly of microbes in the camelina rhizosphere and endosphere. For example, several *Sphingomonas, Fusarium*, and *Mortierella* ASVs were overrepresented (high occupancy, lower abundance) in our dataset, meaning they are (i) present in soils collected throughout eastern Washington regardless of soil origin and (ii) selected for in camelina-associated habitats. Their high occupancy across the region (i.e., as native, core soil taxa), enrichment in camelina-associated habitats (i.e., efficient colonization), and putative plant growth-promoting traits (for *Mortierella* and *Sphingomonas*) make them good candidates for agricultural biostimulants in eastern Washington (Moore et al. 2022). Identifying the exact species/strains enriched across this region is a priority.

Moreover, we found that although Actinobacteria are a dominant component of the camelina-associated microbiome, likely due to their putative role in alleviating drought stress (Rao et al. 2022), our abundance–occupancy analysis showed that the camelina rhizosphere selects for a different community of Actinomycetes than the endosphere: *Aeromicrobium* and *Marmoricola* were more abundant in the camelina rhizosphere, but *Actinoplanes, Promicromonospora, Streptomyces*, and *Umezawaea* were more abundant in the endosphere. Further, most of these taxa were identified as underrepresented (high abundance, low occupancy), suggesting that their presence in soils from specific origins is due to dispersal-limitation or environmental-mediated legacy effects. Other underrepresented taxa include the bacterial genera *Clostridium, Rhizobium*, and *Bradyrhizobium*, as well as Agaricales fungi known to be lignin decomposers such as *Agrocybe, Coprinellus*, and *Macrocystidia*. High abundances of these taxa only at certain sites combined with the significant effect of previous crop identity on microbial composition suggest a strong plant-mediated soil legacy effect (i.e., the effect of past plants on current soil microbiomes; Fox et al. 2020) from historical wheat cropping.

It is worth noting that taxa with high abundance and high occupancy (found in the top right of distributions from Fig. 6), such as the bacteria *Micrococcaceae, Xanthomonadaceae, Sphingomonas*, and *Caulobacter*, as well as the fungi *Penicillium, Mortierella, Solicoccozyma*, and *Pseudogymnoascus*, appear neutrally assembled due to their placement at the edge of our model limits. In general, neutral taxa represent those whose occupancy is expected given their mean abundance, but confirming neutrality versus determinism becomes difficult for those taxa that fall near the edge of the 95% confidence interval (which is an inevitable weakness of the model). Given this, it is difficult to determine the ecological mechanisms guiding their assembly, even though their abundance and occupancy suggest they are dominant members of the camelina microbiome. In addition, we identified several differentially abundant taxa that were not considered part of the camelina core because they were “conditionally rare,” but they may ultimately be important for understanding the camelina microbiome due to their outsized contributions to microbiome assembly or community interactions (Shade et al. 2014).

Specific environmental and soil drivers of camelina communities across eastern Washington

Because we had site-specific soil chemical data for each site and extrapolated environmental data from a climate model, we could identify factors that correlated with microbiome assembly. Overall, across each compartment, a relatively small amount of variation was explained by our CCA model, from 14 to 20% for each axis, which

suggests weak but persistent legacy effects. Despite this, the model consistently grouped high precipitation zone samples separately from low precipitation zone samples. Precipitation and OM were the strongest vectors for the high precipitation zone. These sites receive from 450 to 600 mm of precipitation each year, compared with sites from the low precipitation zone, which receive less than 300 mm/year. The soils of the high precipitation areas are wind-deposited loess soils, originally a bunchgrass-mixed forb prairie, and have OM levels of 2.37 to 5.78% (Morrow et al. 2016). The soil in the low precipitation zone is also wind-deposited loess but was originally a mixed sagebrush steppe/bunchgrass prairie. In these low precipitation areas where OM is 1.4 to 1.8% (Morrow et al. 2016), average temperature was the strongest predictor of OM. In fact, the ratio between the mean annual temperature and mean annual precipitation (cm/mm) was a very strong predictor of soil OM and carbon in eastern Washington ($r = 0.81$, from Morrow et al. 2017).

We also found higher Mantel correlations between environmental factors and microbial composition in the bulk soil as compared with the rhizosphere and endosphere, and, as expected, the contribution of zone-specific soil ASVs to plant-associated microbial diversity weakened from the rhizosphere to the endosphere. It is worth noting that the use of SourceTracker to identify zone-specific ASVs has its limitations and can be substantially influenced by intra-group variability in source communities and by which samples are considered "sources" initially (Brown et al. 2019), which may partially explain the large contributions of an "unknown" source or contributions from geographically distant zones. Still, these results support our hypothesis that soil and environmental factors have stronger effects in the bulk soil compared with the plant-associated compartments, which may be more buffered from environmental effects while simultaneously experiencing a greater influence from the host plant through provision of carbon and other nutrients, as supported by Schlatter et al. (2020) in wheat. Interestingly, we seemed to find stronger relationships between environmental factors and bacterial composition as opposed to fungal composition, which is the opposite of what other studies have found (Hannula et al. 2021; Schmid et al. 2021). However, we believe this could be due to the mechanical disturbance and transplantation of soil into the greenhouse, which may have disproportionately affected certain fungal groups in the short term. We also cannot rule out that soils may have changed in composition throughout the experiment (e.g., before versus after) due in part to a greenhouse effect, nor can we comment on how persistent/long-lasting soil legacy effects may be—both of which would be worth studying in future experiments. Still, the combined results of our CCA model, Mantel test, and SourceTracker analysis suggest that soil legacy effects exist in this system and can exert a measurable effect on camelina microbiome assembly in the greenhouse.

Conclusion

Our study revealed that although both the environment and cropping zone can exert legacy effects on the assembly of the camelina microbiome, there is a core subset of microbes that associates with camelina roots regardless of soil legacy. Several members of Actinobacteriota and Alphaproteobacteria and the fungus *Pseudogymnoascus* were enriched in the plant-associated core microbiome. Some taxa of bacteria and fungi, such as *Sphingomonas* and *Exophiala*, seem to be deterministically selected by camelina. Overall, this study shows that the soil reservoir in eastern Washington is dynamic: Soils are influenced by local climatic/soil physicochemical factors as well as the biotic legacy of previous crops. In turn, differences in the soil reservoir may create historical contingencies during plant microbiome assembly, resulting in distinct microbial assemblages in/on future crops, affecting plant health and performance. This research contributes to our understanding of the assembly of microbes in and around camelina roots while also highlighting the potential lasting impact cropping can have on soil microbial communities.

Acknowledgments

We thank Bryan Carlson for his assistance with making the map of soil sampling locations.

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